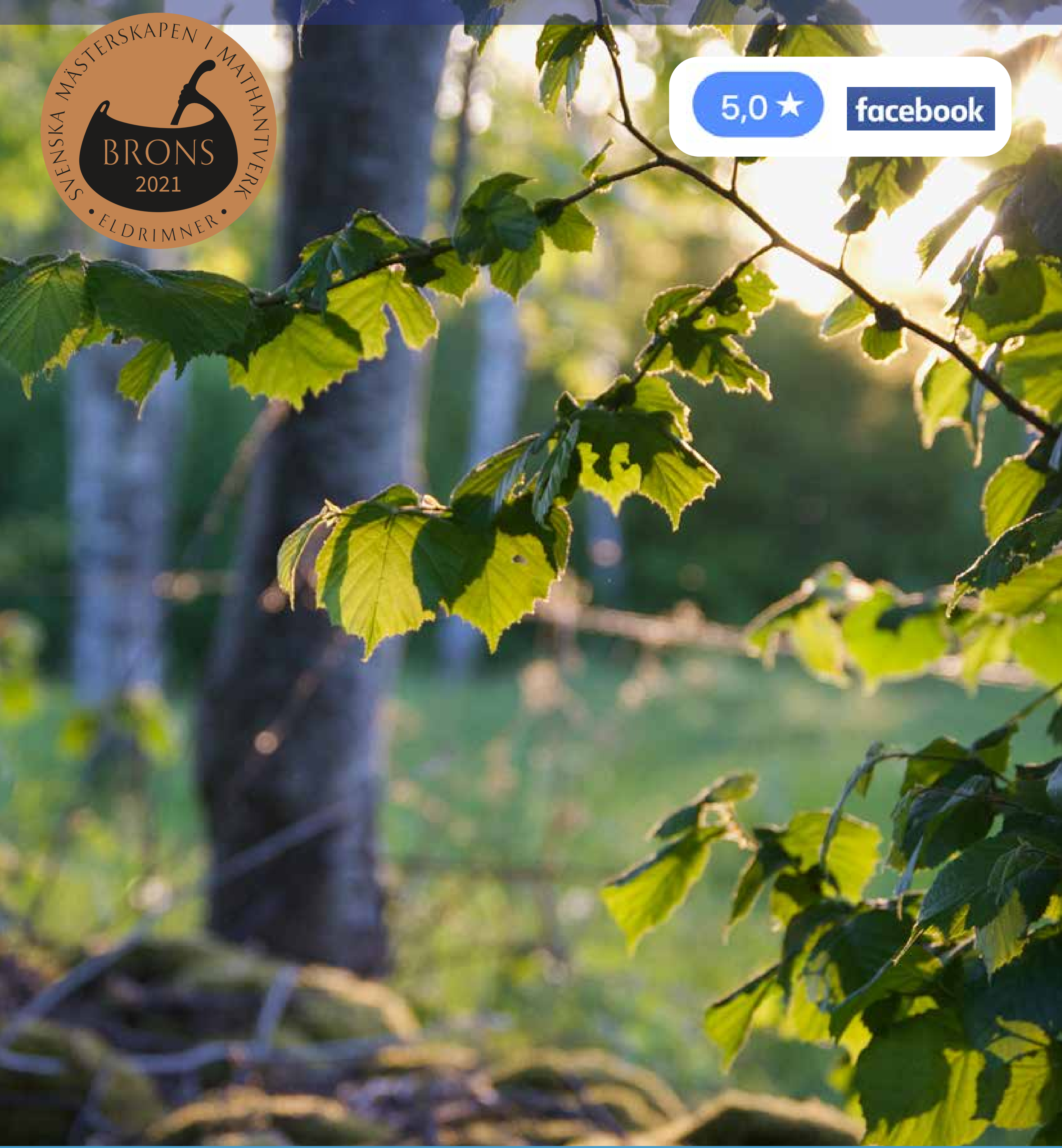


# SÅNNAHULT KURREBO 4

## Tackle the environmental challenges – Improve health and happiness – Create a space for everyone.

*Application by Livskvalitets Svenska Filial*

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> *"The location Småland is therefore a natural choice for us with it's great houses, rich fields and before anything else nature of unmatched beauty."*

# EXECUTIVE SUMMARY

**Sånnehult Kurrebo 4 is for sale and the county has asked for suggestions for projects that can fulfil the four criteria: long term perspective, openness, innovation and experience. The land is absolutely wonderful, placed next to the amazing lake Åsnen, but it is polluted with DDT as well as heavy metals.**

We suggest that the place becomes a culture house – with café, restaurant, youth hostel, bed & breakfast/hotel, shop, art gallery, and fruit tree plant school. We are successfully running a similar project in Asarum, Karlshamn: *Mormors Bakeri*, with café, vegetarian restaurant and 15 bed & breakfast rooms.

We are also running Blekinge Frukttträd Plantskola – a striving organic plant school. Doubling our turnover every year, the plant school is growing to be a significant part of our business. It has now used up its 3 hectares of land and needs desperately more space. 80.000 trees are waiting to be sold or planted.

Since 2014 these projects are run in Sweden by Livskvalitets Svenska Filial, a branch of the Danish mother company Livskvalitet ApS, established 1990 in Copenhagen, with the mission to improve the quality of life for people in general.

The little project „*Mormors Bakeri*" has grown to be a café and restaurant visited by tens of thousands of guest every summer, and rated 5,0 stars on Facebook. The food consists of vegetarian and vegan dishes, made from local products if possible, and with love and care. The cakes are famous, and people often drive from Tingsryd, Karlskrona or Kristianstad for a "fika". Busses are even coming from Germany with tourists. Our style is simple, genuine, honest, homey and cozy; we want you to relax and feel happy. We appreciate nature and the old Swedish architecture. Our garden is big, wild and full of flowers, popular especially amongst families with kids.

Our 15 bed and breakfast rooms are full all summer. We can host 150 people for lunch on a warm July day. And still more people are coming every year, giving us big problems with parking – we are growing out of this place; it is getting too small. We need more space, more houses, more garden, and more land. Nature is essential for our project; Långasjönäs Naturreservat is neighbor to our site and people come for a walk in nature as much as they come for a cup of coffee. To be close to nature, and to protect nature is very much a part of our culture.

Sånnahult Kurrebo is obviously perfect for our project; all parts of it could benefit from this wonderful location, with its precious nature, wonderful houses and unique garden with the best view in the world.

The only real snake in the paradise is the DDT, which can be a real burden, both economically and in limiting the permitted use of the place. To solve the DDT problem is therefore essential if the place shall come to use in its full right.

The Danish company Livskvalitet ApS has from its very beginning been leading research in chemistry, biology and medicine. It has used its competency to analyse the DDT situation on Sånnahult Kurrebo 4. We are very pleased to share the positive conclusion with you: we think there is a simple and useful solution for the DDT problem, which we share with you in a rapport including in this material. As always in research you never know if what works in a lab also works in reality, out there in the fields. But we are willing to give it our best try, and see if we can make it work. Can it work in Kurrebo, then we might have a general strategy for dealing with DDT pollution in Sweden. We expect this to be good news, as the problem we face at Kurrebo with DDT pollution without any doubt is a general problem we find in all the old orchards around Åsnen. It is a problem that needs a solution.

**There we say: Give us the chance to show that we can do it. We will create a wonderful place for locals as well as for people and tourists coming from far away.**

We will of course make a business also, without money we cannot pay the bills for all the tests etc. needed for the DDT project. But we know how to do that, after 10 years in Sweden. With these words we, Livskvalitets Svenska Filial, bid 4,000,000-SEK in cash for Sånnahult Kurrebo 4. If we are accepted, we expect to take over the property around June 1, 2022.

*Søren Ventegodt*
*Director Livskvalitet ApS*

*Paulina Kordova*
*Director Livskvalitets Svenska Filial*

***KEY WORDS: sustainable, nature, DDT degradation, community service, healthy living, happiness, quality of life***

# MISSION & VISION

Livskvalitet Aps
Denmark

Livskvalitets
Svenska Filial

## An Introduction

We are a branch of “Livskvalitet ApS”, established in 1990 and based on quality of life research. We started with the aim to take scientific knowledge of quality of life and health and bring it into practical use for the public. The Initiative was well received and the main activity consisted of publishing books, giving courses and running a culture house and café in the center of Copenhagen.

Further research in the connection between quality of life and the use of nature motivated us to create the Swedish branch, “Livskvalitet svenska filial” in 2012. Which is today running the **culture house “Mormors Bakeri”** – a restaurant and café in Asarum, Karlshamn, Bed and Breakfast, an art gallery and event center. Our **organic fruit tree plant school** runs as a complementary business that celebrates the traditional varieties of apples, plums, pears and cherries.

Thanks to competency and experience gained from the Copenhagen project, we could successfully established the culture house in Nötabråne. Customers appreciate the great vegetarian food, the amazing cakes, the unique beauty of the old renovated house, the amazing location close the nature reservation, Långasjönäs. The culture house focuses on the family, offering both indoor and outdoor play grounds and family oriented events.

In 2013, we started grafting fruit trees at our plant school, focussing on the many wonderful old varieties of apple, plum, pear and cherry. To our surprise this product was very sought after. The great interest led to the establishment of an online shop and which many-doubled the sales in 2018. Ever since the plant school has doubled its turnover every year.

The café has also been increasingly busy. The combined turnover of the activities grew to about 2,4 million SEK in 2021, reaching the limit for what is possible on the present location.

The rapid growth has led to an obvious **need for another location** to fulfil the public’s need for great vegetarian food and organic fruit trees. The increasing production of our medal winning organic apple juice also calls for a better sales place.

**The location Småland is therefore a natural choice for us with its great houses, rich fields and before anything else nature of unmatched beauty.**

# Mission: Improve quality of life

To inspire and motivate people to seek and realize happy and healthy living – a good quality of life – by creating a beautiful space where people can relax, enjoy, meet and learn ... to be more themselves.

We offer simple, yet high quality products; we share a friendly and including philosophy, and provide an inspiring space were you feel our devotion to the project.

# Core Purpose

Driven by love for Swedish nature, architecture, culture and people, we put emphasis on community care, growth and understanding.

# Vision

Our aim is to create a multipurpose culture house for the local community as well as for tourists.
To support the fulfilment of the needs of the people and the community.

We want to raise consciousness and appreciation of the precious and fragile nature both locally and globally.

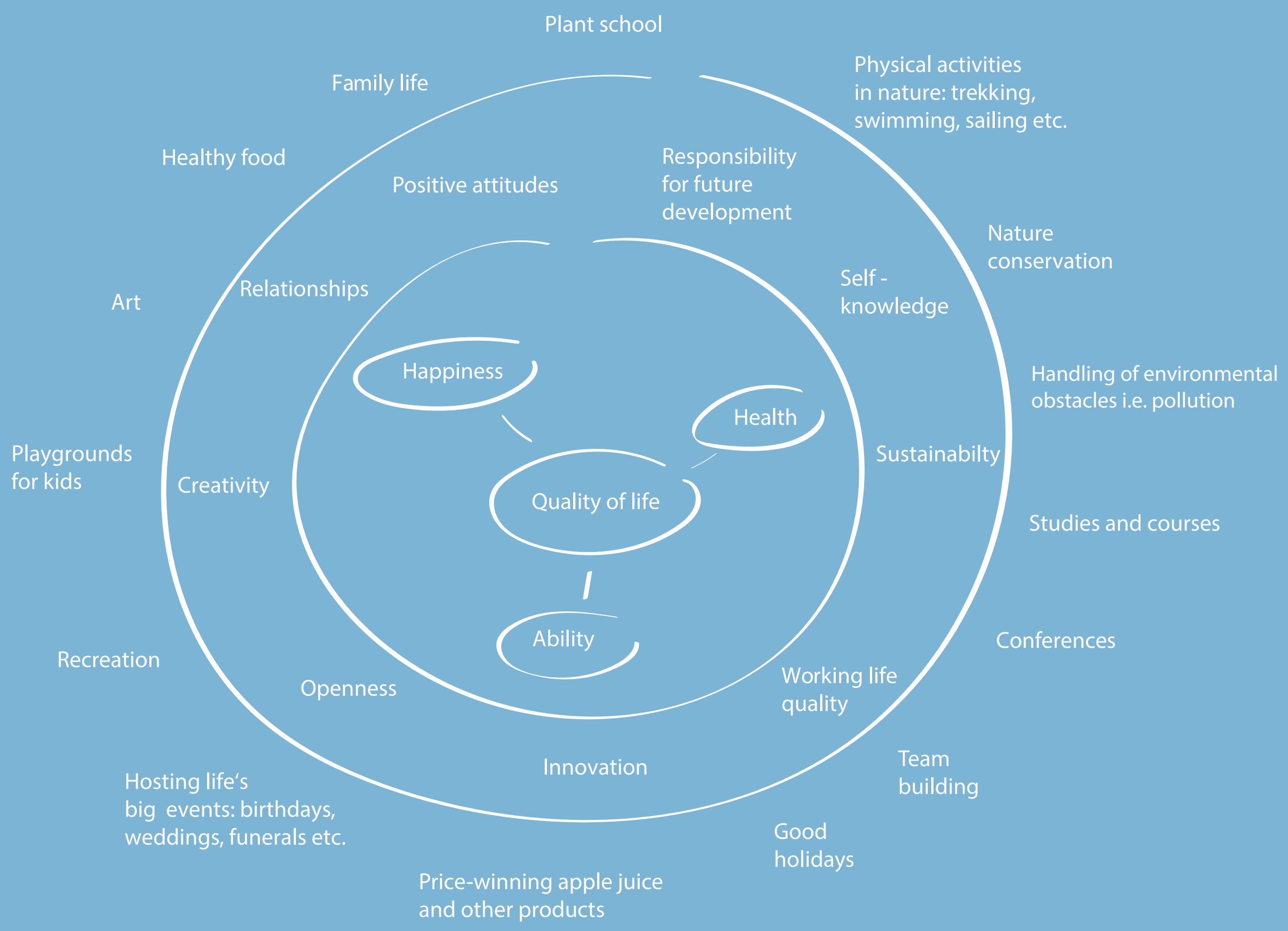
# Core Values

Our company is about taking knowledge and making it useful for people. Our core values is *quality of life* which is expressed in happiness, heath and ability.

These three derived core values are expressed in the good work (quality of working life), good family life, and a good personal life, where talents and character are used to create value for oneself and the world.

The core values give birth to a number of concrete activities like art and gallery projects, garden concerts, handling of environmental obstacles like pollution, wedding celebration, play grounds for kids, bed & breakfast, fruit tree production, production of apple juice and of course wonderful vegetarian food in our restaurant.

**Quality of life is such a broad and open subject that you can approach in 100 different ways. You can see from our business that we constantly renew ourselves and add more wonderful things to the broad spectrum of products, services and activities.**



# Serving the people | Customer Profile

In spite of us coming from rather far fetched concepts, our project is not abstract and far from earth. We have the following user profile in *Mormors Bakeri*, please note who our core target group is:

Our core target group in *Mormors Bakeri* is Swedish, woman, and around 40-45 years. And why? Because we have emphasize on the family and many mothers come to us with their children to socialize and give their children a good time.

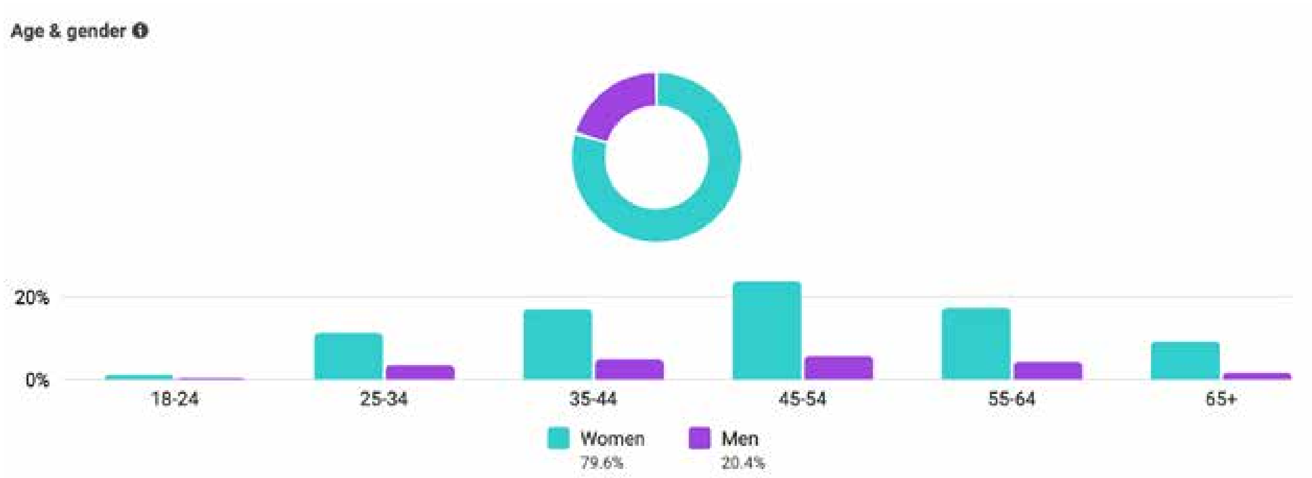



Table 1: Age and gender distribution of guests in *Mormors Bakeri* (data from Facebook)


Table 2: Nationality distribution of guests in *Mormors Bakeri* (data from Facebook)

# PROJECT DESCRIPTION

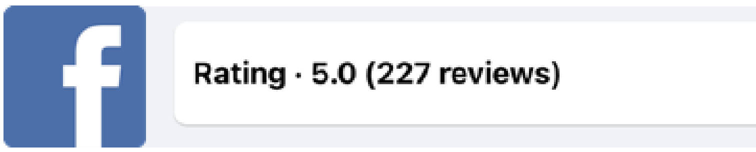
**An introduction to our running businesses and how we plan to develop and expand them for Sånnahult Kurrebo 4.**

# INTRODUCTION TO OUR BUSINESSES

- 1. Café & Vegetarian Restaurant
- 2. Bed & Breakfast /Hotel
- 3. Farm shop & art gallery
- 3. Fruit tree plant school

On the following pages we a give short introductions to our different existing project lines – all of which will be further developed and expanded if we are lucky to be given the chance to do it in Kurrebo.

Our most popular endeavour is undoubtedly *Mormors Bakeri*, a cozy café and vegetarian restaurant that during it's five years of existence have won both the hearts of tourists and locals.




Our bed & breakfast/hotel activity is born out of the need of many travellers who naturally have been attracted to our place. Hundreds of times we were asked if we had a room, because people like to stay with us. Today we have 15 rooms and during the summer they are fully booked.

The farm shop grew naturally out of the big flow of visitors being interested in our organic apples, our apple juice and preserves, honey and other products.

The art gallery was another enterprise that spontaneously happened out of cooperation with young artists. A long row of local artists and musicians have been giving exhibitions and concerts at *Mormors Bakeri* over the years.

The  fruit tree plant school has a story of it's own. On Elsebråne Organic farms grew a lot of old fruit trees of surprisingly good quality. It was just James Grieve, Cox Orange and many other well know apples – but they were undoubtedly tasty. Inspired by our findings we decided to expand the orchard and in the end we had several hundred different apples, as well as pears, plums and cherries. Many people wanted their own traditional apple tree and the fruit tree plant school was a reality. A cooperation with the national fruit collection FAST in the UK ultimately led to a unique collection of fantastic fruit trees.

All these projects are original and complementing each other.

# THE CAFÉ & RESTAURANT

A VEGETARIAN RESTAURANT AND CULTURE HOUSE

# PROJECT DESCRIPTION

*Mormors Bakeri* is a vegetarian café and restaurant, as well as culture house in a beautiful historic building from 1875 with a big garden full of roses and flowers, where you also find the bed & breakfast cottages.

We want to provide a space for people where they can take a break from their stressful daily life, taste wonderful food, enjoy cozy surroundings, art, a walk in nature, a great cake, and even a sleepover.

All our work stems from a basic feeling of love for people. We want to serve and inspire people to live a better life. Improving the quality of life has been our mission from the very beginning.

With happiness comes also health and new resources, and out of these responsibility, sustainable thinking, innovation, creativity, and a will to make the world a better place.

# Overview of Services

*Mormors Bakeri* is essentially a place where you can relax and be yourself. You can just sit and read a book, sip your tea for hours, or you can have a business meeting in our restaurant room and argue with your colleagues to find the best solution to immanent problems. You can come here with your child or partner, you can celebrate your wedding here, you can invite your parents for your birthday, or you can say good bye to a loved one over a strong beer. *Mormors Bakeri* is for all seasons and for all of life's important times.

MORMORS BAKERI

### RESTAURANT – WHY OUR VEGETARIAN FOOD IS POPULAR

It's no secret that vegetarian and vegan food has become trendy. And not only amongst the young people, many people reflect on the importance of food and believe that we need to change our ways and eat less meat. The challenge is to make vegetarian food tasty. Many guests say after a visit: "I didn't know that vegetarian food could make me happy. I didn't even miss the meat".

When it comes to food the following is important:

**TASTY** - A meal of food is playing with nuances of salty, sweet, sour, bitter, aromatic, umami, spicy, surprising, crisp and fresh etc.
**HEALTHY** – A meal contains all the body needs – carbs, proteins, fats including essential fats, vitamins, minerals including essential trace elements, fiber, sufficient water etc. .
**AFFORDABLE** – We want to compete with the low price food chains MAX, Burger King , Mc Donalds, Pizza Hut etc. except that we want to be better, healthier, cheaper and faster and before anything more sustainable…
**SUSTAINABLE** – made from local, seasonal ingredients – minimising the use of plastic and paper, using our own pro-

duced energy through electric solar panels – which we already have in *Elsebråne Organic Farm* – and plan to implement on all our properties. We also plan to use electric cars, bicycles and sustainable means of transportation.

**AESTHETICS** – *beautiful food in an interesting, cozy environment.* A major reason for our 5,0 stars on facebook and 4,5 star on Trip Advisor is the cozy and homey atmosphere we are creating both indoors in our café/restaurant and outdoors in our gardens. We are putting emphasis on traditional Swedish refurbished furniture, old Swedish paintings, lamps, cutlery, porcelain etc. Our garden is kept natural and full of flowers. The romantic, classy and homey is also the base for our wedding decorations, or our popular Christmas julbord events etc.

**MANAGEABLE** – We are using buffés to reduce the time used for serving the food and for keeping the price low. During summer our buffé is placed in the garden in specially designed outdoor furniture: a small open house where people can get food without any risk of infections and good distance to other guests as there is plenty of space in the garden for queues.

## ALCOHOL POLICY

*Mormors Bakeri* has full rights and manages alcohol responsibly. The culture is that alcohol is always following the consumption of food. Alcohol, like wine and spirits, is for celebration, not for everyday consumption. At *Mormors Bakeri* we offer a selection of fine wines from all over the world.
To make our menu complete we also offer refreshments like cocktails (with or without alcohol) from our custom designed bar.

*Homemade apple juice and other products*
Thanks to our plant school we are able produce hundreds of litres of fresh apple juice or syrups (cherry, plum, strawberry etc.) for alc. free lemonades and fine alcoholic cocktails.

## BAKERI

We are developing our own bread and cake recipes. Bread is made by hand and we offer a changing variety also with vegan and gluten free alternatives.

Paulina is leading the baking of cakes and is creating a big selection with something for everyone. Again we have a high demand in cakes for special diets like gluten free, vegan etc. and we have been recommended by many in the scene.
Cakes like our vegan and gluten free chocolate-banana cake with peanut butter topping have turned out to be most popular – not only for people with special diets.

Besides our production for the café we also prepare cake orders on demand for our customers and since two years also big wedding cakes.

Our favourite cake is apple cake made from our plant school's organic apples. We have developed 7 different kinds of apple cake which continues to be the most popular cake in our café. The layered almond cream cake is our signature creation which comes also in nougat and hazelnut versions.

We take pride in serving the most delicious cakes in the region, if possible in Sweden. To illustrate our ambition we participated in the Swedish championship in food art and won the bronze medal with our organic apple juice.




## CATERING & PRIVATE EVENTS

We are also hosting weddings and other private events like baptisms, birthday parties etc. directly at our premises. The wedding couples choose us for our unique charm, delicious food and affordable prices. Besides the location we also offer wedding planning and decorations.

Besides the in-house events we have developed a wide range of catering options. Starting with mini-caterings, frozen food options and take-away that have been especially popular during the pandemic – all possible with non-contact pick up or delivery. After many requests we even provided a catering version of our festive julbord and portions of our famous lasagne if people are too busy to cook for themselves.

Our classic catering packages make it simple for people to order either for a small get-together or a big party at home or a rented location.

People are happy for our personal service that helps them to fit the catering exactly to their needs and diets.


## GARDEN AND CULTURE EVENTS

Every spring we open the garden to our guests with the possibility to sit outside and enjoy the awakening flowers. In summer the garden becomes a beautiful oasis full of blossoming roses and trees. Thousands of people from all over the world are then finding a spot on the many tables or our balcony.

We are also hosting our own festivals. Our "Apple Festival" has become an established event in the region every September and is growing bigger every year. Since two years we also host a "Spring Festival" with plants, crafts for kids, loppis, and mini-workshops. We have invited several artists that have exhibited their work and made life-demonstrations for our visitors.

Concerts are an important part of our culture in the Summer. We support young local artists but have also hosted international bands and musicians. In 2019 we partnered with "Studiefrämjandet " to host as series of concerts (Musik i Nötabråne) for young musicians to present their talent and get experience on stage.

In the winter we are becoming famous for our vegetarian take on the traditional Swedish "julbord".

**KIDS**

Kids are honoured guests at *Mormors Bakeri*. So of course we offer half price kids menus, both at our restaurant and the bed and breakfast –children under 4 years are even for free!

We want to empower the family, creating a place where parents can eat and relax, enjoy nature, social exchange while the kids are playing in our indoor play room full of different toys or the handmade outdoor playground with swings, trampoline, tree house etc.. The safety of children has highest priority and is considered in all aspects.

# Problems, Solutions & Benefits

### HISTORIC RESTORATION

The main house was built in 1875 and the bakery house with a big baking oven was built next to it a few years after. The historic bakery closed officially around 1944, when the new road to Karlshamn opened and led the traffic away.

When we took over the place it was an abandoned and condemned ruin. The real estate agent told us the house could not be saved. We only paid for the parcel, not for the house.

We saved this amazing place, we restored it so today you can see the historical building, built in a quality of craftsmanship no longer available. Every piece of wood was cut or axed by hand, you will not find two of the same pieces of wood in this house. That gives the place a magical charm.

We have **gathered expertise** in taking care of old houses and keeping them in their original state. We will be most happy to bring this expertise to Kurrebo.

*It's hard to imagine this being the state of the property before our renovations*

## OUTDOOR KITCHEN

The house only had a small kitchen so we had a problem when we wanted to transform it into a café and restaurant. Either we were rebuilding the house – changing the architecture and adding another section – or we were building a new kitchen somewhere else. We found an original solution in the form of an outdoor kitchen, with seven big wok burners, combined delivering an effect of 70,000 watt. This allows us to make food for the 150+ guests that visit us every summer day for lunch.

We like to be in contact with our customers so our kitchen is placed in the garden where everybody can see how we prepare our wonderful vegetarian food with many vegan and gluten free dishes that we serve for breakfast, lunch and dinner buffé which we have outside in summer and in winter inside the cozy house with classical old ovens to keep our guests warm.

## WASTEWATER PURIFICATION FACILITY

*Mormors Bakeri* is placed amongst other houses with wells that take the water from the ground. Because of that you can not make a simple infiltration, meaning you can not dispose of your grey water. This meant we could not make a restaurant. We were again meeting an impossible problem, which took a great deal of research to solve.

To solves this problem we had to draw on international expertise and we managed to get the leading developer of grey and black water solutions to Nötabråne.

The solution was to use plants (Sieve  and Iris) to oxidate the water so bacteria could take care of the waste material coming from the Bakeri. Today our grey water well is visited often by people who would like a similar solution on their land.

Planted filters can be used all over the world to purify waste water. They are simple to operate, have very low operational costs and are very reliable facilities. Beside that they are easy to fit in as a part of the garden since they contain green plants themselves and can contain flowers even in the filter.

## CORONA

In cooperation with Karlshamn county and the environmental department we have dealt with all the challenges regarding corona. Our a little less than 2000m$^2$ big garden allows us a good distance between the tables as recommended by the health authorities.

It also allows us to have an outdoor buffé – and even an outdoor kitchen as mentioned above - where the staff can work with several meters between them, and little risk of infection because of the outdoor scene.

# Product & Service Advantages

### NOMINATIONS

*Mormors Bakeri* has been nominated as the best vegetarian restaurant in Karlshamn by RestaurantGuru in 2021.

We have been recommended in several articles and by Visit-Blekinge, several big blogs have praised our food, cakes and services. VisitSweden.de has written about us and we have appeared in several newspaper articles.

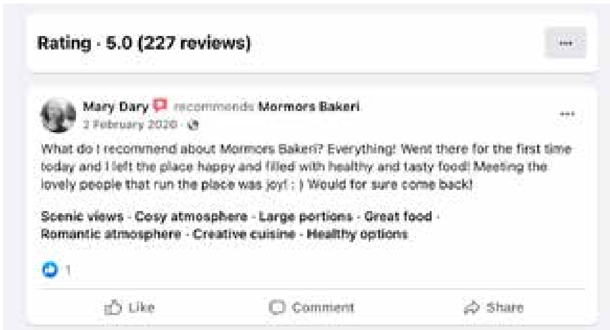
### PRICES

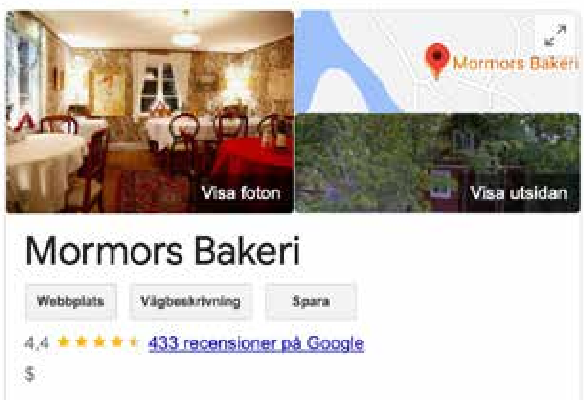
2021 we won the Eldrimner brons medal for our organic apple juice.

### FEEDBACK

Thousands have given ratings on Facebook, Google and trip advisor, which has made us one of the highest rated restaurant and bed and breakfast in southern Sweden. The low price is of course a contributing factor in this, as it keeps our guest's expectations on a humane level.

You can see a selection of feedback here or on Google, Facebook, Trip advisor and our website www.mormorsbakeri.se.




# Implementation at Sånnahult Kurrebo

The existing concept of Mormos Bakeri will be used, re-used, developed and expanded at Kurrebo. We will here give an idea about how this could look like.

**1. Expansion of the existing concept**
- fitting to meet the needs of the premises

**2. Value for the local community**
- integration in local culture
- cooperation with associations and institutions

**3. A tourist spot**
- hikers, bikers, tourists in cars and groups in buses

**4. Events**
- culture ( concerts, theatre,  local, festivals, etc. )
- weddings
- courses
- picnics, afternoon teas
- Christmas markets, harvest markets

**5. Corona/ pandemics**

## 1. Expansion of existing concept

The larger and ideally located point of sale at Sånnahult Kurrebo will increase our range in the South of Sweden. We will adjust and develop the concept. The wonderful view, the big inner and outer spaces and the uniqueness of the location invites for a very special setting. It must be romantic, classy in a relaxed way, traditional Swedish and yet modern, homey, cozy and yet with a uniqueness that make you feel that you have come to a very special place.

The many hundreds of well dimensioned square meter give space to the café, restaurant, youth hostel, bed & breakfast/hotel, art gallery, shop, and much more. Our basic idea is to keep the place open for the public and to share it with everybody who has a thirst for beauty, peace, nature–  and maybe even recreation.

We plan to grow a large number of vegetables for use at *Elsebråne Organic Farm*, where we are in process of expanding the vegetable garden to cover more than 5000 $m^2$.  In the future we might also grow vegetables at Kurrebo.

Based on our experiences at *Mormors Bakeri* we have no doubt that this place will be a profitable business. Also without having high prices for food and accommodation – we would like this place to be affordable so everybody can enjoy it. Of course we will develop products in both the cheap and the expensive end so people in need of luxury can be happy here as well, with a good room with a view and a bottle of champagne.

If you would like a budget we can provide it, based on our present business.

## 2. Value for the local community

In Asarum, Karlshamn it has been very important for us to meet the local culture and offer something understandable and attractive. We have been working in a close dialogue with the neighbours and the county. Our aim has been to understand the needs and wishes of both the private and the public segment. The success of our business has come from the local people liking what we do and we are very grateful for that.

At Kurrebo we will continue this good tradition and work hard for finding good solutions that give win-win to both neighbours and the county.

We want to inspire people to live more healthy and eat more vegetables and less meat. We want to show that the local products are great and that you can find all you need locally.

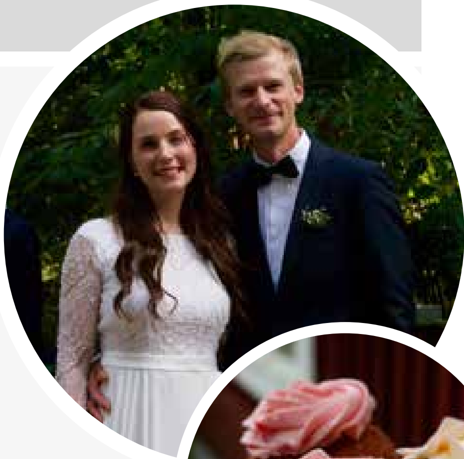
## 3. Tourist spot

We welcome locals, travellers, hikers, bikers and tourists in cars and groups in buses. It's not a secret that *Mormors Bakeri* has attracted tourists in great numbers from Denmark, Norway and even Germany and the Netherlands. It is surprising that such a small place can have such an impact, just because of a good name. We want to keep this up and we expect Sånnahult Kurrebo 4 to be a famous place for the wonders it can offer tourists.

We have actually been very sad that we had to reject a number of tourist buses that wanted to come to our place for "fika" or a good meal. We look forward to the day that we can host also these masses of tourists.

We expect a full house over the Summer in spite of Corona and restrictions.

# 4. Events



The interest in our events has been enormous which made us reach the maximal capacity of what we can do with our space in Asarum, Karlshamn. The location at Kurrebo will solve this problem and allow us to host a wide variety of happenings from big weddings to birthdays to work meetings or annual assemblies.

We plan to offer a wider range of events from cultural (concerts, theatre plays, poetry readings, local festivals etc.) to private events (weddings, birthdays, etc.).

For the café and restaurant we also see the possibility for new creative offers like “Afternoon Tea” events, Summer picnics etc.

We also plan on expanding our public events where people can participate very directly, like Christmas- or harvest markets, flea markets or similar gatherings.

# 5. Corona

In *Mormors Bakeri* we have followed the instructions and recommendations of the health authorities and we plan to do the same at Kurrebo. We have found creative solutions with “non-contact take-away”, “frozen food” and “mini-caterings” in the toughest times. We have had a good cooperation with the local authorities and the dialogue has led to a “one-way walk” through the house and a number of similar solutions necessary in a small house with many visitors. The much bigger space at Kurrebo will make it much easier to follow the recommendations in the future and the big outdoor space is ideal for spreading groups out.

# Summarized
# The Café & Restaurant at Kurrebo

## Perspective for the future

We believe that vegetarian and vegan food will be increasingly popular in the future. We also believe we need to cook more from the local products. We are therefore happy to show the way and inspire all our guests to rethinking food, patterns of consumption and lifestyle in general.

If this is true then we also believe that our guests will reward us for our continuous effort to make wonderful vegetarian food out of the local products.

## Openness

A wonderful place like Sånnahult Kurrebo 4 must be shared. We know that the area is considered to have national importance and it is exceptionally beautiful. We intend to keep the place open and welcome everybody in the future.

## Innovation

Innovation is at the heart of our project. From the beginning our aim has been to use scientific knowledge about happiness and health to improve the quality of life.

We constantly face new problems which we continue to solve in creative and original ways.

## Experience

We have 30 years of experience in project management in Denmark, and 8 years of experience with business in Sweden... in short, we see ourselves as experienced. During the last 5 years of business in our café and restaurant, with an explosive demand during the Summer, we have shown that we are able to manage even the most stressful and demanding situations.

We have never run out of food and we have rarely received a complaint. A staff of about 10 people has been able to work together constructively so we know that we can lead people and rely on our team.

## THE BED & BREAKFAST / HOTEL

SLEEPOVERS MADE SIMPLE FOR
TOURISTS AND HOME-TRAVELERS

# PROJECT DESCRIPTION

Besides the café and restaurant, *Mormors Bakeri* is also established as a Bed & Breakfast since 2018.

## Overview of Services

We have 15 cozy rooms in our summer cabins at the *Mormors Bakeri*, as well as a small guest house. Each decorated simply with wooden floors, beautiful old windows and furniture.

Not having the focus on anonymous comforts, but on personal service, we welcome our guests, to relax in nature and come back to themselves without distractions.

The classic booking is a sleepover with bedlinen, towels and breakfast buffet with fresh pancakes etc. in the morning.

### TARGET GROUP

Besides being a great resting spot for hikers and bikers along the "Blekingeleden" we also welcome tourist from all over the world.

We are specially popular with families who enjoy the kids friendly environment at *Mormors Bakeri*.

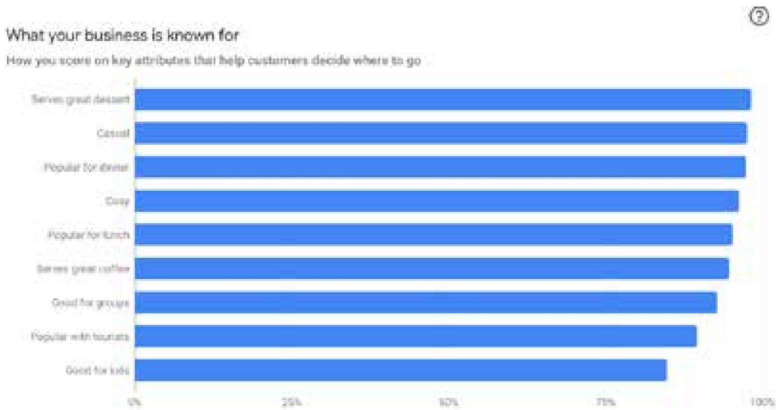
Because of our speciality in providing vegetarian food as well as plenty of vegan and gluten free choices we noticed a big interest of people with special diets who are enjoying this extra service.

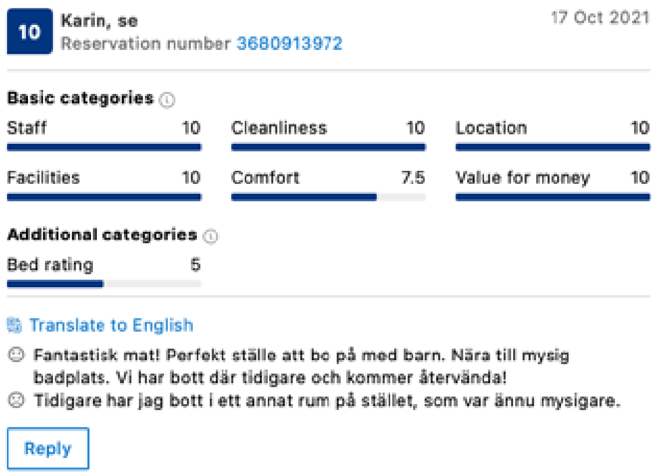
### PRICING

Also here we keep it simple. Affordable prices for simple comforts and great service.

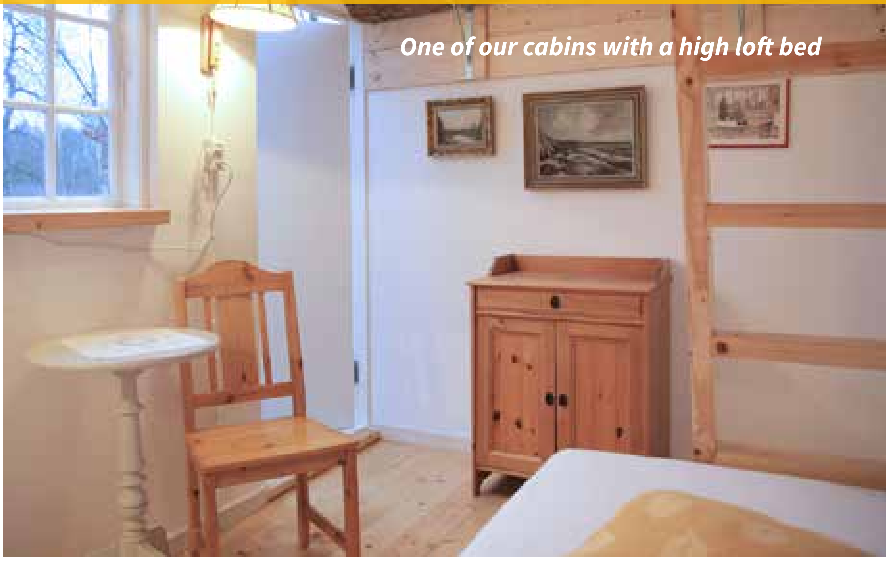
MORMORS BAKERI

Bed & Breakfast



*Breakfast included*



*One of our cabins with a high loft bed*

**SOMETHING SPECIAL**

Our guests are coming for the experience. Our unique room solutions are appealing to singles and families. Our "glamping" lavvu tent is one of our top-sellers with it's own oven and panorama windows.

**ONLINE BOOKING**

We are partners of booking.com, expedia and other online providers to reach people simply and effectively, which has led to great results and increase in bookings.

*The lavvu*

*The guest house*

# Problems, Solutions & Benefits

Our Summer cabins at *Mormors Bakeri* are uniquely fitted for the limited space of the property. We developed a loft-bed solution with high ceilings to house our guest comfortably on little space.

Our exclusive lavvu tent, the garden view, big vegetarian breakfast buffet and emphasize on personal service helps us to stand out.

From the beginning we were met with a high demand specially during the high summer seasons. Developing our own booking system helped us to meet demands of the property and guests and kept the budget low as well.

Last year we expanded the bed & breakfast with a guest house. In the theme of *Mormors Bakeri* we restored the old house and are now offering it also on Airbnb for guests who wish for more independence and privacy. We found that people are renting it also for bigger family get-togethers and we started offering package deals for sleepovers with dinner catering and birthday arrangements.

The guest house also allowed us to prolong the booking season with it's independent setting and heating system which brings more comfort in cold weather then our summer cabins which are only available during the warmer months.

We have grown with the feedback and wishes from customers. Being in dialogue with guests helps us to adjust to their needs quickly and often reveals new ways of services and income.

# Implementation at Sånnahult Kurrebo

The existing concept of *Mormors Bakeri* Bed & Breakfast will be used, re-used, developed and expanded at Kurrebo. We will here give and  idea about how this could look.

**1. Expansion of existing concept**

**2. A new wholesome experience**
- swimming
- sauna
- boating
- sports and relaxing

**3. Tourism & local cooperation**
- bus tours to local points of interest and events at the museum celebrating local heritage
- round trips and  talks

**4. Corona/ pandemics**

*foto: fastighetsbyran*

## 1. Expansion of existing business

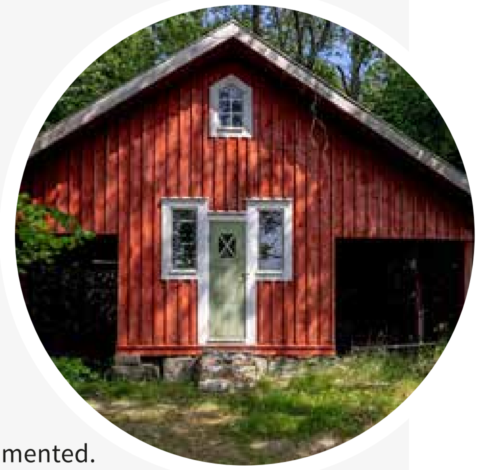
Again, the spaciousness of Kurrebo will allow us to provide bigger and more comfortable rooms for our guests. Besides our classic set up of cozy rooms with a wonderful breakfast we could offer bigger common areas and a wide range of sights close by – just to mention the natural reservation, lake and heritage museum.

There will also be more possibility for package deals, long stays and bus groups for e.g. with schools, elderly or tourists. We already see that demand currently at *Mormors Bakeri*.

More rooms and more comfort – will also be transferred to our pricing strategy, which will increase the overall profit and range of service.

# 2. A new wholesome experience

The location brings many opportunities for new services for our guests.
We wish to give our visitors a great experience.



### SWIMMING

The lake brings a whole new dimension. We would like to create an easy **access to the water** to create pleasant swimming opportunities.

### SAUNA

The boat house is an ideal location for a traditional wooden **lake sauna** to relax.

### BOATING

**Both boat and sailing tours** as well as small boat- and **canoe rentals** can be implemented.

### BIKING

We are already now **renting out bicycles** on demand to our customers and are offering equipment if one of our many biking guests needs to fix their gear. We would like to expand this service also at Kurrebo.

### TREKKING

Give access to hiking trails and day tours in the area.

### OTHER IDEAS

Besides sport **courses like Yoga, Aerobics** etc. we can also imagine cooperation with professional massage or aroma therapists etc.. We rent out **sports equipment** to our guests as well as inspire personal growth in this wonderful, supportive environment.

*fotos: web*

*foto & foto up: fastighetsbyran*

Fotos: hembygd.se/hembygdsforeningen-gamla-urshult

## 3. Tourism & local cooperation

The scenic location and many points of interest in the region bring new wonderful experiences we can offer to our guests. New adventures might range from guided tours through nature and the area, to organized bus tours to local points of interest like museums, historic churches, towns, businesses and of course nature.

We would like to cooperate closely with the local tourist office, the Hembygdsföreningen and others to offer our guests a glimpse into the local Swedish heritage and traditions.

## 4. Corona and pandemics

We expect the trend of “home vacations” to grow in the future. With an expanded offer and new services we are convinced to keep attracting guests – regional, national and international.

With our Bed & Breakfast we have followed the instructions and recommendations of the health authorities and we plan to do the same at Kurrebo. Besides special cleaning routines we also offer non-contact check-in on demand. The much bigger space at Kurrebo will make it easier to follow the recommendations in the future.

# Bed & Breakfast Summarized

## Perspective for the future

We believe that travel will be more expensive and difficult in the future. This means that great experiences you can have in Sweden will be more attractive. To create a wonderful place were even a local can be happy to sleep over, just because of the beauty and the peace and the amazing view over the lake, might therefore be a very good and long lasting idea.

## Openness

Our vision is to keep the prices low so everybody can enjoy a stay at Kurrebo, the same way as everybody can enjoy a stay at “Mormors Bakeri” in Asarum.

## Innovation

The traditional youth hostel (Vandrarhem), with it’s 6 bunk bed rooms, might seem a bit boring and uninspiring. If you come to “Mormors Bakeri B&B” you can see that we offer a bed at the same price as a normal Vandrarhem and yet the experience is much more cozy and much more inspiring. Indoor decoration with art, alternative solution of use of space etc. just give a better experience.

## Experience

We have done this for many years and we have shown that we can manage the bed & breakfast/hotel activities also under pressure during summer where everything is rented out. We are confident that we an do the same at Kurrebo. Even if the number of rooms are the double of what we have now.

## FARM SHOP & ART GALLERY

CULTURE WITH SOCIAL LIFE AS IT’S CORE


# FARM SHOP


## PROJECT DESCRIPTION

In our shop people can choose from a variety of products and bring "*Mormors Bakeri*" with them home. We have many types of syrups, jams and compotes made from organic cherries, plums, apple etc. from our 2 ha orchards. We also sell our national bronze winning juice, a jar of regional honey or a guide book to the "Blekingeleden". If our backpackers have only little space in their luggage they can buy our own organic mint tea or a beautiful postcard to send home. When guests are falling in love with our art exhibitions they can purchase an art print to take with them. We are also offering sacks of firewood for the winter months.

## Outlook

A shop will be part of Kurrebo – changing with the seasons you will find plants, fruit and vegetables, homemade jams, honey, apple juice, sweets, art prints, local arts and crafts as well as other wonderful products from farms and people in the area, books and manuals, tour guides, home goods, garden supplies and much more.

The shop will be closely interwoven with the planned info-center as well as events like Christmas-, harvest- or flea markets.

### PERSPECTIVE FOR THE FUTURE

Our conviction is that to be sustainable we need to shop locally which means **supporting local businesses**.

In case of pandemics we are able to offer non-contact pick-ups as well as **online ordering and delivery** (on minimum purchase).

### OPENNESS

We want to **celebrate the traditions of local markets** where people come together, and present their goods and talents. It can be a simple „loppis" or a cozy Christmas market. We wish to include a selection of local crafts and goods permanently in our shop.

### INNOVATION

As with our other projects the shop will not be a stiff or fixed entity. We **analyse the products** that works and constantly adjust our offer to the market.

Changing with the seasons the shop reflects our environment and by that helps to educate about seasonal products and sustainable shopping.

### EXPERIENCE

During the last years we have made a lot of experiences with our shop at *Mormors Bakeri*. We know what products works and what doesn't. We know how to **adjust fast** to new circumstances. With our **in-house design team** we are able to create appealing packaging – fast and at a low cost.

# ART GALLERY

foto: fastighetsbyran + edited

## PROJECT DESCRIPTION

Art is already a big part of “*Mormors Bakeri*” with a permanent exhibition in the small gallery, sales of prints in our shop, paintings from local artists in the house as well as changing exhibitions and performances of Swedish artists during events like festivals and concerts. We also host creative courses for young people like several print workshops.

## Outlook

Art is part of culture and we plan to make it an important part of the “Kurrebo experience”. The **gallery** will be a place for local and international artists with changing exhibitions, vernissages, lectures and performances.

The large garden, forest and lake area leave endless space for the imagination and seems perfect for **outdoor exhibitions** of sculptures and installations.

In combination with the **shop**, art prints and books can be sold as well as **local arts and crafts**.

**Painting and sculpting courses** for all ages are planned and we even imagine to host the now popular **art retreats** in combination with our hotel and restaurant for beginners and established artists. We also plan to **support artists with scholarships** like a sponsored art retreats and workspace.

### PERSPECTIVE FOR THE FUTURE

Art is and will always be part of our lives. We want to keep a fresh approach with curated high quality exhibitions and establish Kurrebo as “to go place” for art enthusiasts and creative people from all around. We already experience a big interest of tourist for “art retreats” which we plan to realize now. Even in times of pandemics art can be enjoyed outside and courses can be given online if necessary.

### OPENNESS

Art only lives with people so it is essential to keep a dialogue and fluid exchange of ideas and artistic expressions. Local and international, young and old, abstract and concrete. Everyone comes together at Kurrebo to inspire each other and dream a future that is only limited by our imagination.

### INNOVATION

The unique space of Kurrebo brings unique possibilities. Specially the dialogue and integration of art and nature opens a wide spectrum unusual exhibitions beyond a “classic” gallery exhibition.

### EXPERIENCE

We have always worked closely with artists and can rely on their expertise in the implementation of the art center at Kurrebo.


# THE FRUIT TREE PLANT SCHOOL

EVERYBODY'S PATH TO SELF-SUSTAINABILITY

# EKOLOGISKA FRUKTTRÄD

Made in Blekinge!

## PROJECT DESCRIPTION

Blekinge Fruktträd Plantskola is an **EU certified organic fruit tree nursery** that specializes in **climate-resilient** apples, pears, cherries and plums that are:

1. Very resistant to disease, drought and cold
2. Fast-growing and bears plenty of fruit
3. Can thrive under ecological growing conditions
4. Can be 100 years old

Currently located in Asarum, Blekinge, it was established in 2013. The large and growing demand of fruit trees from both private persons and businesses has been met by the plant school with **constant expansion** of the yearly tree production as well as by offering broader services (web shop, orchard planning, courses and counselling, European wide shipping solutions, etc). With a comprehensive selection of fruit trees of ca. 200 different kinds – for eg. cider production, eating and baking or preserving – everyone can find their tree(s).

BLEKINGE
FRUKTTRÄD
PLANTSKOLA

## Overview of Services

The trees of the plant school are sold bare-rooted or in pots. 50,000 fruit trees are in stock ready for sale, and in the grafting season around 20,000 trees are produced every year.

**Fruit tree production (grafting) over the years:**

| | |
|---|---|
| 2013: | 500 fruit trees |
| 2014: | 5000 fruit trees |
| 2015: | 12000 fruit trees |
| 2016: | 6000 fruit trees |
| 2017: | 10000 fruit trees |
| 2018: | 15000 fruit trees |
| 2019: | 20000 fruit trees |
| 2020: | 22000 fruit trees |
| 2021: | 18000 fruit trees |

The plant school **strives to preserve the old fruit tree varieties**. The grafting material comes from our own arboretum ("Elsebråne Pommet") where we have more than 300 different types of fruit trees, which we grow under ecological conditions, to be able to select the varieties that do best in organic plantations. Elsebråne Pommet has joined collaborations with the nursery "De Gamle Sorter" of Boi Jensen in Denmark and "FAST" in England, which has more than 7000 varieties of apple trees from around the world.

Coming to know the market during the recent year, the plant school has **recognized and acted upon the growing demand** of (organic) juice and cider trees, of eg. cider factories. Therefore increasing focus has been paid to providing cider


*For several years we have been in an ongoing cooperating with* **Stora Coop in Karlshamn**

www.blekingefrukttradplantskola.se

and juice trees, eg. Foxwhelp red cider, Major cider, Tremletts Bitter, Ellis Bitter, Tom Putt cider, and more.

Besides producing fruit trees for a wide spectrum of usage, the plant schools offers services such as **consulting and planning** of gardens and orchards, **transport** of trees, s**oil and fertilizer**, **grafting courses and teachings** in fruit tree growing.

The trees can be ordered (with the possibility of shipment) via the plant school's web shop. In the years from 2019 to 2020 there was a five fold increase of orders made via the web shop. From 2020 to 2021 there was a **70 percent increase of orders** made via the web shop, with the average order being for 1438 SEK.

Besides the possible purchase of trees via the **web shop** and directly at the **sales place** at *Mormors Bakeri*, Asarum, the plant schools hosts a trees exhibition in front of **Stora Coop in Karlshamn** twice a year.

The big yield of fruit every year allows us to **sell fruit directly** in our shop or process them as **apple juice or compote** for reselling.

## THE ADVANTAGE OF VARIETY

The pant school offers many different old and modern sorts of fruit trees in sizes of 70cm to 5 m, which can be fitted to and combined by customers according to their needs, in relation for instance to

- the conditions on site
- timing of ripening of fruit
- use of fruit (cider, baking, preserving)
- budget
- expected and manageable yield


Examples of available fruit trees:

**Cherries:** Stella, Sunburst, Regina, Merton late, Merton Glory, Lapins, Ulster, Sunburst

**Apples:** Aroma, Ellis Bitter, Rubinola, Tremlett's Bitter, Adams Pearmain, Ire Kungsäpple, Stonehenge, Belle de Boskoop, Spigold, Vejlø, Astrakan, Major cider, Tom Putt, Arthur Turner fast, Katy, Summerred, Discovery, Transparante Blanche, Holsteiner Cox, Elrödduva, Langeland hvid pigeon

**Plums:** Ungarsk plommon, Oulliance Reine Claude plommon, Althans Reine Claude plommon, Victoria plommon Violette Reine Claude plommon, Green Reine Claude plommon, Bornholmsk kæmpe plommon, Gul Eggeplommon, Rosinplommon, Stanley plommon, Exp. Fæltets sveske plommon, Ponds seedling, Rivers early

**Pears:** Ananas de Curltail päron, Conference päron, Packhamns Triumph päron, Hochfeine Butterbirne päron, Gråpäron, Clara Friis päron, Williamspäron, Coloree de Juilette

We have also started to sell **quinces, medlars and peaches.**

## Problems, Solutions & Benefits


Bee keeping


grafting


Quinces

**Growing fruit trees organically is uncommon for a reason**. Factors of growth, pest, weed, yield etc. have to be considered and new solutions need to be found that neither include pesticides nor artificial fertilizer.

We are happy to say that we found organic solutions for these challenges:
**Constant pest and disease control** is mandatory. Regarding worms and other insect larvae which are attacking the various species of fruit trees consuming their leaves an organic solution has been found: an extensive amount of (homebuilt) bird boxes around the fields. The bird boxes attract families of small birds, which are eating and therefore containing the spreading of larvae in an non-invasive way.

Finding and **providing the optimal conditions** for the different fruit trees in terms of warmth, sun, water supply, grouping of tree sorts, most favourable soil quality and fertilizing will make the containment and eliminating of disease easier to handle by strengthening the trees' immune system. Essential was the development of a manageable and handy watering- and fertilizer system for 3 ha of fruit trees orchard especially in the summer months.

To avoid the development and spreading of disease, old and **up to date knowledge** in eg. chemistry had to be acquired. To provide the trees with **all the nutritions**, the optimal mixture of composted horse manure with the correct percentage of chalk had to be found and is now applied.

The **pollinations** of the fruit trees is managed by keeping bees close to the orchards plus by the right combination of neighbouring fruit trees.

To process the growing yield, a part of the harvest is used for apple juice production. In the year 2021, for instance, 1400 kg of apples have been juiced in cooperation with a local juice factory, which led to 850 l of organic- ready to sell- apple juice. Now, the plant schools' own juicing facilities are initiated to be built. Another part of the harvest has been used to manufacture fruity multi-purpose compotes and syrups.

### HARDY, CLIMATE-PROTECTED TREES

The trees are of the old well-known as well as modern varieties and are grafted on wild root stocks or with selected root stocks, which in addition to the good and strong properties of the wild trunk have e.g. high survival against extreme cold. We use the Antonovka apple from Siberia that survives -40 degrees.

The plant school believe that the climate will change in the future; it looks like the weather will be more extreme, with more heat and drought in the summer and more cold in the winter. This means that **fruit trees must be able to withstand heavy frost and dehydration** to produce a lot of good fruit in the future. We therefore choose to graft our trees on roots, which give the trees exactly these characteristics. They are fast-growing tree types ( wild types or similar ) with very large deep roots.

### PRICING

The ambition of the plant school is to **offer affordable or-**

**ganic trees**. A lot has been done to develop the production method so that the plant school can be competitive.

The store is located at *Mormors Bakeri* in Nötabråne near Karlshamn, where customers can pick up the trees after ordering or where visitors can choose from the fruit trees exhibited in the garden sales place. The plant school provides know-how about orchard management, old and modern fruit types and root types.

### DEVELOPMENT

Besides, with the help of the **Blekinge Fruit Initiative** the goal of the plant school is to create about 1000 hectares of orchards in Blekinge, and thus being established as one of the leading fruit producers in Sweden. We hope to get around 100 large and small fruit producers to join the collaboration. According to experience, an orchard of 10 hectares is large enough to create an income for a family.

### WARRANTY

As a speciality, the plant school offers a **1 year growing guarantee**. Under the precondition that customers are taking well care of the bought trees (watering, fencing, fertilizing etc.), a warranty applies which give customer the right for one year after the purchase to receive money back for a tree which had died or to choose a new tree.

### WEB-SHOP AND SHIPPING

Online sales have increased dramatically and we have teamed up with shipping partners like DSV and suitable sales interfaces. From **packing and shipping of large trees**, to **processing** orders and extended **customer service** and **consulting**. Our expertise has been growing with the challenges.

## Product & Service Advantages

The plant school has developed solutions for private gardens, for resellers of trees, and for farmers with land who want to make orchards.

For **private persons**, who want fruit in their garden, the plant schools offers fruit trees, which can be chosen according to the trees properties and the customers wishes. There is always the options of the plant school advising on which trees to choose. The purchase can happen via the plant schools web shop, or order can be made by email and phone. Self pick up and payment of orders can happen at the plant schools sales place at *Mormors Bakeri*, Asarum.

The plant schools is also open for **cooperation with resellers**. There are many trees in stock. The usual deal is that trees are sold by the reseller for 40 percent of the sales place.

The plant schools is also eager to **increase the amount of fruit trees orchards in (south) Sweden**. Under the motto ”Let’s grow apples together! You contribute the soil, and we contribute the trees!¨” the plant school has set in motion

the Blekinge Fruit Initiative - a new collaboration between “Blekinge Fruktträd Plantskola” and fruit producers in Blekinge.

Blekinge Fruktträd Plantskola has for several years investigated which root stocks and fruit types are **best suited for organic production in Blekinge**. This experience is now made available to those who want to be fruit producers in Blekinge. Blekinge Fruktträd Plantskola is good at delivering fruit trees and its arboretum has access to grafting material from over 200 different types of fruit, mainly apples. The nursery will for the next 10 years be able to deliver trees to anyone who wants them.

For instance farmers, which want to turn the land into an orchard, the plant schools offers  a collaboration where the farmer contribute with soil and where the plant school delivers and plant the fruit trees that are requested. The land owners´ job is to put up fences, mow the grass around the trees etc. The harvest and the selling of the fruit can be done together and the income  will be divided in proportion to how the efforts are distributed.

# Implementation at Sånnahult Kurrebo

The one thing we are lacking for our plant school is space. The setup at Kurrebo is ideal to expand, given that it was already used as a plant school previously.

**1. Expansion of existing business**
– Website
– Cooperation

**2. Apple picking and fruit sales**

**3. Juice manufacturing**

**4. National clone archive**

**5. Local cooperation and teachings**
– Information center
– Cooperation with local institutions
– Cooperation with local businesses

## 1. Expansion of existing business

In truth we have reach the limit of the fruit trees we can produce on the limited space we have today. If we are allowed to buy Sånnahult Kurrebo 4 we plan to raise the production to 50,000 trees in 2023. We also plan to make an international online shop that sells trees to all Europe.

The larger and ideally located point of sale will increase our range in South Sweden. With the area's fame for apple production come a lot of opportunities for cooperation with other local businesses and new customers.

## 2. Apple picking and fruit sales

We also have a dream that in the future our guests will pick their own fruit at Kurrebo. Because nothing is more wonderful then picking your own apples.
Again in a dialogue with authorities we will develop the place. We imagine be both to have self picking areas (with focus on experience and sense engaging ) and the tradition-al harvest by the organization for the purpose of sale or production.

# 3. Juice manufacturing

We will expand our already existing juicing production.

**EXPERIENCE**

For creating the most tasteful juice, knowledge of the right combination of the available sorts and their qualities had to be collected and tried out. Dimensions which have to be acknowledged in this process are: ripening times of fruits, their sourness, bitterness and sweetness as well as the perfect blending of fruits with their different properties.

In short: The development of an outstanding juice recipe had to be made. This recipe was awarded with the bronze medal in October 2021 during the national food championship.

SVENSKA MÄSTERSKAPEN I MATHANTVERK

BRONS 2021

• ELDRIMNER •

**WINNER OF THE ELDRIMNER BRONZE MEDAL 2021**

"En frisk och syrlig äppelmust med härligt fruktig eftersmak!" is what Eldrimner called our juice at the SVENSKA MÄSTERSKAPEN I MATHANTVERK where we won the third place competing nationally in Sweden in 2021.

**VISION**

We plan to expand the juice production in the years to come and increase the quality so our organic juice one day, will be the best in Sweden.

We also have an ambition about establishing our own juice factory, hopefully a place that will offer good jobs to the community.

*foto: fastighetsbyran*

## 4. National clone archive

We plan a national clone archive of all the old varieties so they are preserved for the future and people can come, taste and explore the wonders of the old apple and fruit types. Today most people know 5 different types of apples from the supermarkets and these have often been stored for many months in basements, preserved with carbon dioxide. A naturally ripe fruit, picked directly from the tree of one of the great old varieties is an unmatched experience. There will be a map for visitors that allows them to identify the different fruit trees and taste the fruit in the season.

## 5. Teachings and courses

The new space will allow more room for bigger grafting workshops as well as teachings in planting, historic fruit sorts, etc. A place for everyone to come and learn about sustainable fruit growth and gardening.

## 6. Expansion of the Orchard

Today we have 2,5 ha of orchard at *Elsebråne Organic Farm*. We would like to expand this to 5,0 ha (with 2,5 ha more in Kurrebo) so we have a sufficient apple production for a small juice factory.

# Plant school summarized

## Perspective for future

**We believe that Swedish grown fruit has a big future. For many years fruit has come from Southern Europe and America. But with the increasing prices for fossil fuels and transportation it will be less attractive in the future. And locally produced fruit will be in high demand.**

**We also want a cooperation with local farmers for establishing common orchards. We hope to reach 100 ha of new plantation in Southern Sweden.**

**We have meet an increasing demand for our organic fruit trees and believe the market to be even bigger in the future.**

## Openness

**We are happy to share our knowledge about the varieties of fruit, how to grow fruit and how to keep the trees healthy and free of pests in organic and sustainable ways. We plan to have maps where interested can find the trees and explore the different varieties in the fruit season.**

## Innovation

**In our project we have researched the different types of root stocks and have found great qualities in the Antonovka root stocks from Siberia. Apples grafted on this rootstock are healthy, resilient to draft and frost, fast growing and in modest need of fertilizer. They seem to be perfect for organic farming. They also last 100 years.**

## Experience

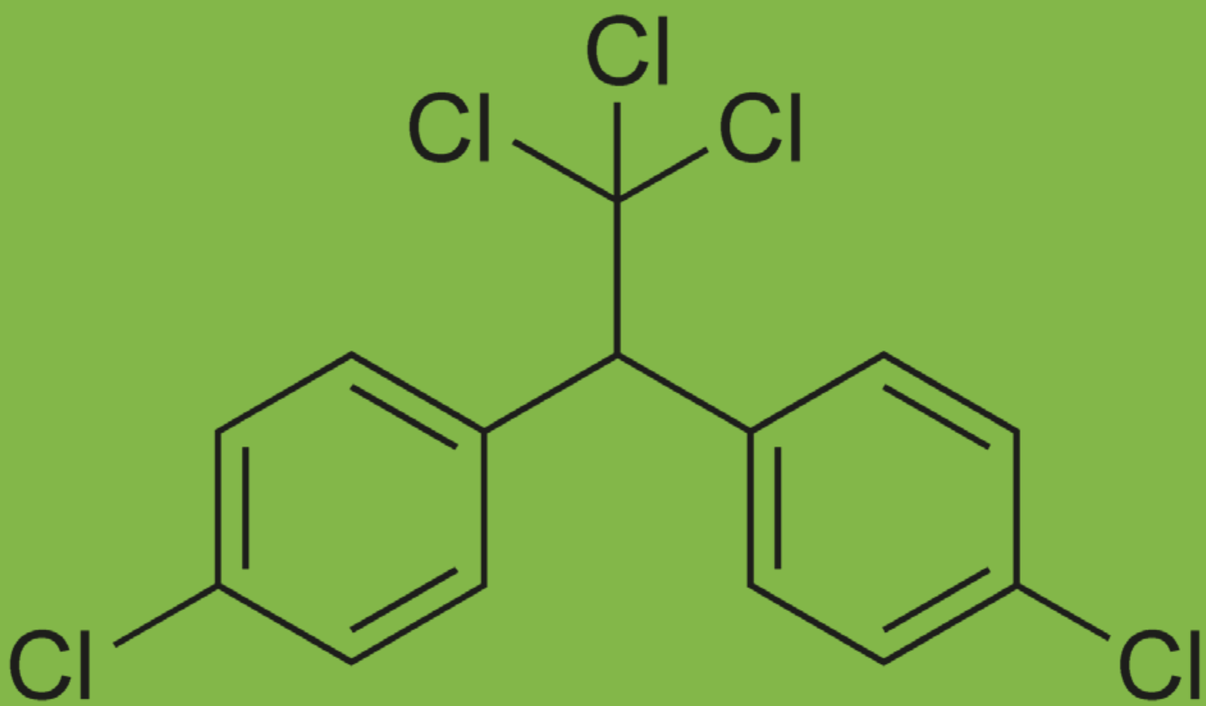
**9 years of experience have taught us a great deal about growing, grafting, selling etc. of fruit trees. We have developed many new methods for production, to keep effectivity high and the production cost low. This has been necessary to compete with the producers from Poland and Southern Europe. We are happy to say we have managed and we expect to be even better in the future.**

# DDT HANDLING

## Rapport on handling the DDT pollution in old orchards around lake Åsnen treated with substantial amounts of DDT between 1940 and 1974.

**By Søren Ventegodt MD**

**2022-01-16**



# Rapport on handling the DDT pollution in old orchards around lake Åsnen treated with substantial amounts of DDT between 1940 and 1974.

**By Søren Ventegodt MD**

**2022-01-16**


## ABSTRACT

DDT is an insecticide used in many orchards in Sweden; it is toxic and stays in the soil for many years, where it is hard bound and insoluble in water. It takes typically 20-30 years before half the DDT is out of the soil, meaning that a pollution can stay for hundred years or more. When high concentration of DDT is found in the soil, the soil is often removed and taken to a land dump, leaving a sad scar on the land that now misses its good soil.

During the last decades substantial progress has been made; we have gained a lot of scientific knowledge allowing us **in principle** to accelerate the half-life of DDT in the polluted field, from decades to years, or even months or weeks. In the lab where the circumstances are ideal can half-times of 22 days or even for DDT less be seen. Still, DDT breaks down to DDD and DDE, which degrades slower, nobody knows how slow.

The important factors that will allow an acceleration of DDT and its sisters DDE an DDD, always following DDT and as toxic and DDT itself, is air, water, iron combined with rich amounts of a broad variety of micro-organisms, both bacteria and mushrooms.

The perfect solution seems to be to mix 10% manure into the soil, ad iron to it, then cover it with straw or hay, and keep it appropriately wet while the process is running.

This is a lot of manure, limiting how much land can be cleansed ever year, as there is a limit for how much manure (containing much K, P and N) you according to the law can add to a piece of land.

If you identify the areas that needs to be cleansed; if you accept that you only work with the top soil; if you accept that this procedure will take many years, then there should be a simple, cheap, effective and sustainable solution for the DDT pollution problem.

We would like to test this in a research project at Kurrebo 4. We believe that, if successfully done, this can lead a way to a general strategy for dealing with DDT pollution in Sweden and elsewhere.

## PREFACE

- Reuters, Sep 16, 1999:

**Cow manure to mop up DDT (70)**

Sheffield, England - Canadian researchers are using chicken droppings, cow manure and waste paper in a new technique to clean up land contaminated with DDT and other dangerous pesticides.

The new technology, which has been tested in Florida, uses bacteria in the soil that feed on organic waste to break down DDT, one of the so-called "dirty dozen" toxic pollutants, into harmless by-products. Dr Neil Gray of Sheridan Park Environmental Laboratory, an Ontario subsidiary of the Anglo-Swedish drugs group AstraZeneca, told a British science conference that the new bioremediation technology is cheaper than other methods of dealing with contaminated soil, such as incinerating it or burying it in a landfill.

"This is a new technology. We've just received the first of three (US) patents on the technology, and we've had three more approved just last week. So it is fairly new off the books," he told a news conference.

"This new process is not only cost-effective, but also has an excellent overall environmental profile," he added.

Rom
The system uses microflora or bacteria that thrive on organic waste such as cow manure and chicken feed. Although DDT is banned in most western countries, it is still being used to control malaria in many developing nations.
"It is a chemical that is fairly persistent in the soil until you actually treat it," Gray added.

In pilot studies in the laboratory in Canada, the researchers were able to get 97 percent degradation of the DDT in eight weeks. Further tests on a contaminated site in Tampa, Florida were also promising. But Gray said the system would have to be customised for each site based on what is in the soil.

DDT is one of 12 persistent organic pollutants or POPs - dubbed the "dirty dozen" – that are targeted for restriction or elimination in a global treaty being negotiated in Geneva under the auspices of the UN Environment Programme.
In talks on Monday, negotiators exempted DDT on public health grounds. The World Health Organisation says it remains the most effective way to deal with mosquitoes that transmit malaria.

Environmental groups say DDT can travel long distances in the air and water, that it builds up in the fatty tissues of living beings and that it builds up in the sub-soil because of its non-biodegradable character. – Reuters

*While this promising project never lead to the final solution to the DDT problem as many hoped around year 2000, some of the results from the project. 97 percent degradation of the DDT in eight weeks means a half-life for DDT of only 11,2 days! Again we have not seen the numbers for DDD and DDE, so we don't know the reality of this wonder yet, but of course it is promising. On the other hand, as we still 22 years after do not have a good solution, the conclusion can only be that the Canadians did not make it work after all…*

*The challenge stands; we still need a good solution for the DDT problem. Often the problem of DDT these days is solved with brute force: When too much DDT is found in soil, is it filled in a land dump. This is not a satisfying solution; it is expensive and it leaves a brutal scar on the land now deprived of its good and fertile soil. As a consequence, farmers with old apple orchards do not want to test for DDT, which potentially put their families in danger.*

## INTRODUCTION

In this rapport we look at the recent reviews of the biodegradation of DDT (1-7), the theory about how DDT is broken down in soil (8-13), the major laboratory experiments done in this regard (14-60), and finally suggesting or experiments done in situ, where worms, bacteria, compost or manoeuvre has been added to the fields in the intention to remove DDT from the soil (61-87).

The presents of DDT and its sister compounds with similar properties, DDE and DDE, is a major problem in old orchards and other places where DDT was used massively from 1940 to about 1970 where its use was banned in most countries, also Sweden.

Around lake Åsnen is a large number of farms that have used DDT, and because of the hard binding to soil particles, DDTs hydrophobic character, and its extreme stability, it takes up to 30 years to half the amount of DDT and its sister compounds in the soil.

Recently important scientific progress has been made, and our understanding of how to increase bio-degradation directly in the field have grown to such an extent that it is justified to make experiments on how to most effectively accelerate the biodegradation of DDT in situ.

The DDT-pollution at Kurrebo, an old apple farm next to Åsnen, is after 50 years still severe. While most places tested have shown acceptable levels of DDT, a few places have shown up to 3 mg DDT and derivatives pr. kg soil, which must be considered a treat to nature as well as to human health. It is therefore mandatory that action is taken that acceleration the degradation of DDT and derivatives.

In the most promising experiments, the half-life of DDT, DDE and DDE are found to go from 20 years to about 22 days. If that could be copied in a general procedure applicable for the old apple farms around Åsnen, this could be of major importance for the whole area.

Needless to say, there is also a great need of such methods internationally, especially in regions where DDT is still in use, in spite of the treat this is to the global environment, where even the eggshells of birds in the Arctic area are said to getting thinner because of the global DDT pollution.

In this report we review the science and give suggestions to experiment that could be done at Kurrebo.

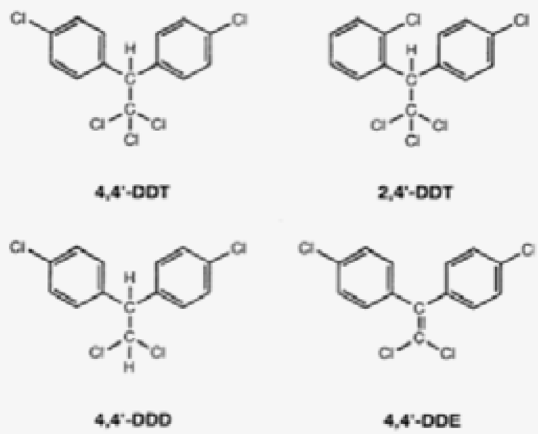
## THE POSSIBILITY OF ACCELERATED BIODEGRADATION OF DDT IN SOIL

Studies have clearly showed that there are micro-organisms in the soil that can degrade DDT and that the rate of degradation is dependent on the presence and numbers of microbes in the soil with the required degradative ability, environmental factors and access of the microbes to DDT (9).

Wango et al (9) writes: One of the removal processes with significant impact on the fate of DDT in the environment is biodegradation *Corresponding author. E-mail: hboga@fsc.jkuat.ac.ke. (You et al., 1995). Biodegradation and bioremediation are matching processes to an extent that both of these are based on the conversion or metabolism of pesticides by micro-organisms (Hong et al., 2007). A successful bioremediation technique requires an efficient microbial strain that can degrade largest pollutant to minimum level (Kumar and Philip, 2006).The rate of biodegradation in soil depends on four variables: (i) Availability of pesticide or metabolite to the micro-organisms (ii) Physiological status of the micro-organisms (iii) Survival and proliferation of pesticide degrading micro-organisms at contaminated site and (iv) Sustainable population of the micro-organisms (Dileep, 2008). Therefore, to attain an achievable bioremediation, it requires the creation of unique niche or microhabitats for desired microbes, so they can be successfully exploited.

### General background

D.D.T. (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane) is one of the best synthetic pesticides. First synthesized in 1874, its properties were not discovered until 1939. It was used to control mosquitoes spreading malaria, typhus and other insect borne diseases among both military and civilian populations. After the war DDT was made available for use as an agricultural insecticide. It is an organo-chlorine insecticide highly hydrophobic, colorless, crystalline solid with a weak, chemical odor. It is nearly insoluble in water but has a good solubility in most organic solvents, fats and oils.(8) Commercial DDT is a mixture of several related compounds. It has significant amount of (15%) dichloro-diphenyl-dichloro-ethylene (DDE) and dichloro-diphenyl-dichloroethane (DDD).




Since DDT residue are lipophilic they tend to accumulate in the fatty tissues of the ingesting organism along the food chain. (8) It is reported to be a potential endocrine disruptor in both avian and mammals. Resulting egg shell thinning, impaired male reproductive ability, interference with sex hormones, causes cancer and many other nervous diseases. (8) Biodegradation is the process by which organic substances are broken down by the enzymes produced by living organisms.

Some micro-organism have the astonishing, naturally occurring, microbial catabolic diversity to degrade or transform hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), and poly aromatic hydrocarbons (PAHS); (8) amongst the known micro-organisms capable to degrade DDT we find:

**Bacteria:**

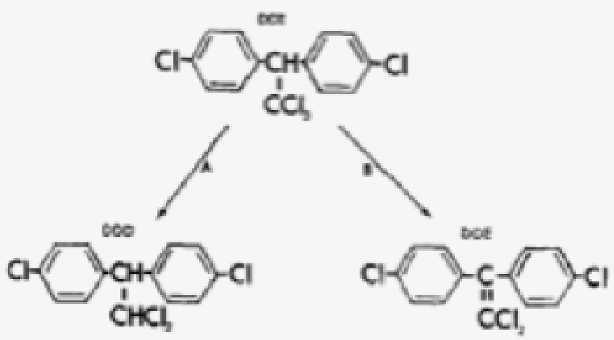
*Escherichia coli*
*Enterobacter aerogens.*
*Enterobacter cloacae.*
*Klebsiella pneumonia.*
*Pseudomonas aeruginosa.*
*Pseudomonas putida.*
*Bacillus species.*
*Hydrogenomonas.*

**Fungi:**

*Saccharomyces cervisiae.*
*Phanerochaete chrysosporium.*
*Trichoderma viridae.*

Many of these organisms if not all are common in soil and in horse, cow and chicken manure.

Although DDT is metabolised through co-metabolism pathway by a great number of facultative and obligate micro-organism under suitable conditions (8). DDD and DDE are metabolized product of DDT that are produced by microbial degradation, chemically or by photochemical reactions. (8)
There are various method for biological degradation of DDT like fugal degradation, aerobic degradation and anaerobic degradation. Comparison to Fugal degradation, aerobic degradation and anaerobic degradation has various advantage such as economical, easy, simple and needs no extra efforts. Chemical representation of DDT degradation by aerobic and anaerobic method is as follows (8):



De-chlorination of DDT: A is Anaerobic de-chlorination (DDD); B is aerobic Dehydro-chlorination (DDE).

The research, motivated by commercial interest, has been a search for the “wonder bacterium” that could be sold to farmers and spread on the soil to remove DDT (i.e. 18,26,28,46). No such wonder-bacterium has yet been found – or no wonder-chemical like a magical surfactant giving more oxygen to the deep soils.

What seems effective in the field is the combination of a long row of micro-organisms, both bacteria and fungi, that seems to cooperate in complicated ways in the processing of the organic material in the soil, and in degrading large and complicated, “annoying” lipophilic organic molecules like DDT, DDE and DDD.

Many experiments have tried to take DDT out of the soil in mechanical processes; one effective method is to use Ozone (O3) (20). Again, to treat all the soil in a mechanical system (a reaction chamber) out of the field with Ozone and other chemicals is very expensive and not realistic for the normal farmer, who wants to take DDT out of his fields.

[8. Chauhan and Singh, J Textile Sci Eng 2015, 5:1 DOI: 10.4172/2165-8064.1000183]

## EXPERIMENTS

Table 1 shows the result from an experiment where bacteria have been added to a soil containing DDT; the time scale can be understood from Figure 6 from the article brought below: after only 25 days (!) a significant amount of the DDT (30-50%) was degraded; but more importantly it was found that a combination of different bacteria dramatically accelerates the process (here 83% was degraded!).

This means that instead of cultivating a single, specific bacteria, one should find and add a growth media to the soil where large amounts of many different bacteria are present.

**A thing worth trying would thus be to mix large amounts of cow or horse manure into the DDT-polluted soil. If that solves the problem, we have found an easy, cheap method that at the same time add value to the soil in the field – a perfect and sustainable solution.**

**Of course this needs to happen with some caution, not to pollute the ground water with N, P and K from the manure. A simple way to do this is by "spot-treatment" so only the most polluted places are treated with big amount of manure, while the average use on the field is not going over the amount you can legally use.**

**Table 1.** Sources and amount of DDT degraded by isolates in pure and mixed culture.

| Isolates | % DDT degraded | Source | |
|---|---|---|---|
| | | Cultivated | Uncultivated |
| 101 | 44.31 | | √ |
| 102 | 58.08 | | √ |
| 103 | 39.72 | √ | √ |
| 104 | 30.33 | √ | |
| 105 | 28.97 | √ | |
| 110 | 28.48 | √ | √ |
| Six (mixed) | 82.63 | √ | √ |

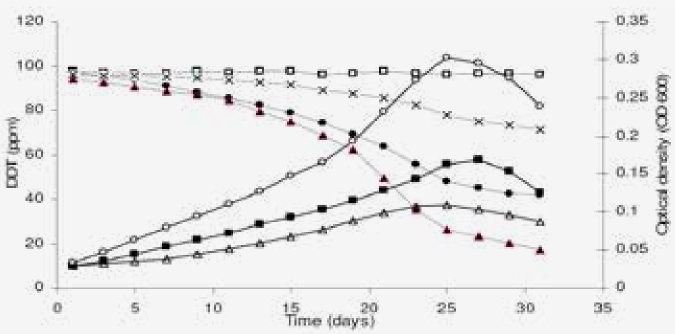



**Figure 6.** Bacterial growth and DDT degradation. Results are means where n=2 and SE <5 % of means in all cases. The symbols represents: -□- Control, -Δ- growth curve for isolate 110, -■- growth curve for isolate 102, -o- growth curve for the six mixed isolates , -x -DDT degradation curve for isolate 110, • -DDT degradation curve for isolate 102, ▲- DDT degradation curve for the six mixed isolates.

A word of caution: This experiment was done with tropical soil, which as different from the soil around lake Åsnen. Nobody can therefore know if it works, before it is tried. Furthermore, DDT was in this experiment only broken down to DDD, which has about the same properties of DDT.

> We qote from (9): *"This study shows that as the DDT peaks were reducing, the DDD peaks were increasing and at no particular point did the DDD peak start to decrease even with the mixed cultures. This shows that under the conditions, DDD was probably the end product hence this was not a complete break down of DDT to CO2 or to non chlorinated compounds like phenylacetic, phenylpropionic and salicylic acids. In various ecosystems, microorganisms cause only modest changes in the DDT molecule (Alexander 1985). Complete degradation of DDT is possible only through a cometabolic process (Pfaender and Alexander, 1976) and that only the first step in the process, the dechlorination of DDT to DDD can take place without an additional substrate, as was the case in this study. The major transformation products, DDD and DDE, are more toxic and recalcitrant than the parent compound. This is of concern as these compounds are metabolized slowly, if at all (Aislabie et al., 1997)."*

Again, it is fair to assume that DDD also with time will be bio-degrated; we can fear that this is a process that takes years, not days. A new study (14) by Suman and Tanuja as well as elder studies (15) also failed to look at the DDD. We are sorry that Mwangi et al. didn't follow the development in this experiment over longer time, so we could learn what eventually happens with the DDD; but again this will be more clear from practical experiments in the field.

The abstract from this important article can be found below.

### Degradation of dichlorodiphenyltrichloroethane (DDT) by bacterial isolates from cultivated and uncultivated soil

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Accepted 3 December, 2009

The re-introduction of dichlorodiphenyltrichloroethane (DDT) to control mosquitos was recommended by the World Health Organization in 2007. In this study, the potential for biodegradation of DDT by soil microorganisms through enrichment and isolation of DDT biodegraders from soils without a history of prior exposure to DDT was done. Microorganisms from cultivated and uncultivated soils grew in minimal media with DDT (100 ppm) as the only carbon source. Six bacteria coded as isolates 101, 102, 103, 104, 105 and 110 degraded DDT to l, l-dichloro-2, 2-bis (p-chlorophenyl) ethane (DDD). None of the isolates degraded DDT into l, l-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE). Degradation by the mixed culture of the six isolates was higher (82.63%) than that of any individual isolates whose range was 28.48 - 58.08%. The identity of the isolates was determined through biochemical, morphological, physiological and molecular techniques. Isolate 101 was a member of the genus *Bacillus*; isolates 102 and 110 belonged to the genus *Staphylococcus* while isolates 103, 104 and 105 clustered with members of the genus *Stenotrophomonas*. This study showed that there are microorganisms in the soil that can degrade DDT and that the rate of degradation is dependent on the presence and numbers of microbes in the soil with the required degradative ability, environmental factors and access of the microbes to DDT.

**Key words:** DDT, biodegradation, bacterial isolates, phylogenetic analysis.

Abstract from (9)

Zhao et al (19) published in 2010 promising research with an enzyme laccase derived from white rot fungi (see also 76).

Laccase is a multi-copper oxidase that catalyzes the oxidation of one electron of a wide range of phenolic compounds. The enzyme is considered eco-friendly because it requires molecular oxygen as co-substrate for the catalysis and it yields water as the sole by-product. The abstract can be found below.

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Received 17 October 2009; revised 2 November 2009; accepted 18 November 2009; available online 1 March 2010

**ABSTRACT:** This research describes application of laccase from white-rot fungi (polyporus) to remove dichlorodiphenyltrichloroethane in soil. The degradation kinetics of dichlorodiphenyltrichloroethane in soil was also investigated by laboratory batch experiments. The results showed that laccase from white-rot fungi can effectively degrade dichlorodiphenyltrichloroethane and the degradation of total dichlorodiphenyltrichloroethane (the sum of the four dichlorodiphenyltrichloroethane compounds in a sample) was pseudo-first-order kinetics. The residues of almost all the dichlorodiphenyltrichloroethane components and total dichlorodiphenyltrichloroethane in soils treated with laccase decreased rapidly during first 15 days and then kept at a stable level during next 10 days. The residues of total dichlorodiphenyltrichloroethane in soils with different dosages laccase decreased by about 21-32 %, 29-45%, 35-51 % and 36-51 % after 5, 10, 15 and 25 days of incubation, respectively. The half-life of total dichlorodiphenyltrichloroethane in soils with different dosages laccase ranged from 24.75 to 41.75 days. The residues of total dichlorodiphenyltrichloroethane in three different types of soils decreased by 25-29 %, 39-43 %, 44-47 % and 47-52 % after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane in different types of soil ranged from 24.71 to 27.68 days. The residues of total dichlorodiphenyltrichloroethane in soils with different pH levels decreased by 18-24 %, 29-39 %, 36-39 % and 39-50% after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane ranged from 25.63 to 36.42 days. Laccase can be an efficient and safe agent for remediation of dichlorodiphenyltrichloroethane-contaminated soil.

**Keywords:** *Bioremediation; Enzyme; Half-life; Organochlorine pesticide; Residue*

Figure 1 from this paper is shown here:

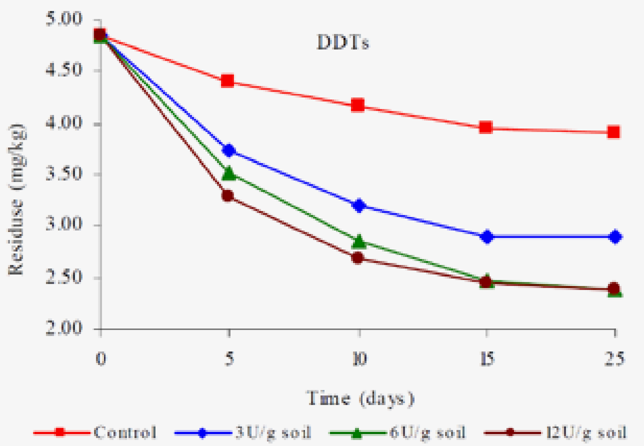


Fig. 1: Dynamic changes of residues of 4 components of DDT and DDTs in soil with different dosages laccase

What again is remarkable is that half the DDT in the lab experiment is degraded in just a few weeks. Unfortunately, it is not so simple that you can just transfer this procedure to soil in a field; in the field DDT is hard bound to soil particles and only a little down there is no oxygen, which is needed by the enzyme. Also, the enzyme itself is costly and must be used in so large quantities, that it is not a possible method in normal farming. Therefore, the discovery of this interesting chemical process did not lead to effective commercial solutions. Nevertheless the discovery of fungal enzymes able to take DDT downs fast and effectively is very important. Zhao also found that laccase has a pH optimum around 3 i.e. in the acidic spectrum. It might therefore be wise to test processes in situ that take the pH down.

**Practical note. Addition of simple manure to the land, i.e. horse manure, is known to take the pH down; it is common procedure to rebalance the soil with chalk in farming but it might be worth trying not to do so, to facilitate the fungal degradation of DDT and its derivates. (Unfortunately apple trees like chalk.)**

**An other discovery that might have great practical importance is done** by Li et al in 2010 (43), where they presented the paper „Effects of Different Fertilizers on Soil-borne DDTs Dynamics and Its Impacts on DDTs Uptake by Ipomoea aquatica“ on the 19th World Congress of Soil Science „Soil Solutions for a Changing World 89“ 1 – 6 August 2010, in Brisbane, Australia.
They found that fertilisers with iron dramatically accelerated the biodegradation of DDT.

**Practical note: It seems important to add iron to the field, when processing the DDT with manure.**

Another experiment done by Zhao and Yi (48) showed that the soil should not be so wet that is stops the diffusion of oxygen into it. They conclude: “The residue of DDTs in soil under the atmosphere of oxygen decreased by 28.1% compared with the atmosphere of nitrogen at the end of the incubation with laccase. A similar pattern was observed in the remediation of DDT contaminated soil by laccase under different flooding conditions, the higher the concentrations of oxygen in soil, the lower the residues of four DDT components and DDTs in soils. The residue of DDTs in the non-flooding soil declined by 16.7% compared to the flooded soil at the end of the incubation. The residues of DDTs in soils treated with laccase were lower in the pH range 2.5–4.5.” Again we see the importance of a low pH for the degradation process.

**Practical note: It seems important to keep the soil wet yet sufficiently dry to allow the free diffusion of oxygen into the soil , when processing the DDT with manure.**

A way to get more oxygen into the soil is using a surfactant (58). This method seems not realistic in farm practice for economical reasons. The effectiveness of surfactant in lab experiments underline the necessity of a good air flow to the degradation process.

**Practical solution – research implemented in the field**

Interestingly. a method to clear DDT from soil was sought patented in 1997 (69) by Roger L. Bernier, Neil C. C. Gray, Lori

E. Moser under the title "Compost decontamination of ddt contaminated soil", International application published under the Patent Cooperation Treaty (PCT), International Publication Number WO 97/11794, 3 April 1997. It is not clear if the patent was granted, but it seems that it was not.

Nevertheless, Bernier et als. composting method seems to work and work well, but is a highly complicated and work-consuming procedure, with e row of complicated steps that need to be repeated again and again, and the method seems therefore never to have been used.

*But the project is definitely a step in the right direction, strongly indicating that there is a simple solution to the DDT problem; we just have to find it.*

**Practical note: What is most important to the information coming from this project is that you need to mix at least 10% manure (weight %) into the soil for the process to work! This is an important discovery, as this might explain why normal fertilizing of fields, where you typically ad max 10 kg manure pr m2 (about 2% in weight) do not lead to the fast biodegradation of DDT and its derivates.**

In 2010 a promising paper by Purnomo et al. called "DDT degradation potential of cattle manure compost" appeared (73). I was made by a Japanese research group, who had interest in manure as a biotool for degrading because of its ability to stimulate both bacterial and fungal growth, found promising results from stimulation biodegradation of DDT with manure. Unfortunately, the Japanese group found that the process they discovered runs best about 60C where DDT is broken down in a matter of days. Again, in this study, we see no accounting for the DDD and DDE produced in the biodegradation processes. Also, we would like to see how the composing of manure at normal temperature works when mixed into soil in a field.

Another experiment with chicken manure was done by Deng et al.(71). In 2016. The idea was here basically the same: By adding manure the presence of active bacteria and fungi in the soil could go up thousands of times, effectively accelerating the process of biodegradation of DDT and its even more toxic derivatives DDE and DDD.

The group concluded that chicken manure effectively could accelerate the production of DOC and the degradation of p, p'-DDT in loam soil. They noticed that a high concentration of water further accelerated the process, which might be an important observation; they found that the concentrations of the degradation product DOC gradually increased with increasing of the added proportion of chicken manure in clay soils – the more the better for short. The degradation rate of p, p'-DDT gradually increased both with increasing of the added proportion of CM in clay soils, but also with increasing the concentration of water.

**Practical note: Deng et als. experiment suggests that we to acceleration biodegradation using manure need to work with high concentrations of manure, under very wet conditions.**

## CONCLUSION

A large number of experiment have been done the last two decades, where acceleration of biodegradion process fo DDT and its toxic derivatives DDE and DDD has been hunted. The painful half-life of 10-30 years of DDTs in soil means that the pollution from 1940-1974 where DDT was carelessly used in farming, especially in Sweden in apple orchards, needs to go down so we don't have this pollution forever.

Surprisingly we see that DDTs out of the soil can be degraded in a few weeks or months. In this report we have systematically gone through the literature to identify the key factors that must be taken into consideration for a new experimental attempt to make this happen.

The most promising, affordable and sustainable solution seems to be the simple use of manure from chicken, cow or horse, to accelerate the biodegradation process.

*From the studies we have reviewed we have collected the following important information:*

1. *DDD and DDE are at least as toxic as DDT and even more resilient.*

2. *What seems effective in the field is the combination of a long row of micro-organisms, both bacteria and fungi, that seems to cooperate in complicated ways in the processing of the organic material in the soil, and in degrading large and complicated, "annoying" lipophilic organic molecules like DDT, DDE and DDD.*

3. *Mixing in manure in the soil is therefore a highly likely method for accelerating the process, as many studies have shown.*

4. *A thing worth trying would thus be to mix large amounts of cow or horse manure into the DDT-polluted soil, more*

*than 10%. If that solves the problem, we have found an easy, cheap method that at the same time add value to the soil in the field – a perfect and sustainable solution.*

*5. Of course this needs to happen with some caution, not to pollute the ground water with N, P and K from the manure. A simple way to do this is by "spot-treatment" so only the most polluted places are treated with big amount of manure, while the average use on the field is not going over the amount you can legally use.*

*6. Addition of simple manure to the land, i.e. horse manure, is known to take the pH down; it is common procedure to rebalance the soil with chalk in farming but it might be worth trying not to do so, to facilitate the fungal degradation of DDT and its derivatives. (Unfortunately apple trees like chalk.)*

*7. It seems important to add iron to the field, when processing the DDT with manure*

*8. It seems important to keep the soil wet yet sufficiently dry to allow the free diffusion of oxygen into the soil , when processing the DDT with manure.*

*9. What is most important to the information coming from this project is that you need to mix at least 10% manure (weight %) into the soil for the process to work! This is an important discovery, as this might explain why normal fertilizing of fields, where you typically ad max 10 kg manure pr m2 (about 2% in weight) do not lead to the fast biodegradation of DDT and its derivatives.*

*10. Deng et als. experiment suggests that we to accelerate biodegradation using manure need to work with high concentrations of manure, under very wet conditions.*

*11. Several studies have shown that the rate of biodegradation of DDT and derivatives are strongly dependent of earthworms(62,66,67,72), maybe even proportional with the number of earthworms in the soil; therefore anything that increases the number of worms, like leafs and other organic material, compost or manure will accelerate the degradation speed. The strategy of adding cow and horse manure and cover with organic material that can function as food for worms therefore seems optimal also in this respect.*

## DESIGNING THE RESEARCH PROJECT

The first important thing is to establish where the land is so polluted that it make sense to intervene strongly and systematically. This must be established from the international and national safety values for DDTs in the soil.

In the case of Kurrebo 4 there are only 3 of 18 tested spots that contain threatening levels of DDTs; we are thus talking about all in all maybe 50x50 m or in total 250 m2.

The next thing to establish is the significance of the DDTs in the different layes of the soil. It is obvious that DDTs are strongly hydrophobic and hard bound to the soil, so DDTs are not going into the ground water or moving around after the chemical laws of diffusion.

The art is to find the correct depth for the process, maybe only 20 cm, as the DDTs in the lower layer do not constitute a problem and eventually will break down by it self.

Opening the soil brings the dangerous DDTs in contact with the environment, why all soil digged or ploughed up must be taking under treatment with manure right away, and maybe covered with straw, hay, or leaves or black fresh compost soil, so the polluted soil is not in contact with the environment.

If we go 20 cm down we need at least 30 kg manure per m2 for the process to work, according to the knowledge we have accumulated above. This means that we need to add 250 m2 x 50 kg = 12,5 tons of horse manure on the polluted spot. Of course this must be done in steps so we are not adding more manure to the fields than is safe for the environment and within the limits set by the law.

The content of earthworms, water, iron, the acidity and the accessibility must be controlled carefully, together with the other important factors mentioned above.

Realistically the process will take 3-5 years. It might also be wise to repeat the process in the years to come. We are talking about a 10 year plan.

Interestingly we did not find any experiment in the literature where such an approach has been attempted.

If we are allowed to buy Kurrebo, we will, together with the county and the responsible authorities, make this project, and document its effect. If we are lucky we will come up with a solution to the DDT problem, that exist and is important to solve, to secure hundreds of farmers and their families, around Åsnen and elsewhere in Sweden. It might even be that we find a general solution that can be used also in the pour countries, as our solution is both cost-effective, sustainable, and at the same time improves the soil.

# List of references

## REVIEWS


Ref.1 – review

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Xiao Deng, Chunyuan Wu, Yi Li, Jingkun Liu, Qinfen Li, Effects of Chicken Manure Compost on the Production of Dissolved Organic Carbon and the Degradation of p, p'-DDT in Loam Soil, Atlantis Press, Available Online October 2016.

Link: https://doi.org/10.2991/iccahe-16.2016.22

Implementation → coffee grain ?
Ref 72
R. Rodríguez-Vázquez, D.S. Acosta-Ramírez, REGENERATION OF DDT CONTAMINATED SOILS BY CO-COMPOSTING, Acta Horticulturae 1076 II International Symposium on Organic Matter Management and Compost Use in Horticulture, Article number 1076_25, Pages 217-224.

Link: https://doi.org/10.17660/ActaHortic.2015.1076.25

Implementation
Ref 73
Adi Setyo Purnomo, Futoshi Koyama, Toshio Mori, Ryuichiro Kondo, DDT degradation potential of cattle manure compost, Chemosphere
Volume 80, Issue 6, July 2010, Pages 619-624

Link: https://doi.org/10.1016/j.chemosphere.2010.04.059

Implementation
Ref 74
Brian S. Anderson, John W. Hunt, Bryn M. Phillips, Matt Stoelting, Jonathon Becker, Russell Fairey, H. Max Puckett, Mark Stephenson, Ronald S. Tjeerdema, Michael Martin, Ecotoxicologic change at a remediated superfund site in San Francisco, California, USA, Environmental Toxicology, Volume19, Issue 4, April 2000, Pages 879-887
Link: https://doi.org/10.1002/etc.5620190414

Implementation
Ref 75
Biao Fan, Yuechun Zhao, Ganhui Mo, Weijuan Ma, Junqin Wu, Co-remediation of DDT-contaminated soil using white rot fungi and laccase extract from white rot fungi, J Soils Sediments 13, 1232–1245, 30 April 2013

Link: https://doi.org/10.1007/s11368-013-0705-3

Implementation
Ref 76
Pengfei Xiao, Toshio Mori, Ichiro Kamei, Ryuichiro Kondo, A novel metabolic pathway for biodegradation of DDT by the white rot fungi, Phlebia lindtneri and Phlebia brevispora, Biodegradation 22, 859–867 (2011)

Link: https://doi.org/10.1007/s10532-010-9443-z

Implementation → Slurry system?
Ref 77
Ricardo Dalla Villa, Raquel F. Pupo Nogueira, Oxidation of p,p'-DDT and p,p'-DDE in highly and long-term contaminated soil using Fenton reaction in a slurry system, Science of The Total Environment Volume 371, Issues 1–3, 1 December 2006, Pages 11-18

Link: https://doi.org/10.1016/j.scitotenv.2006.05.010

Implementation → fungus → white rot
Ref 78
JOHNA.BUMPUS, STEVEN D. AUST, Biodegradation of DDT [1,1,1-Trichloro-2,2-Bis(4-Chlorophenyl)Ethane] by the White Rot Fungus Phanerochaete chrysosporium, Applied and environmental microbiology, Sept.1987,p.2001-2008

Link: https://doi.org/10.1128/aem.53.9.2001-2008.1987

Implementation → bacteria
Ref 79
Jie Qu, Yang Xu, Guo-MinAi, Ying Liu, Zhi-Pei Liu, Novel Chryseobacterium sp. PYR2 degrades various organochlorine pesticides (OCPs) and

achieves enhancing removal and complete degradation of DDT in highly contaminated soil, Journal of Environmental Management Volume 161, 15 September 2015, Pages 350-357

Link: https://doi.org/10.1016/j.jenvman.2015.07.025

Implementation → Fungi
Ref 80
Yi Huang, Jie Wang, Degradation and mineralization of DDT by the ectomycorrhizal fungi, Xerocomus chrysenteron, Chemosphere Volume 92, Issue 7, August 2013, Pages 760-764

Link: https://doi.org/10.1016/j.chemosphere.2013.04.002

Implementation → fungi → white rot
Ref 81
Guanyu Zheng, Ammaiyappan Selvam, Jonathan W.C.Wong, Oil-in-water microemulsions enhance the biodegradation of DDT by Phanerochaete chrysosporium, Bioresource Technology Volume 126, December 2012, Pages 397-403

Link: https://doi.org/10.1016/j.biortech.2012.02.141

Implementation → washing (cosolvents and surfactants)
Ref 82
Smith, E., Smith, J., Naidu, R. et al. Desorption of DDT from a Contaminated Soil using Cosolvent and Surfactant Washing in Batch Experiments, Water, Air, & Soil Pollution 151, 71–86 (2004)

Link: https://doi.org/10.1023/B:WATE.0000009899.03630.78

Implementation → Bacteria
Ref 83
Beunink, J., Rehm, HJ. Synchronous anaerobic and aerobic degradation of DDT by an immobilized mixed culture system, Appl Microbiol Biotechnol 29, 72–80 (1988).

Link: https://doi.org/10.1007/BF00258354

Implementation → Bacteria
Ref 84
Sun, G., Zhang, X., Hu, Q. et al. Biodegradation of Dichlorodiphenyltrichloroethanes (DDTs) and Hexachlorocyclohexanes (HCHs) with Plant and Nutrients and Their Effects on the Microbial Ecological Kinetics, Microb Ecol 69, 281–292 (2015).

Link: https://doi.org/10.1007/s00248-014-0489-z

Implementation → Biostimulation and surfactant addition
Ref 85
Bibiana Betancur-Corredor, Nancy J.Pino, Santiago Cardona, Gustavo A. Peñuela, Evaluation of biostimulation and Tween 80 addition for the bioremediation of long-term DDT-contaminated soil, Journal of Environmental Sciences Volume 28, 1 February 2015, Pages 101-109

Link: https://doi.org/10.1016/j.jes.2014.06.044

Implementation → Bacteria → sewage sludge
Ref 86
Qi Liang, Mei Lei, Tongbin Chen, Jun Yang, Xiaoming Wan, Sucai Yang, Application of sewage sludge and intermittent aeration strategy to the bioremediation of DDT- and HCH-contaminated soil, Journal of Environmental Sciences Volume 26, Issue 8, 1 August 2014, Pages 1673-1680

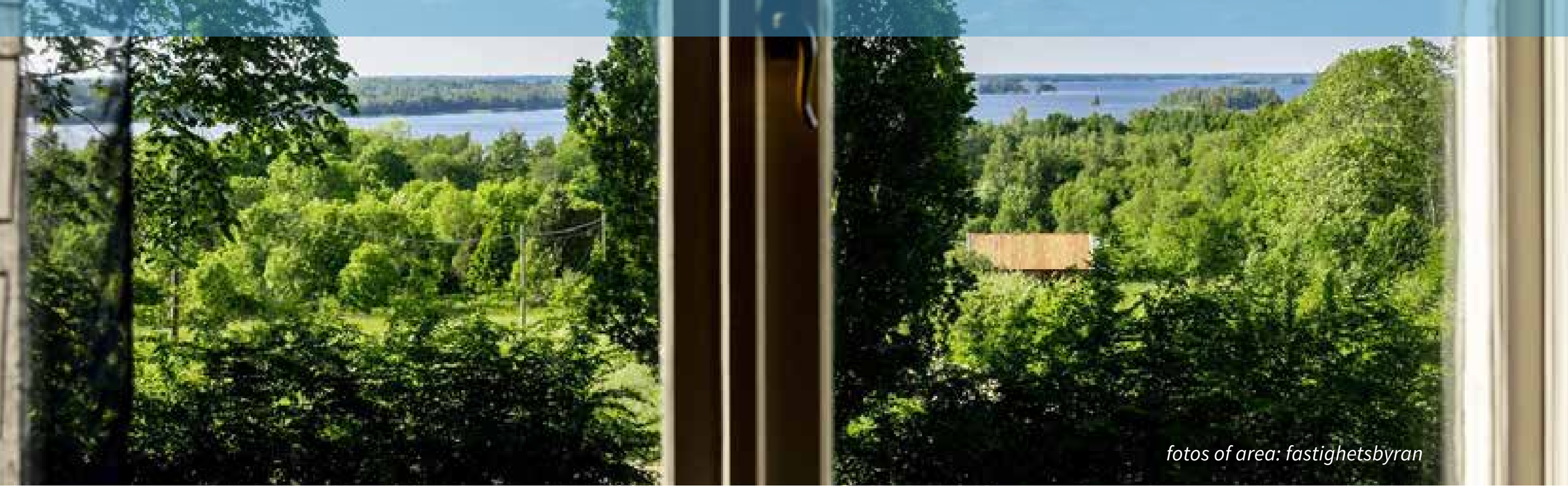
Link: https://doi.org/10.1016/j.jes.2014.06.007

Implementation → Stimulation of native microbes
Ref 87
Ortíz, I., Velasco, A., Le Borgne, S. et al. Biodegradation of DDT by stimulation of indigenous microbial populations in soil with cosubstrates, Biodegradation 24, 215–225 (2013).

Link: https://doi.org/10.1007/s10532-012-9578-1

# EXPANSION

## New projects fitted to a new place



*fotos of area: fastighetsbyran*

# A UNIQUE SPACE WITH MANY OPPORTUNITIES

We see enormous potential at Kurrebo. Of course it comes with a lot of work and a lot of problems like the DDT pollution that demand our focus. Nevertheless we see this as a long term project – not for the next few years, but for decades to come. And with that there is space for much more. Here a brief look into the projects we would like to realize in time:

**1. Together sustainable**
- info-, vistorcenter / school
- community garden
- teachings about pollution
- botanical garden
- mushrooms growth and tours
- biological teaching lab

**2. Congress center**
- meetings
- courses in cooking, sustainability, environmental protection, nature care, handling pollution
- teachings
- sports like yoga, aerobic, judo etc.
- opportunities for development of talent and personal growth

**3. Animals**
- Bees
- Sheep
- Chicken

**“WE SEE THIS AS A LONG TERM PROJECT – NOT FOR THE NEXT FEW YEARS, BUT FOR DECADES TO COME. “**


foto: fastighetsbyran

# TOGETHER SUSTAINABLE

Under the motto „together sustainable“ we want to start a project we have been dreaming about for a while. One of our core aims is to show people how to live in a sustainable, environmentally friendly way and close to nature. Kurrebo offers a unique space for this to manifest.

## INFO CENTER

An **info and visitor center** will be the center point. Informations about nature, the local environment and sustainable living will be displayed here. We will link to the already existing **natural reserve** and information as well as the **museum** and other points of interest close by.

## EDUCATION

One of the most important goals will be to **educate people about pollution**, it's effects and solutions. Besides informations in the visitor center we plan on reaching schools and giving lectures and courses on the topic.

We imagine **guided tours** through the forest, explaining, foraging, exploring with kids but also for adults. The already established **botanical garden** seems like an ideal starting point. Even **workshops** on making your own insect hotel, birdhouse, compost, planting etc. are possible. Regular „tidy up“ actions in the forest and at the water can be implemented.

With time we would also like to establish a **teaching laboratory** where school classes can explore the microscopic lifeforms you can find everywhere in nature. These life forms give an amazing view into the living cell and are a great inspiration for exploration of life, health and biology.

## COMMUNITY GARDEN

In the future a **community garden** could be at the entrance of the property to invite everyone to join in, plant, grow and harvest organically. People will come together, exchange experiences and even seeds or plants. Ideal for school kids and families.

## MUSHROOM FACILITY

A mushroom growing facility, is one of the ideas to expand this project even further in the future.

foto: fastighetsbyran

# CONGRESS CENTER

So far our events have been hosted through the café and restaurant. In the future we plan to expand this popular service even more by opening a congress center as well as offering extensive event planning.

fotos up: web

## STANDING OUT

It is our experience that people and also business are attracted to events like team building, annual meetings etc. at *Mormors Bakeri* because of the beautiful and relaxed atmosphere.

Cold practicability is not appealing and our customers are searching for peaceful atmosphere ideally close to nature, fitted service, and reasonable pricing.

## RANGE OF EVENTS

We plan to host anything from work meetings to private events and annual gatherings.

Courses and teachings from external providers can be hosted and even include food and sleepovers. If it's an educational course on environmental issues, a creative course or talent development – the space makes it possible.

Also outdoor courses in canoeing, sailing etc. are possible. Maybe even cooking classes!


# ANIMALS

Another possible expansion lies in the keeping of animals. Bees are a must and we imagine also sheep and chicken at the property – both for practical and social reasons.

## BEES

Bees are vital for food production. Our orchard has been accompanied by our own hives since 2019. In 2021 we harvested our experience with bee keeping in form of the first honey production for sale – in cooperation with the bee-keeper Association in Karlskrona. We find bee-keeping both beneficial and joyful.

Driven by our passion we aim to grow happy and healthy colonies also in Urshult. We wish to explore the wide range of activities bee-keeping offers and share this joy with public. This could also be combined with the info center and different courses.

## SHEEP

A new aspect of farm living to conquer is sheep keeping.
As we can learn from similar projects, grazing sheep seems to be an environmentally-friendly way of how to control weeds, to keep land open and to preserve landscapes. In the meantime their small hooves limiting soil compaction and erosion. As we want ourself and others to enjoy a rural lifestyle, we see sheep as an additional aspect of agricultural production.

As we have noticed that there is already an ongoing sheep husbandry,  and we are interested in continuing an existing cooperation or otherwise to have an sheep herd ourselves in the future.

## CHICKEN

From keeping chicken we have learned that they come with a lot of benefits. You are getting supplied with fresh and healthy eggs plus they are lively and social pets, interesting for adults and children. They keep the areas of their reach low in weed, turn around soil and they give great fertilizer for fruit trees and co.. As they can eat most kitchen scraps, they are helping handling waste. In this way, chicken act as a part in self-sufficient lifestyle and local food systems.
Chicken are also great teachers for children in regards to the importance of caring for others and responsibility.
We can imagine also chicken at Kurrebo.


## ABOUT US:

The managing team consist of our founder Søren Ventegodt MD and Pavlina Kordova.

Søren Ventegodt grew up with a burning interest in chemistry, only 13 year old he already owned a chemical lab in the basement of his parents house. It was only natural that he studied chemistry at the University of Copenhagen after he graduated from high school –18 years old and the best from his school of that year.

His growing interest in medicine was pursued and he graduated from med school, again with top marks. After graduation he accepted the position as the research director of the department for quality of life at the University Hospital, Copenhagen. Søren soon started his own  company, Livskvalitet ApS, which amongst other activities ran a culture house in central Copenhagen with the aim to inspire people to improve the health and happiness - the quality of life. These days Søren together with Paulina Kordova runs the Swedish branch “Livskvalitets svenska filial”.

Paulina Korodova, Sørens partner, is the director of the Swedish branch that aims to improve the quality of life in Sweden. Paulina, thanks to her mother, who was a restaurant and hotel manager, spent a big part of her youth in the staff area of the restaurant and hotel – observing, learning and helping with jobs from kitchen assistant to cleaning, serving of customers and sales.

Paulina successfully finished business school and after that she studied Theater Science at The Charles university in Prague. Paulina changed her path, met Søren and came to Sweden to become manager of the cafe, restaurant and plant school and finally manifesting her passion for baking.

The team of *Mormors Bakeri* and the plant school is built from passionate young people who all share the same interest in sustainable philosophy.

The company employs professionals in the area of electrical and technical engineering, construction, social science, accounting, hotel-, kitchen- and restaurant management, graphic design, concept development and curation as well as photography.

High trades of the team are openness, creativity, flexibility, friendliness, cooperation, consideration and most of all love for Sweden.

**Søren Ventegodts extensive CVs can be provided if wanted.**

**In the moment we saw the property we knew this is the place we need and the place that needs us!**

”

# Conclusion

Do we fulfil your criteria? Let's take a last look. But before we do so, let us just assure you that we put our heart and our best efforts into all our projects, including this one.

The Swedish allmansright is an amazing thing that gives everybody access to all nature. This makes Sweden very special. If you live here you might not notice it much, but if you come from abroad you will be amazed. The world is shared and we are here together. Everybody is welcome. It is an inclusive attitude and this is an attitude we want to stand for if we get the opportunity to take over Kurrebo.

As mentioned in our Mission Statement the main goal of our company is to improve the life quality of the people around us. This is at the core of all we do.

## Perspectives for the future

Yes, we are all responsible for the future.
We will do our best to inspire everybody to feel the same way.

## Openness

Yes, we love people and we love nature. And we welcome everyone here.
It is our intention to share this space with the community.

## Innovation

Yes, we are innovative. We are actually on the way to solve the DDT problem. If you take your self time to read the report you will notice that we have a fair chance.

## Experience

30 years is a long time to run a company. We have made dozens if not hundreds of projects. This might be the biggest project yet, but we are confident that we can handle it.

**Livskvalitets**
Svenska Filial

## We are happy to provide further and in depth information, just give us a call!

### For any questions please contact Søren Ventegodt

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# APPENDIX

## Scientific references for the DDT project with abstracts

# REVIEWS

**Ref.1 – review**

Julia Foght, Trevor April, Kevin Biggar &Jackie Aislabie/Bioremediation of DDT-Contaminated Soils: A Review/Bioremediation Journal /Volume 5, 2001 - Issue 3

Abstract
The insecticide 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane (DDT) has been used extensively since the 1940s for control of agricultural pests, and is still used in many tropical countries for mosquito control. Despite a ban on DDT use in most industrialized countries since 1972, DDT and its related residues (DDTr) persist in the environment and pose animal and human health risks. Abiotic processes such as volatilization, adsorption, and photolysis contribute to the dissipation of DDTr in soils, often without substantial alteration of the chemical structure. In contrast, biodegradation has the potential to degrade DDTr significantly and reduce soil concentrations in a cost-effective manner. Many bacteria and some fungi transform DDT, forming products with varying recalcitrance to further degradation. DDT biodegradation is typically co-metabolic and includes dechlorination and ring cleavage mechanisms. Factors that influence DDTr biodegradation in soil include the composition and enzymatic activity of the soil microflora, DDTr bioavailability, the presence of soil organic matter as a co-metabolic substrate and (or) inducer, and prevailing soil conditions, including aeration, pH, and temperature. Understanding howthese factors affect DDTr biodegradation permits rational design of treatments and amendments to stimulate biodegradation in soils. The DDTr-degrading organisms, processes and approaches that may be useful for bioremediation of DDTr-contaminated soils are discussed, including in situ amendments, ex situ bioreactors and sequential anaerobic and aerobic treatments.

https://www.tandfonline.com/doi/abs/10.1080/20018891079302

**Ref.2 - review**

J. M. Aislabie, N. K. Richards & H. L. Boul/Microbial degradation of DDT and its residues—A review/ New Zealand Journal of Agricultural Research / Volume 40, 1997 - Issue 2

Abstract
Microbial degradation of DDT residues is one mechanism for loss of DDT from soil. In this review pathways for biodegradation of DDT, DDD, and DDE by bacteria and fungi are described. Biodegradation of DDT residues can proceed in soil, albeit at a slow rate. To enhance degradation in situ a number of strategies are proposed. They include the addition of DDT-metabolising microbes to contaminated soils and/or the manipulation of environmental conditions to enhance the activity of these microbes. Lignolytic fungi and chlorobiphenyl degrading bacteria are promising candidates for remediation. Flooding of soil and the addition of organic matter can enhance DDT degradation. As biodegradation may be inhibited by lack of access of the microbe to the contaminant, the soil may need to be pre-treated with a surfactant. Unlike DDT, little is known about the biodegradation of DDE, and this knowledge is crucial as DDE can be the predominant residue in some soils.

https://www.tandfonline.com/doi/abs/10.1080/00288233.1997.9513247?src=recsys

**Ref.3 - review**

H. L. Boul/DDT residues in the environment—A review with a New Zealand perspective/ New Zealand Journal of Agricultural Research / Volume 38, 1995 - Issue 2

Abstract
The source, form, and fate of DDT residues in the environment are reviewed. Discussion is primarily from a New Zealand perspective, where a major use of DDT was the control of soil-dwelling pasture pests. Reasons for the persistence of DDT residues, the association between residues and soil components, and possible degradative and non-degradative losses from soils are discussed.

https://www.tandfonline.com/doi/abs/10.1080/00288233.1995.9513126?src=recsys

**Ref.4 - review**

John E Thomas , Li-Tse Ou, Abid All-Agely/DDE remediation and degradation/Rev Environ Contam Toxicol/2008/194:55-69.

Abstract
DDT and its metabolites, DDD and DDE, have been shown to be recalcitrant to degradation. The parent compound, DDT, was used extensively worldwide starting in 1939 and was banned in the United States in 1973. The daughter compound, DDE, may result from aerobic degradation, abiotic dehydrochlorination, or photochemical decomposition. DDE has also occurred as a contaminant in commercial-grade DDT. The p,p'-DDE isomer is more biologically active than the o,p-DDE, with a reported half-life of -5.7 years. However, when DDT was repeatedly applied to the soil, the DDE concentration may remain unchanged for more than 20 yr. Remediation of DDE-contaminated soil and water may be done by several techniques. Phytoremediation involves translocating DDT, DDD, and DDE from the soil into the plant, although some aquatic species (duckweed > elodea > parrot feather) can transform DDT into predominantly DDD with some DDE being formed. Of all the plants that can uptake DDE, Cucurbita pepo has been the most extensively studied, with translocation values approaching "hyperaccumulation" levels. Soil moisture, temperature, and plant density have all been documented as important factors in the uptake of DDE by Cucurbita pepo. Uptake may also be influenced positively by amendments such as biosurfactants, mycorrhizal inoculants, and low molecular weight organic acids (e.g., citric and oxalic acids). DDE microbial degradation by dehalogenases, dioxygenases, and hydrolases occurs under the proper conditions. Although several aerobic degradation pathways have been proposed, none has been fully verified. Very few aerobic pure cultures are capable of fully degrading DDE to $CO_2$. Cometabolism of DDE by Pseudomonas sp., Alicaligens sp., and Terrabacter sp. grown on biphenyl has been reported; however, not all bacterial species that produce biphenyl dioxygenase degraded DDE. Arsenic and copper inhibit DDE degradation by aerobic microorganisms. Similarly, metal chelates such as EDTA inhibit the breakdown of DDE by the extracellular lignolytic enzymes produced by white rot fungi. The addition of adjutants such as sodium ion, surfactants, and cellulose increased the rate of DDT aerobic or anaerobic degradation but did little to enhance the rate of DDE disappearance under anaerobic conditions. Only in the past decade has it been demonstrated that DDE can undergo reductive dechlorination under methanogenic and sulfidogenic conditions to form the degradation product DDMU, 1-chloro-2,2'-bis-(4'-chlorophenyl)ethane. The only pure culture reported to degrade DDE under anaerobic conditions was the denitrifier Alcaligens denitrificans. The degradation of DDE by this bacterium was enhanced by glucose, whereas biphenyl fumes had no effect. Abiotic remediation by DDE volatilization was enhanced by flooding and irrigation and deepplowing inhibited the volatilization. The use of zero-valent iron and surfactants in flooded soils enhanced DDT degradation but did not significantly alter the rate of DDE removal. Other catalysts (palladized magnesium, palladium on carbon, and nickel/aluminum alloys) degraded DDT and its metabolites, including DDE. However, these systems are often biphasic or involve explosive gases or both. Safer abiotic alternatives use UV light with titanium oxide or visible light with methylene green to degrade DDT, DDD, and DDE in aqueous or mixed solvent systems. Remediation and degradation of DDE in soil and water by phytoextraction, aerobic and anaerobic microorganisms, or abiotic methods can be accomplished. However, success has been limited, and great care must be taken that the method does not transfer the contaminants to another locale (by volatilization, deep plowing, erosion, or runoff) or to another species (by ingestion of accumulating plants or contaminated water). Although the remediation of DDT-, DDD-, and DDE-contaminated soil and water is beset with myriad problems, there remain many open avenues of research.

https://pubmed.ncbi.nlm.nih.gov/18069646/

**Review Ref 5**

Ahlem Mansouri, Mickael Cregut, Chiraz Abbes, Marie-Jose Durand, Ahmed Landoulsi & Gerald Thouand, The Environmental Issues of DDT Pollution and Bioremediation: a Multidisciplinary Review, Applied Biochemistry and Biotechnology volume 181, pages 309–339 (2017), published 03 September 2016

Abstract
DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane) is probably the best known and most useful organochlorine insecticide in the world which was used since 1945 for agricultural purposes and also for vector-borne disease control such as malaria since 1955, until its banishment in most countries by the Stockholm convention for ecologic considerations. However, the World Health Organization allowed its reintroduction only for control of vec-

tor-borne diseases in some tropical countries in 2006. Due to its physicochemical properties and specially its persistence related with a half-life up to 30 years, DDT linked to several health and social problems which are due to its accumulation in the environment and its biomagnification properties in living organisms. This manuscript compiles a multidisciplinary review to evaluate primarily (i) the worldwide contamination of DDT and (ii) its (eco) toxicological impact onto living organisms. Secondly, several ways for DDT bioremediation from contaminated environment are discussed. For this, reports on DDT biodegradation capabilities by microorganisms and ways to enhance bioremediation strategies to remove DDT are presented. The different existing strategies for DDT bioremediation are evaluated with their efficiencies and limitations to struggle efficiently this contaminant. Finally, rising new approaches and technological bottlenecks to promote DDT bioremediation are discussed.

Link: https://doi.org/10.1007/s12010-016-2214-5

**Review**
**Ref 6**
Adi Setyo Purnomo, Toshio Mori, Ichiro Kamei, Ryuichiro Kondo, Basic studies and applications on bioremediation of DDT: A review, International Biodeterioration & Biodegradation Volume 65, Issue 7, October 2011, Pages 921-930

Abstract
The persistent insecticide DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) has been widely used for pest control in the management of mosquito-borne malaria and is still used for that purpose in some tropical countries. Considering the potential for negative effects due to DDT contamination, it is necessary to determine effective methods of remediation. Several methods have been used to degrade or transform DDT into less toxic compounds. Bacteria and white-rot fungi (WRF) have been shown to enhance the degradation process in soil using both pure and mixed cultures. Recently, a biological approach has been used as an environmentally-friendly treatment, using new biological sources to degrade DDT, e.g. brown-rot fungi (BRF), cattle manure compost (CMC) and spent mushroom waste (SMW). In this review, the abilities of BRF, CMC and SMW to degrade DDT are discussed, including the mechanisms and degradation pathways. Furthermore, application of these sources to contaminated soil is also described. The review discusses which is the best source for bioremediation of DDT.

Link: https://doi.org/10.1016/j.ibiod.2011.07.011

**Review**
**Ref 7**
J. M. Aislabie , N. K. Richards & H. L. Boul, Microbial degradation of DDT and its residues—A review, New Zealand Journal of Agricultural Research Volume 40, 1997 - Issue 2, Pages 269-282, Published online: 17 Mar 2010

Abstract
Microbial degradation of DDT residues is one mechanism for loss of DDT from soil. In this review pathways for biodegradation of DDT, DDD, and DDE by bacteria and fungi are described. Biodegradation of DDT residues can proceed in soil, albeit at a slow rate. To enhance degradation in situ a number of strategies are proposed. They include the addition of DDT-metabolising microbes to contaminated soils and/or the manipulation of environmental conditions to enhance the activity of these microbes. Ligninolytic fungi and chlorobiphenyl degrading bacteria are promising candidates for remediation. Flooding of soil and the addition of organic matter can enhance DDT degradation. As biodegradation may be inhibited by lack of access of the microbe to the contaminant, the soil may need to be pre-treated with a surfactant. Unlike DDT, little is known about the biodegradation of DDE, and this knowledge is crucial as DDE can be the predominant residue in some soils.

Link: https://doi.org/10.1080/00288233.1997.9513247

# THEORIES

**Ref.8 - theories**

Ashish Chauhan and Jashwant Singh/Biodegradation of DDT/Journal of Textile Science & Engineering/ ISSN: 2165-8064 JTESE, an open access journal

Abstract
Some microorganisms that are capable to degrade DDT are: Bacteria: Escherichia coli Enterobacter aerogens. Enterobacter cloacae. Klebsiella pneumonia. Pseudomonas aeruginosa. Pseudomonas putida. Bacillus species. Hydrogenomonas. Fungi: Saccharomyces cervisiae. Phanerochaete chrysosporium. Trichoderma viridae.

https://www.hilarispublisher.com/open-access/biodegradation-of-ddt-2165-8064.1000183.pdf

**Ref.9 - theories**

Kinuthia Mwangi, Hamadi I. Boga, Anne W. Muigai, Ciira Kiiyukia and Muniru K. Tsanuo/Degradation of dichlorodiphenyltrichloroethane (DDT) by bacterial isolates from cultivated and uncultivated soil/African Journal of Microbiology Research Vol. 4 (3) pp. 185-196, 4 February, 2010

Abstract
DDT is still one of the first and most commonly used insecticides for indoor residual spraying because of its low cost, high effectiveness, persistence and relative safety to humans (Hecht et al., 2004). It is therefore a viable insecticide in indoor residual spraying owing to its effectiveness in well supervised spray operation and high excito-repellency factor. Although DDT is very effective in killing or repelling mosquitoes its use has been severely reduced and restricted to indoor residual spraying, due to its persistence in the environment and ability to bioconcentrate in the food chain (Cousins et al., 1998; Hickey, 1999). One of the removal processes with significant impact on the fate of DDT in the environment is biodegradation *Corresponding author. E-mail: hboga@fsc.jkuat.ac.ke. (You et al., 1995). Biodegradation and bioremediation are matching processes to an extent that both of these are based on the conversion or metabolism of pesticides by microorganisms (Hong et al., 2007). A successful bioremediation technique requires an efficient microbial strain that can degrade largest pollutant to minimum level (Kumar and Philip, 2006).The rate of biodegradation in soil depends on four variables: (i) Availability of pesticide or metabolite to the microorganisms (ii) Physiological status of the microorganisms (iii) Survival and proliferation of pesticide degrading microorganisms at contaminated site and (iv) Sustainable population of the microorganisms (Dileep, 2008). Therefore, to attain an achievable bioremediation, it requires the creation of unique niche or microhabitats for desired microbes, so they can be successfully exploited. So far, no micro-organisms have been isolated with the ability to degrade DDT as a sole carbon and energy 186 Afr. J. Microbiol. Res. source (Jacques et al., 2008), but organisms may degrade the organochlorine via co-metabolism under aerobic or anaerobic conditions. Most reports indicate that DDT is reductively dechlorinated to DDD under reducing conditions (Lai and Saxena, 1999). Extensive biodegradation of DDT and DDT metabolites in some bacteria has been demonstrated (Aislabie et al., 1998). The major bacterial pathway appears to involve an initial reductive dechlorination of the trichloromethyl group to form DDD. Further dechlorination to other intermediaries occurs resulting finally into non chlorinated compounds which are not harmful to the environment. This study was based on the need to clean soil containing DDT in case it finds its way there and initiation of an assessment on impact of DDT on the tropical soil environment. The great versatility of microorganisms offers an inexpensive, simpler and more environmentally friendly strategy to reduce environmental pollution than non biological options (Jacques et al., 2008). The aim of this research was to isolate and characterize microorganisms that could biodegrade DDT from the tropical soil. Knowledge of the genetics, physiology and biochemistry of these microbes could further enhance the microbial process to achieve bioremediation of DDT with precision and in a short time. The standard method for isolating microorganisms with the ability to degrade environmental pollutants is to enrich them from contaminated soils. This process has not been very successful for the isolation of microorganisms that can mineralize DDT. A novel approach for isolating DDT-degrading microorganisms is to screen alternative sources like uncontaminated soil and other materials.

https://elibrary.pu.ac.ke/bitstream/handle/123456789/556/Degradation%20of%20dichlorodiphenyltrichloroethane%20(DDT).pdf?sequence=1

**Ref.10 - theories**
Cynthia Zook, Jingfeng Feng/ 1,1,1-Trichloro-2,2-bis-(4'-chlorophenyl)ethane (DDT) Pathway Map/ University of Minnesota/December 28, 1998

Abstract
DDT Pathway Map
Map: http://eawag-bbd.ethz.ch/ddt/ddt_image_map.html

http://eawag-bbd.ethz.ch/ddt/ddt_map.html

**Ref.11 – THEORY (EDUCATIONAL POWER-POINT)**
Educational power-point about Bioremediation

https://www.slideshare.net/vanithagopal/bioremediation-41934065?fbclid=IwAR13wNeqzIqfG63hOER-2Spn8_BTc9e8NOQ832FaUGx9b6p1nigkv2sq6Tok

**Theories**
**Ref 12**
Suiling Wang, Catherine N.Mulligan, An evaluation of surfactant foam technology in remediation of contaminated soil, Chemosphere Volume 57, Issue 9, December 2004, Pages 1079-1089

Abstract
Soil contamination is notoriously difficult to treat because the contaminants are often tightly bound to the soil particles. Conventional remediation technologies are becoming less popular due to the high treatment costs. This paper gives a comprehensive overview and evaluation of an emerging promising alternative, surfactant foam technology. Different from other approaches, surfactant foam technology may be designed either to remove contaminants or/ and simultaneously act as an augmentation for the existing technologies such as pump-and-treat systems and bioremediation processes to improve the contaminant removal efficiency and cost effectiveness. Encouraging results were achieved from laboratory and field demonstrations. However, as an innovative technology, there are many factors to be investigated with the future development. Special attention is paid to the selection of the most appropriate foaming surfactant and surfactant concentration, which are critical to the success of the implementation of the remediation process and have significant effects on the treatment costs. Moreover, development of predictive mathematical models in for future research is helpful to optimize the remediation process.

Link: https://doi.org/10.1016/j.chemosphere.2004.08.019

**Theories (Study book)**
**Ref 13**
Ramesh C. KuhadAtul K. JohriAjay SinghOwen P. Ward, Bioremediation of Pesticide-Contaminated Soils, In: Singh A., Ward O.P. (eds) Applied Bioremediation and Phytoremediation. Soil Biology, vol 1. Springer, Berlin, Heidelberg.

Abstract
About 4 million tonnes of pesticides are applied to agricultural crops annually for pest control worldwide. It is estimated that less than 1% of total applied pesticides generally gets to the target pests and most of the pesticides remain unused and enter into the ecosystem. The ultimate sink for excessive pesticides is soil and water. Despite their persistence in the environment, with a tendency of residues to bioaccumulate and be toxic to non-target organisms including humans, the use of chemical pesticides cannot be discontinued. Among various soil remediation technologies available today for decontamination and detoxification of pesticide-contaminated soils, bioremediation seems to be one of the most environmentally safe and cost effective methods (Fogarty and Tuovinen 1991; Häggblom 1992; Alexander 2000). Most of the pesticides generally fall under the major classes of chlorophenoxy acids, organochlorines, organophosphates, carbamates and s-triazines. This chapter focuses on microorganisms having the potential to degrade pesticides and on factors affecting pesticide biodégradation in contaminated soils.

Link: https://doi.org/10.1007/978-3-662-05794-0_3

# LABORATORY EXPERIMENT

**Ref.14 - lab experiment**

Tanuja Sonal Suman/Isolation and Characterization of a Bacterial Strain Enterobacter cloacae (Accession No. KX438060.1) Capable of Degrading DDTs Under Aerobic Conditions and Its Use in Bioremediation of Contaminated Soil/PubMed/2021/June 14

Abstract
DDT is one of the most persistent pesticides among all the different types of organo-chlorine pesticides used. Among all the degradation methods, bacterial degradation of DDT is most effective. The present study was conducted to isolate different bacteria present in waste samples which have the ability to degrade DDT present in the soil in the minimum possible period of time and to observe the effect of different physical and chemical properties of the soil samples. Many pesticide degrading bacteria were isolated and identified through cultural, biochemical tests and further identified by 16S RNA sequencing method. The most potent strain DDT 1 growth in mineral salt medium supplemented with DDT as the only source of carbon (5-100 PPM) and was monitored at an optical density of 600 nm. The growth parameters at different physio-chemical conditions were further optimized. The result showed that Enterobacter cloacae had maximum growth in 15 days. FTIR analysis of the residual DDT after 15 days incubation showed that Enterobacter cloacae was able to degrade pesticide into its further metabolites of DDD, DDE, DDNU and other components can be used for biodegradation of DDT present in contaminated soil and water ecosystems.

https://journals.sagepub.com/doi/full/10.1177/11786361211024289

**Ref.15 – lab experiment**

L J Nadeau, F M Menn, A Breen, and G S Sayler/Aerobic degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by Alcaligenes eutrophus A5/ Appl Environ Microbiol. 1994 Jan; 60(1): 51–55.

Abstract
Biotransformation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by Alcaligenes eutrophus A5 was demonstrated by analysis of ethyl acetate-extracted products from resting cell cultures. Gas chromatography-mass spectrometry characterization of the neutral extracts revealed two hydroxy-DDT intermediates (m/z = 370) with retention times at 19.55 and 19.80 min that shared identical mass spectra. This result suggested that the hydroxylations occurred at the ortho and meta positions on the aromatic ring. UV-visible spectrum spectrophotometric analysis of a yellow metabolite in the culture supernatant showed a maximum A402 with, under acidic and basic conditions, spectrophotometric characteristics similar to those of the aromatic ring meta-cleavage products. 4-Chlorobenzoic acid was detected by thin-layer chromatography radiochemical scanning in samples from mineralization experiments by comparison of Rf values of [14C]DDT intermediates with that of an authentic standard. These results were further confirmed by gas chromatography-mass spectrometry analysis. This study indicates that DDT appears to be oxidized by a dioxygenase in A. eutrophus A5 and that the products of this oxidation are subsequently subjected to ring fission to eventually yield 4-chlorobenzoic acid as a major stable intermediate.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC201268/

**Ref.16 – lab experiment, electrochemical**

Mohammad Moniruzzaman ,Yoshio Yano ,Toshikazu Ono ,Yoshio Hisaeda ,* and Hisashi Shimakoshi/ Aerobic Electrochemical Transformations of DDT to Oxygen-Incorporated Products Catalyzed by a B12 Derivative/Bulletin of the Chemical Society of Japan, 2021, Vol.94, No.11

Abstract
Electrochemical transformations of DDT into oxygen-incorporated products, amides and esters, catalyzed by a B12 derivative, heptamethyl cobyrinate perchlorate, have been developed under aerobic conditions. The dechlorinative oxygenation of DDT forms the acyl chloride as an intermediate for the synthesis of the amide and ester in the reaction with the amine and alcohol, respectively. This electrochemical method demonstrated with 20 oxygen-incorporated dechlorinated products up to 88% yields with 15 new compounds and was also successfully applied to the conversion of methoxychlor to an amide and ester.

https://www.journal.csj.jp/doi/abs/10.1246/bcsj.20210316

**Ref.17 – lab experiment**

H.C. Agarwal, D.K. Singh & V.B. Sharma/Persistence, metabolism and binding of P,P'-DDT in soil in Delhi, India/Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes/
Volume 29, 1994 - Issue 1

Abstract
The persistence, metabolism and binding of 14C-p,p'-DDT in soil were studied for two years under field sub-tropical conditions in Delhi, India. Two experiments were conducted at initial concentrations of 3.7 and 12.8 ppm and it was shown that about 80% of the DDT was lost from the soil in two years. The half life of DDT was 319 and 343 days in the two experiments. At zero-time p,p'-DDT accounted for 93–95% of the extractable residues. Gradually, the proportion of p,p'-DDT declined with time to 58–71% of the extractable residues after two years. In addition to DDT, the extractable residues contained DDE and DDD. DDE was the major metabolite of DDT in soil and accounted for about 22% of the extractable residue after 245 days and 37% after 18 months. DDD accounted for only 5% of the extractable residues after 18 months. Initially, the amount of bound residues was very small, but it gradually increased reaching a maximum of about 8% after one year. However, the bound residues declined thereafter to 4.5–5.45% after 1.5 years and to 3.4–4.2% after 2 years. The soil-bound residues were chemically released by sulfuric acid treatment and were found to consist of DDT, DDE and DDD.

https://www.tandfonline.com/doi/abs/10.1080/03601239409372860

**Ref.18 – lab experiment**

Guangli WangJi ZhangLi WangBin LiangKai ChenShunpeng LiJiandong Jiang/Co-metabolism of DDT by the newly isolated bacterium, Pseudoxanthomonas sp. wax/Braz. J. Microbiol. 41 (2) • June 2010

Abstract
Microbial degradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) is the most promising way to clean up DDT residues found in the environment. In this paper, a bacterium designated as wax, which was capable of co-metabolizing DDT with other carbon sources, was isolated from a long-term DDT-contaminated soil sample by an enrichment culture technique. The new isolate was identified as a member of the Pseudoxanthomonas sp., based on its morphological, physiological and biochemical properties, as well as by 16S rRNA gene analysis. In the presence of 100 mg l-1 glucose, the wax strain could degrade over 95% of the total DDT, at a concentration of 20 mg l-1, in 72 hours, and could degrade over 60% of the total DDT, at a concentration of 100 mg l-1, in 144 hours. The wax strain had the highest degradation efficiency among all of the documented DDT-degrading bacteria. The wax strain could efficiently degrade DDT at temperatures ranging from 20 to 37ºC, and with initial pH values ranging from 7 to 9. The bacterium could also simultaneously co-metabolize 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), 2,2-bis(p-chlorophenyl)-1,1-dichlorethylene (DDE), and other organochlorine compounds. The wax strain could also completely remove 20 mg kg-1 of DDT from both sterile and non-sterile soils in 20 days. This study demonstrates the significant potential use of Pseudoxanthomonas sp. wax for the bioremediation of DDT in the environment.

https://www.scielo.br/j/bjm/a/xN7NP3GQLqKxVwTyx7by3gF/

**Ref.19 – lab experiment**

Y. C. Zhao; X. Y. Yi; M. Zhang; L. Liu ; W. J. Ma/ Fundamental study of degradation of dichlorodiphenyltrichloroethane in soil by laccase from white rot fungi/Int. J. Environ. Sci. Tech., 7 (2), 359-366, Spring 2010

Abstract
This research describes application of laccase from white-rot fungi (polyporus) to remove dichlorodiphenyltrichloroethane in soil. The degradation kinetics of dichlorodiphenyltrichloroethane in soil was also investigated by laboratory batch experiments. The results showed that laccase from white-rot fungi can effectively degrade dichlorodiphenyltrichloroethane and the degradation of total dichlorodiphenyltrichloroethane (the sum of the four dichlorodiphenyltrichloroethane compounds in a sample) was pseu-

do-first-order kinetics. The residues of almost all the dichlorodiphenyltrichloroethane components and total dichlorodiphenyltrichloroethane in soils treated with laccase decreased rapidly during first 15 days and then kept at a stable level during next 10 days. The residues of total dichlorodiphenyltrichloroethane in soils with different dosages laccase decreased by about 21-32 %, 29-45%, 35-51 % and 36-51 % after 5, 10, 15 and 25 days of incubation, respectively. The half-life of total dichlorodiphenyltrichloroethane in soils with different dosages laccase ranged from 24.75 to 41.75 days. The residues of total dichlorodiphenyltrichloroethane in three different types of soils decreased by 25-29 %, 39-43 %, 44-47 % and 47-52 % after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane in different types of soil ranged from 24.71 to 27.68 days. The residues of total dichlorodiphenyltrichloroethane in soils with different pH levels decreased by 18-24 %, 29-39 %, 36-39 % and 39-50% after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane ranged from 25.63 to 36.42 days. Laccase can be an efficient and safe agent for remediation of dichlorodiphenyltrichloroethane-contaminated soil.

https://applications.emro.who.int/imemrf/Int_J_Environ_Sci_Technol/Int_J_Environ_Sci_Technol_2010_7_2_359_366.pdf

**Ref.20 lab experiment, influence of hydroxyl radicals and ozone**

Maciej Balawejder, Piotr Antos, Sylwia Czyjt-Kuryło, Radosław Józefczyk/262013655_A_Novel_Method_for_Degradation_of_DDT_in_Contaminated_Soil/ Ozone: Science & Engineering: The Journal of the International Ozone Association/April 2014/Volume 36, 2014 - Issue 2

Abstract
A novel method for remediation of DDT contaminated soil based on advanced oxidation processes including reactions with hydroxyl radicals and ozone has been developed. On the basis of the conducted experiments the authors concluded that ozone did not degrade DDT in soil. Analysis of degradation products that were formed during exposure to a stream of gaseous ozone indicated that hydroxyl radicals might play a key role in degradation of this compound. In the modified fluidized bed reactor where hydroxyl radicals could be generated from ozone and water vapor, reduction of over 90% of DDT residue level has been achieved. Authors proposed a pathway of DDT degradation, under the influence of hydroxyl radicals and ozone. As a result of this research, an effective method for remediation of DDT-contaminated soil has been proposed.

https://www.researchgate.net/publication/262013655_A_Novel_Method_for_Degradation_of_DDT_in_Contaminated_Soil

**Ref.21 – lab experiment**

Yei-Shung Wang , Tzu-Chuan Chiu , Jui-Hung Yen/ EFFECTS OF MICROBIAL INHIBITORS ON ANAEROBIC DEGRADATION OF DDT/ORGANOHALOGEN COMPOUNDS – Volume 66 (2004)

Abstract
Introduction Chlorinated insecticide DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] was extensively used for controlling pests in the agricultural field and human-being living environments in the past several decades. Due to the chemical stability, DDT was extremely persistent and recalcitrant in soils and sediments and it was banned by nations. Microorganisms usually play important roles in reducing organochlorine compounds in the environments1.2.3. Under low-oxygen conditions, microbial dechlorination is thought as the onset of highly chlorinated compounds. Methanogenic and sulfate-reducing bacteria participate in microbial dechlorination under anaerobic condition has been reported4,5. In this study, a mixed anaerobic culture enabling to dechlorinate DDT was obtained from river sediment in Taiwan. In order to understand the effect of these microorganisms on DDT dechlorination, microbial inhibitors BESA (2-bromoethanesulfonate) and molybdate, for inhibiting methanogenic and sulfate-reducing bacteria, respectively, were chosen to investigate the interaction between specific microbial communities and their degradation activities. Besides, a molecular technique, denaturing gradient gel electrophoresis6 (DGGE), based on analyzing the 16S rDNA of bacteria, was used for monitoring the bacterial community structure in this study.

https://www.osti.gov/etdeweb/servlets/purl/20828157

**Ref.22 – lab experiment**

Hui Xie, Lusheng Zhu & Jun Wang/Combined treatment of contaminated soil with a bacterial Stenotrophomonas strain DXZ9 and ryegrass (Lolium perenne) enhances DDT and DDE remediation/ Environmental Science and Pollution Research 25, 31895–31905/2018

Abstract
Bioremediation of contaminated soils by a combinational approach using specific bacterial species together with ryegrass is a promising strategy, resulting in potentially highly efficient degradation of organic contaminants. The present study tested the combination of strain DXZ9 of Stenotrophomonas sp. with ryegrass to remove DDT and DDE contaminants from soil under natural conditions in a pot experiment. The strain DXZ9 was successfully colonized in the natural soil, resulting in removal rates of approximately 77% for DDT, 52% for DDE, and 65% for the two pollutants combined after 210 days. Treatment with ryegrass alone resulted in slightly lower removal rates (72 and 48%, respectively, 61% for both combined), while the combination of strain DXZ9 and ryegrass significantly ($p < 0.05$) improved the removal rates to 81% for DDT and 55% for DDE (69% for both). The half-life of the contaminants was significantly shorter in combined treatment with DXZ9 and ryegrass compared to the control. The remediation was mostly due to degradation of the contaminants, as the net uptake of DDT and DDE by the ryegrass accounted for less than 3% of the total amount in the soil. DDT is reductively dechlorinated to DDD and dehydrochlorinated to DDE in the soil; the metabolites of DDE and DDD were multiple undefined substances. The toxicity of the soil was significantly reduced as a result of the treatment. The present study demonstrates that the bioremediation of soil contaminated with DDT and DDE by means of specific bacteria combined with ryegrass is feasible.

https://link.springer.com/article/10.1007/s11356-018-1236-7

**Ref.23 – lab experiment**

RajkumarBidlanH.K.Manonmani/Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by Serratia marcescens DT-1P Volume 38, Issue 1/ September 2002/ Pages 49-56

Abstract
Microbial degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)-residues is one of the mechanisms for the removal of this compound from the environment. A DDT-degrading consortium was isolated by long term enrichment of soil samples collected from DDT-contaminated fields. This consortium was acclimated by repeated passages through a mineral salt medium containing increasing concentrations of DDT. This acclimated consortium could degrade 25 ppm of DDT in 144 h. The consortium consisted of four bacteria. Of these, Serratia marcescens DT-1P was used for further studies. Various factors such as inoculum size, concentration of DDT, pH, temperature, presence of co-substrates, the type of carbon source used influenced the degradation of DDT in shake flasks. Complete degradation was observed up to 15 ppm DDT, followed by inhibitory effects at higher concentrations showing a total loss of degradative ability at 50 ppm DDT. Effective degradation of DDT was obtained with the inoculum pre-exposed to DDT for 72 h. Degradation was inhibited in the presence of auxiliary carbon sources such as citrate, rice straw hydrolysate. However, the presence of yeast extract, peptone, glycerol and tryptone soya broth (TSB) showed complete disappearance of DDT. Mesophilic temperatures (26–30 °C) and near neutral pH (6.0–8.0) were most favourable for degradation. This microbial culture holds the potential for use in bioremediation of DDT-contaminated soils, waste deposits and water bodies.

https://www.sciencedirect.com/science/article/abs/pii/S0032959202000663

**Ref.24 – lab experiment, fenton reaction**

SetyoPurnomo, ToshioMori, RyuichiroKondo/Involvement of Fenton reaction in DDT degradation by brown-rot fungi/International Biodeterioration & Biodegradation,Volume 64, Issue 7/ October 2010/ Pages 560-565

Abstract
In this study, the ability of the brown-rot fungi (BRF) Fomitopsis pinicola and Daedalea dickinsii to degrade DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) was investigated. F. pinicola and D. dickinsii degraded approximately 63% and 47% of total DDT in potato dextrose broth (PDB) medium, respectively. Degradation of DDT by D. dickinsii re-

sulted in several metabolic products; DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene), and DDMU (1-chloro-2,2-bis (4-chlorophenyl) ethylene), whereas degradation by F. pinicola produced only one metabolic product, DDD. Investigation was done to find out whether the Fenton reaction was involved in the degradation process. More DDT was degraded in medium lacking FeSO4 than in that containing FeSO4, suggesting that these fungi lack the benzoquinone-driven Fenton reaction cycle system. In addition, F. pinicola and D. dickinsii produced only very low levels of hydroxyl radicals (1.2 and 5.1 μM, respectively), which provides further evidence that these fungi lack the Fenton reaction cycle system. The addition of mannitol as a hydroxyl radical scavenger did not inhibit DDT degradation. Hydroxyl radical production increased when the medium was supplemented with Fe2+. Higher levels of hydroxyl radicals enhanced DDT degradation by D. dickinsii, but not by F. pinicola. These results indicate that F. pinicola and D. dickinsii have different mechanisms for degrading DDT from that reported for the BRF Gloeophyllum trabeum, in which the Fenton reaction is an important factor in DDT degradation.

https://www.sciencedirect.com/science/article/abs/pii/S0964830510001198

**Ref.25 – lab experiment**

BeibeiWang, WuxingLiuaXiaoyanLiu, Ashley E. Franks, YingTeng, YongmingLuo/Comparative analysis of microbial communities during enrichment and isolation of DDT-degrading bacteria by culture-dependent and -independent methods/Science of The Total Environment, Volumes 590–591/ 15 July 2017/ Pages 297-303
Abstract
Microcosms for enrichment of DDT degrading microorganisms were monitored using culture-dependent and -independent methods. Culture dependent methods isolated several strains with DDT degradation potential, Pseudomonas species being the most frequent. One isolate, Streptomyces sp. strain D3, had a degradation rate of 77% with 20 mg L– 1 of DDT after 7 days incubation, D3 also had degradation rates of 75% and 30% for PCB77 (3,3′ ,4,4′ -tetrachloro biphenyl) and PCNB (pentachloronitrobenzene) respectively. Culture-independent high-throughput sequencing identified a different subset of the microbial community within the enrichment microcosms to the culture dependent method. Pseudomonas, the most frequently isolated strain, only represented the 12th most abundant operational taxonomic unit in the sequencing dataset (relative abundance 0.9%). The most frequently observed bacterial genus in the culture-independent analysis did not correspond with those recovered by culture-dependent methods. These results suggested that deep sequencing followed by a targeted isolation approach might provide an advantageous route to bioremediation studies.

https://www.sciencedirect.com/science/article/abs/pii/S0048969717305090

**Ref.26 – lab experiment**

Noreen Asim, Mahreen Hassan, Farheen Shafique, Maham Ali, Hina Nayab, Nuzhat Shafi, Sundus Khawaja, Sadaf Manzoor/Characterizations of novel pesticide-degrading bacterial strains from industrial wastes found in the industrial cities of Pakistan and their biodegradation potential/ Published October 5, 2021

Abstract
Background
Lack of infrastructure for disposal of effluents in industries leads to severe pollution of natural resources in developing countries. These pollutants accompanied by solid waste are equally hazardous to biological growth. Natural attenuation of these pollutants was evidenced that involved degradation by native microbial communities. The current study encompasses the isolation of pesticide-degrading bacteria from the vicinity of pesticide manufacturing industries.
Methods
The isolation and identification of biodegrading microbes was done. An enrichment culture technique was used to isolate the selected pesticide-degrading bacteria from industrial waste.
Results
Around 20 different strains were isolated, among which six isolates showed significant pesticide biodegrading activity. After 16S rRNA analysis, two isolated bacteria were identified as Acinetobacter baumannii (5B) and Acidothiobacillus ferroxidans, and the remaining four were identified as different strains of Pseudomonas aeruginosa (1A, 2B, 3C, 4D). Phylogenetic analysis confirmed their evolution from a

common ancestor. All strains showed distinctive degradation ability up to 36 hours. The Pseudomonas aeruginosa strains 1A and 4D showed highest degradation percentage of about 80% for DDT, and P. aeruginosa strain 3C showed highest degradation percentage, i.e., 78% for aldrin whilst in the case of malathion, A. baumannii and A. ferroxidans have shown considerable degradation percentages of 53% and 54%, respectively. Overall, the degradation trend showed that all the selected strains can utilize the given pesticides as sole carbon energy sources even at a concentration of 50 mg/mL.
Conclusion
This study provided strong evidence for utilizing these strains to remove persistent residual pesticide; thus, it gives potential for soil treatment and restoration.

https://peerj.com/articles/12211/

**Ref.27 – lab experiment**

Brendan J. Ryan/The Microbial Degradationf the DDT Metabolite Dichlorobenzophenone (DBP) / University of Canterbury Christchurch, N .z. 1995
Abstract
Soil samples which had been previously exposed to chlorinated aromatics were screened for ability to degrade dichlorobenzophenone (DBP). Of the samples tested soil from the Dow Elanco Agricultural Farm and soil from the AgResearch Winchmore Research Station showed apparent degradative capabilities. Degradation was not sustainable inthese soil samples and a time scale study showed DBP was stable in soil over a 24 week period. Samples from Winchmore were further used to establish enrichment cultures capable of degrading DBP and its non-chlorinated analogue benzophenone (BP) through selection pressure. BP proved to be readily degraded but DBP degradation was only achieved after fungal suppressants were used. Degradation of DBP was enhanced with the addition of yeast extract and sodium salicylate to the enrichment cultures. Degradation ofDBP was confirmed by capillary gas chromatography and the detection of the metabolite p-chlorophenyl acetic acid by gas chromatography-mass spectroscopy and thin layer chromatography. The enrichment cultures established on DBP also extensively degraded BP, p-chlorobenzophenone and pchlorobenzoic aid. When inoculated back into soil, the enrichment cultures degradative capabilities were significantly reduced due to competition from other organisms, availability of alternative carbon sources and the bioavailability ofDBP due to binding to soil particles. Two organisms capable of degrading BP as sole carbon source were isolated and identified as a Rhodococcus spp. and a Streptomyces spp. BP degradative capabilities were not maintained by these organisms and lost when subculturing on nutrient media Three presumptive DBP degraders were isolated and presumptively identified as two separate Streptomyces spp. and a Pseudomonas vesicularis. Of the three none were able to degrade DBP as sole carbon source in liquid culture although the P. vesicularis was able to co-metabolise DBP with the addition of yeast extract and sodium salicylate. Preliminary genetical studies of the P. vesicularis isolated were carried out.

https://ir.canterbury.ac.nz/bitstream/handle/10092/8554/ryan_thesis.pdf;sequence=1

**Ref.28 – lab experiment**

Xiong Pan, Dunli Lin, Yuan Zheng, Qian Zhang, Yuanming Yin, Lin Cai, Hua Fang & Yunlong Yu/Biodegradation of DDT by Stenotrophomonas sp. DDT-1: Characterization and genome functional analysis/ Scientific Reports 6, Article number: 21332 (2016)
Abstract
A novel bacterium capable of utilizing 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) as the sole carbon and energy source was isolated from a contaminated soil which was identified as Stenotrophomonas sp. DDT-1 based on morphological characteristics, BIOLOG GN2 microplate profile and 16S rDNA phylogeny. Genome sequencing and functional annotation of the isolate DDT-1 showed a 4,514,569 bp genome size, 66.92% GC content, 4,033 protein-coding genes and 76 RNA genes including 8 rRNA genes. Totally, 2,807 protein-coding genes were assigned to Clusters of Orthologous Groups (COGs) and 1,601 protein-coding genes were mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The degradation half-lives of DDT increased with substrate concentration from 0.1 to 10.0 mg/l, whereas decreased with temperature from 15 °C to 35 °C. Neutral condition was the most favorable for DDT biodegradation. Based on genome annotation of DDT degradation genes and the metabolites detected by GC-MS, a mineralization pathway was proposed for DDT biodegrada-

tion in which it was orderly converted into DDE/DDD, DDMU, DDOH and DDA via dechlorination, hydroxylation and carboxylation and ultimately mineralized to carbon dioxide. The results indicate that the isolate DDT-1 is a promising bacterial resource for the removal or detoxification of DDT residues in the environment.

https://www.nature.com/articles/srep21332

**Ref.29 – LAB (FUNGI)**
Y. C. Zhao, X. Y. Yi, M. Zhang, L. Liu, W. J. Ma/Fundamental study of degradation of dichlorodiphenyltrichloroethane in soil by laccase from white rot fungi/International journal of Environmental Science and Technology/1.March-2010/Pages359-366

ABSTRACT
This research describes application of laccase from white-rot fungi (polyporus) to remove dichlorodiphenyltrichloroethane in soil. The degradation kinetics of dichlorodiphenyltrichloroethane in soil was also investigated by laboratory batch experiments. The results showed that laccase from white-rot fungi can effectively degrade dichlorodiphenyltrichloroethane and the degradation of total dichlorodiphenyltrichloroethane (the sum of the four dichlorodiphenyltrichloroethane compounds in a sample) was pseudo-first-order kinetics. The residues of almost all the dichlorodiphenyltrichloroethane components and total dichlorodiphenyltrichloroethane in soils treated with laccase decreased rapidly during first 15 days and then kept at a stable level during next 10 days. The residues of total dichlorodiphenyltrichloroethane in soils with different dosages laccase decreased by about 21-32 %, 29-45%, 35-51 % and 36-51 % after 5, 10, 15 and 25 days of incubation, respectively. The half-life of total dichlorodiphenyltrichloroethane in soils with different dosages laccase ranged from 24.75 to 41.75 days. The residues of total dichlorodiphenyltrichloroethane in three different types of soils decreased by 25-29 %, 39-43 %, 44-47 % and 47-52 % after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane in different types of soil ranged from 24.71 to 27.68 days. The residues of total dichlorodiphenyltrichloroethane in soils with different pH levels decreased by 18-24 %, 29-39 %, 36-39 % and 39-50% after 5, 10, 15 and 25 days of incubation with laccase, respectively. The half-life of total dichlorodiphenyltrichloroethane ranged from 25.63 to 36.42 days. Laccase can be an efficient and safe agent for remediation of dichlorodiphenyltrichloroethane-contaminated soil.

http://www.bioline.org.br/pdf?st10035

**Ref.30 – LAB (water)**
Azrina Aziz, Mohamad Nasran Nasehir Khan, Mohamad Firdaus Mohamad Yusop, Erniza Mohd Johan Jaya, Muhammad Azan Tamar Jaya, Mohd Azmier Ahmad/"Single-Stage Microwave-Assisted Coconut-Shell-Based Activated Carbon for Removal of Dichlorodiphenyltrichloroethane (DDT) from Aqueous Solution: Optimization and Batch Studies"/International Journal of Chemical Engineering/vol. 2021, Article ID 9331386/8.Oct.-2021/15pages

ABSTRACT
This research aims to optimize preparation conditions of coconut-shell-based activated carbon (CSAC) and to evaluate its adsorption performance in removing POP of dichlorodiphenyltrichloroethane (DDT). The CSAC was prepared by activating the coconut shell via single-stage microwave heating under carbon dioxide, CO2 flow. The total pore volume, BET surface area, and average pore diameter of CSAC were 0.420 cm3/g, 625.61 m2/g, and 4.55 nm, respectively. The surface of CSAC was negatively charged shown by the zeta potential study. Response surface methodology (RSM) revealed that the optimum preparation conditions in preparing CSAC were 502 W and 6 min for radiation power and radiation time, respectively, which corresponded to 84.83% of DDT removal and 37.91% of CSAC's yield. Adsorption uptakes of DDT were found to increase with an increase in their initial concentration. Isotherm study revealed that DDT-CSAC adsorption system was best described by the Langmuir model with monolayer adsorption capacity, Qm of 14.51 mg/g. The kinetic study confirmed that the pseudo-second-order model fitted well with this adsorption system. In regeneration studies, the adsorption efficiency had slightly dropped from 100% to 83% after 5 cycles. CSAC was found to be economically feasible for commercialization owing to its low production cost and high adsorption capacity.

https://www.hindawi.com/journals/ijce/2021/9331386/?fbclid=IwAR1yPZ3CBAakWgqZTFxv1ObNamxREjBH2sh7f-znoQY0lBYUFAd4HnkEzMg

**Ref.31 – LAB (water)**

Sarah Al-Rashed, Najat Marraiki, Asad Syed, Abdallah M Elgorban, Kollur Shiva Prasad, Chandan Shivamallu, Ali H Bahkali/Bioremediation characteristics, influencing factors of dichlorodiphenyltrichloroethane (DDT) removal by using non-indigenous Paracoccus sp/Chemosphere 2021, Volume 270/May-2021

ABSTRACT
The marine bacterium able to consume DDT as the nutrient source was isolated from sea water which was identified as Paracoccus sp. DDT-21 based on 16 S rDNA gene sequence and Gram negative rod, obligate aerobic, non-motile biochemical characteristics. The isolate can degrade over 80% of the DDT, at a concentration of 50 mg/L in MSM in 72 h. Time and pollutant (DDT) dependent growth studies indicated that the isolate Paracoccus sp., DDT-21 significantly degrade the DDT and tolerates under DDT stress up to 50 mg/L. The DDT degradation capability of the strain Paracoccus sp. DDT-21 was found to be 5′ 10′ 15′ 25′ 50 mg/L DDT. The high concentrations (75 and 100 mg/L) of DDT showed significant decrease in DDT degradation. The optimal DDT degradation (′ 90.0%) was observed at 6 g/L of yeast extract, 6% of glucose in pH 7.0 at 35 °C with 72 h of incubation as constant. Furthermore, four metabolites were observed by GC-MS analysis such as, DDE, DDD, DDMU, and DDA. The obtained results indicate that the isolate Paracoccus sp. DDT-21 is a promising candidate for the removal and/or detoxification of DDT in the environment.

https://www.sciencedirect.com/science/article/abs/pii/S0045653520336729

**Ref.32 – LAB**

Sarah Mustafa Ahmed, Mohd Raihan Taha, Omer Muhie Eldeen Taha/Kinetics and Isotherms of Dichlorodiphenyltrichloroethane (DDT) Adsorption Using Soil-Zeolite Mixture/Nanotechnology for Environmental Engineering, Article number 4 (2018)/Published 20.Dec-2017

ABSTRACT
Soil contaminated by organic pollutants such as dichlorodiphenyltrichloroethane (DDT) is an environmental concern due to the strong sorption of organochlorine pesticide onto the soil matrix and persistence in the environment. The remediation of contaminated soils with organochlorine pesticide using nanotechnology is an innovative technology for speeding up this process. This work presents a study of adsorption of DDT onto the zeolite surface. Experiments were conducted using batch adsorption procedures at different DDT concentrations, from 5 to 50 mg/L, and the amount of the zeolite used was 0.1, 0.5, 0.8, and 1.2 g. Results show that the zeolite has a moderate adsorption capacity for the DDT, and the highest adsorption capacity obtained from this study was about 30%. However, the percentage of adsorption can be increased significantly with the increase in the amount of the zeolite in samples. Also, adsorption kinetics and adsorption isotherms were applied. Five different kinetic models, i.e., pseudo-first-order kinetic model, the pseudo-second-order kinetic model, intraparticle diffusion model, Elovich kinetic model, and Bangham kinetic model were used to fit the kinetic data. The result shows that the pseudo-second-order model represented the best fits to the experiments. The adsorption isotherms were determined using three different models as well, i.e., Freundlich, Langmuir, and Temkin. The best-fitted adsorption isotherm models were found to be in both Langmuir and Freundlich. Moreover, results show that the effectiveness of treatment process is highly affected by pH. Increasing the pH has a negative effect on the adsorption process, and best uptake of DDT was noted in acidic media at pH 3.

https://scr.toolsminati.com/document/472532204/Kinetics-and-isotherms-of-dichlorodiphenyltrichloroethane-DDT-adsorption-using-soil-zeolite-mixture?fbclid=IwAR1KA_l02N7kp7KhRQ5g_amBTDZcZgxdKxAlCboDCjct1htdxcN6ETqHcJA

**Ref.33 – LAB (AEROBIC)**

RajkumarBidlan, H.K.Manonmani/Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by Serratia marcescens DT-1P/Process Biochemistry, Volume 38, Issue 1/September-2002/Pages49-56

ABSTRACT
Microbial degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)-residues is one of the mechanisms for the removal of this compound from the environment. A DDT-degrading consortium was isolated by long term enrichment of soil samples collected from DDT-contaminated fields. This consortium was acclimated by repeated passages through a mineral salt medium containing increasing concentrations of DDT. This acclimated consortium could degrade 25 ppm of DDT in 144 h. The consortium consisted of

four bacteria. Of these, Serratia marcescens DT-1P was used for further studies. Various factors such as inoculum size, concentration of DDT, pH, temperature, presence of co-substrates, the type of carbon source used influenced the degradation of DDT in shake flasks. Complete degradation was observed up to 15 ppm DDT, followed by inhibitory effects at higher concentrations showing a total loss of degradative ability at 50 ppm DDT. Effective degradation of DDT was obtained with the inoculum pre-exposed to DDT for 72 h. Degradation was inhibited in the presence of auxiliary carbon sources such as citrate, rice straw hydrolysate. However, the presence of yeast extract, peptone, glycerol and tryptone soya broth (TSB) showed complete disappearance of DDT. Mesophilic temperatures (26–30 °C) and near neutral pH (6.0–8.0) were most favourable for degradation. This microbial culture holds the potential for use in bioremediation of DDT-contaminated soils, waste deposits and water bodies.

https://www.sciencedirect.com/science/article/abs/pii/S0032959202000663?fbclid=IwAR0v8Wf6B-jgn6E211XYscfNAIjoleAmI1f4yrWZ-tuMeM5UD-7nOgSsa0Bdg

**Ref.34 – LAB (NZVI)**

Swatantra Pratap Singh, Purnendu Bose/Degradation of soil-adsorbed DDT and its residues by NZVI addition/RSC Advances/November-2015

ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is a highly persistent and toxic chlorinated pesticide. Market-grade DDT is a mixture of 4,4-DDT (85%), 2,4-DDT (15%) and trace amounts of 4,4-DDD, 2,4-DDD, 4,4-DDE and 4,4-DDMU. This mixture is commonly known as DDT and its residues, i.e., DDTr compounds. Due to their strongly hydrophobic nature, DDTr compounds are mostly partitioned into soil and sediments in the natural environment. Preliminary aqueous phase experiments showed that DDT and DDD were degraded by NZVI, with the degradation rates being 2,4-DDT > 4,4-DDT > 2,4-DDD > 4,4-DDD. NZVI addition to soil contaminated with DDTr compounds resulted in rapid reduction in soil-phase 4,4-DDT and 2,4-DDT concentrations and increase in soil-phase 4,4-DDD and 2,4-DDD concentrations, indicating conversion of 2,4-DDT to 2,4-DDD and 4,4-DDT to 4,4-DDD. Multiple addition of NZVI resulted in complete degradation of soil phase 4,4-DDT and 2,4-DDT and reduction in concentrations of 4,4-DDD and 2,4-DDD. Considering the extremely hydrophobic nature of DDTr compounds and their consequent unavailability in the aqueous phase, only direct soil-phase interaction between DDTr compounds and NZVI can explain these experimental observations. A mathematical model incorporating soil phase DDTr–NZVI interactions could explain and simulate the experimental data adequately. Mass balance on DDTr concentrations in soil indicated that ′ 40 percent of the DDTr initially present in soil could be removed through the first NZVI addition. Further NZVI additions were successively less effective in removing DDTr from soil and after four successive additions of NZVI, ′ 64% reduction in soil-phase DDTr concentration was achieved.

https://pubs.rsc.org/en/content/articlelanding/2015/ra/c5ra18282d?fbclid=IwAR1mA6weIlgVexFcRSsb-ZU37argazDqkDdbzNhihVezpJKASg1g8tyL05SA#

**Ref.35 – LAB (BACTERIA)**

Geetika Pant, Sandeep Kumar Mistry, G. Sibi/Isolation, Identification and Characterization of p, p’-DDT Degrading Bacteria from Soil/Journal of Environmental Science and Technology/7. November-2013/Pages130-137

ABSTRACT

DDT (dichlorodiphenyltrichloroethane), one of the organochlorine pesticides was widely used in agriculture and healthcare. Though its usage is banned in most of the countries, DDT residues cause varying negative effects through bioaccumulation and bioamagnification. Biodegradation is an potential method to detoxify the recalcitrant compounds and this study is an investigation to isolate and characterize the p, p’-DDT degrading bacteria from DDT contaminated soil. An p, p’-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] degrading bacterium was isolated and identified by 16S rRNA studies. The role of temperature, pH and DDT concentration in the degrading ability of the isolate has also been investigated. Based on the analysis of the phenotype, biochemical characteristics and 16S rRNA, the strain was identified to belong to the bacterial genera Bacillus and was named as Bacillus strain GSS. The isolate had an optimum pH of 7.0 at 35°C and was able to degrade DDT at a wide range of concentrations with complete degradation of the DDT (10 mg L-1) and 89.3% of 15 mg L-1 in 120 h, whereas 100% degradation of 5 mg L-1 concentra-

tion was observed within 48 h. Significant degradation was observed at 72 h and 96 h for 15 and 20 mg L-1, respectively. At the end of 120 h, 73% of 20 mg L-1 and 34% of 25 mg L-1 was recorded by the isolate Bacillus strain GSS.

https://scialert.net/fulltext/?doi=-jest.2013.130.137&fbclid=I-wAR0-JpuM9ev_3Y0QpeaT9KZX_XLufMak0WxA-viys4KQ3PlJ2Znk_nwwBK8c

**Ref.36 – LAB (BACTERIA)**

Gary Wedemeyer/Biodegradation of dichlorodiphenyltrichloroethane: intermediates in dichlorodiphenylacetic acid metabolism by aerobacter aerogenes/ Applied Microbiology, Volume 15, Issue 6/1967/Pages1494-1495

ABSTRACT
The final product of dichlorodiphenyltrichloroethane (DDT) degradation by vertebrates is commonly considered to be dichlorodiphenylacetic acid, DDA. Recently, certain organisms have been found to degrade further DDA to dichlorobenzophenone (DBP), but the possibility that such degradation was due to microbial action could not be excluded. Significantly, dichlorobenzhydrol (DBH), dichlorophenylmethane (DPM), and dichlorodiphenylethylene (DDE) have been tentatively identified in rats fed DDA. Since DDA as well as DDT is degraded by the ubiquitous microorganism Aerobacter aerogenes, it seemed reasonable that the intestinal microflora might be involved in DBP formation, DPM and DBH being intermediates in its pathway from DDA. Since DDA is a (3,y-unsaturated acid, ketone formation via an alkene and an alcohol would be expected.

https://journals.asm.org/doi/epdf/10.1128/am.15.6.1494-1495.1967

https://pubs.er.usgs.gov/publication/70171221?fbclid=IwAR2EpCxfwd9z44DY9crqKeCbaGuD-A07b-7NMKHlr15-fdYGudFT3im2gKjI

**Ref.37 – LAB (BACTERIA)**

Koji Ito, Ahmad Mahmood, Ryota Kataoka, Kazuhiro Takagi/Dichlorodiphenyltrichloroethane (DDT) degradation by Streptomyces sp. isolated from DDT contaminated soil/Bioremediation Journal/ Febuary-2021/Pages148-158

ABSTRACT
Bioaccumulation, toxicity and long-range transport of persistent organic pollutants (POPs) is a threat to soil and other ecosystems, therefore removal of such pollutants from soil is required. This study thus aimed to isolate and identify bacteria capable of biodegrading a persistent organic pollutant; dichlorodiphenyltrichloroethane (DDT) from a former DDT production unit. The soil was sampled, transported to Japan through standard protocols and bacteria were isolated and assayed for dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) degradation. The concentration of DDTs in a composite soil sample was 0.7 mg kg′ [1]. Twenty-four bacterial strains were isolated from DDT-contaminated soil, and one of them, strain 885, showed degradation of DDT but could not degrade DDE after 14 days of incubation. This strain also showed significant degradation of 15.6 µM DDD (degradation rate 55.9%) after 28 days incubation. Further metabolites of DDT including 2,2-bis(p-chlorophenyl) ethanol (DDOH) and 4,4-dichlorobenzophenone (DBP) were detected from a 14-day incubation culture with DDD as initial substrate. Strain 885 also showed degradation of metabolites of DDT including those of DDOH, and DBP. In conclusion, strain 885 successfully degraded DDT; therefore, it can be used for remediation of DDT-contaminated soil.

https://www.researchgate.net/publication/349092718_Dichlorodiphenyltrichloroethane_DDT_degradation_by_Streptomyces_sp_isolated_from_DDT_contaminated_soil

**Ref.38 - LAB**

A Researcher Was Attempting To Quantify The Amount Of Dichlorodiphenyltrichloroethane (DDT) In Spinach Using Gas

A researcher was attempting to quantify the amount of dichlorodiphenyltrichloroethane (DDT) in spinach using gas chromatography mass spectrometry (GCMS). First attempts using external calibration proved difficult, as reproducibility and external standard agreement were poor. To overcome these problems, the researcher used the internal standard calibration method with chloroform as the internal standard. To begin, the researcher analyzed a sample containing 6.53 mg/L DDT standard and 2.15 mg/L chloroform as the internal standard, which produced peak areas of 4415 and 10119 for the DDT and chloroform, respectively. Determine the response factor

(F). F= After establishing the response factor of the instrument, the researcher collected 13.57 g of spinach, homogenized the sample, and extracted the DDT using an established method, producing a 2.60 mL solution containing an unknown amount of extracted DDT. The researcher then prepared a sample for analysis that contained 0.750 mL of the unknown DDT solution and 1.00 mL of 10.15 mg/L chloroform, which was diluted to a final volume of 25.00 mL. The sample was analyzed using GCMS, producing peak areas of 6463 and 11979 for the DDT and chloroform, respectively. Calculate the amount of DDT in the spinach sample. Express the final answer as milligrams of DDT per gram of spinach. amount of DDT: mg DDT /g spinach

https://www.chegg.com/homework-help/questions-and-answers/researcher-attempting-quantify-amount-dichlorodiphenyltrichloroethane-ddt-spinach-using-ga-q45096918?fbclid=IwAR2ZqbNfUA_r41jURhxcTM1IrJLYWBOWmIslDQD7ffHk7UPf0NFgs_9fmbY

**Ref.39 - LAB**

A process for the degradation of dichlorodiphenyltrichloroethane (DDT) using an improved strain of Serratia marcescens

The present invention provides a process for degradation of dichlorodiphenyltrichloroethane (DDT) using an improved bacterial strain of serratia marcescens. A novel improved strain of serratia marcescens having ability to degrade DDT was isolated by enrichment of DDT contaminated soil and culturing in medium containing 5ppm DDT as the sole source of carbon. The obtained strain was cultivated in a 0.5% basal glucose-minimal medium cotaining 10 ppm DDT and a co-substrate at a temperature of 20-300C for 2 h. The said sulture acclimatized in shake culture for a period of 48 hours the acclimatized culture degrade DDT within 72 h.

https://aamkunhi.com/a-process-for-the-degradation-of-dichlorodiphenyltrichloroethane-ddt-using-an-improved-strain-of-serratia-marcescens/?fbclid=IwAR2frz1GfBqmxqNukIEML6ksdWydDpdrCuGhtJeoJLgmBxFq4Zxfeqedit8

**Lab experiments**

**Ref 40**

Adi Setyo Purnomo, Toshio Mori, Kazuhiro Takagi, Ryuichiro Kondo/Bioremediation of DDT contaminated soil using brown-rot fungi/International Biodeterioration & Biodegradation, Volume 65, Issue 5/August 2011/Pages 691-695

ABSTRACT

The ability of brown-rot fungi (BRF) to eliminate DDT in artificially and historically contaminated soil was investigated to determine whether the BRF would be suitable for the bioremediation of DDT in soil. Gloeophyllum trabeum, Fomitopsis pinicola and Daedalea dickinsii showed an ability to eliminate DDT in artificially contaminated sterilized (SL) and un-sterilized (USL) soils. The addition of Fe2+ to the soil system enhanced the ability of some BRF to eliminate DDT. In the contaminated SL soil, the DDT was eliminated by approximately 41%, 9% and 15% by G. trabeum, F. pinicola and D. dickinsii, respectively. Compared with the controls, in the USL soil approximately 43%, 29% and 32% of DDT was eliminated and approximately 20%, 9% and 26% of DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) was detected as a metabolic product with G. trabeum, F. pinicola and D. dickinsii, respectively. Of the BRF, G. trabeumdemonstrated the greatest ability to eliminate DDT both in the SL and USL soils. G. trabeum was applied to a historically contaminated soil which had a DDT concentration more than three times the artificially contaminated soil. G. trabeum remediated about 64% of the initial DDT with the addition of Fe2+. There were no significant differences in the results with or without the addition of Fe2+, indicating that G. trabeum can be used directly for the degradation of DDT in soil without any other additional treatment. This study identified that G. trabeum is the most promising BRF for use in the bioremediation of DDT contaminated soil.

https://www.sciencedirect.com/science/article/abs/pii/S0964830511000680?via%3Dihub

**Lab experiment**

**Ref 41**

Adi Setyo Purnomo, Toshio Mori, Ryuichiro Kondo/Involvement of Fenton reaction in DDT degradation by brown-rot fungi/International Biodeterioration & Biodegradation, Volume 64, Issue 7/October 2010/Pages 560-565

ABSTRACT

In this study, the ability of the brown-rot fungi (BRF) Fomitopsis pinicolaand Daedalea dickinsii to degrade DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) was investigated. F. pinicola and D. dickinsii degraded approximately 63% and 47% of total DDT in potato dextrose broth (PDB) medium, respectively. Degradation of DDT by D. dickinsii resulted in several metabolic products; DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene), and DDMU (1-chloro-2,2-bis (4-chlorophenyl) ethylene), whereas degradation by F. pinicola produced only one metabolic product, DDD. Investigation was done to find out whether the Fenton reaction was involved in the degradation process. More DDT was degraded in medium lacking FeSO4than in that containing FeSO4, suggesting that these fungi lack the benzoquinone-driven Fenton reaction cycle system. In addition, F. pinicolaand D. dickinsii produced only very low levels of hydroxyl radicals (1.2 and 5.1 μM, respectively), which provides further evidence that these fungi lack the Fenton reaction cycle system. The addition of mannitol as a hydroxyl radical scavenger did not inhibit DDT degradation. Hydroxyl radical production increased when the medium was supplemented with Fe2+. Higher levels of hydroxyl radicals enhanced DDT degradation by D. dickinsii, but not by F. pinicola. These results indicate that F. pinicola and D. dickinsii have different mechanisms for degrading DDT from that reported for the BRF Gloeophyllum trabeum, in which the Fenton reaction is an important factor in DDT degradation.

https://www.sciencedirect.com/science/article/abs/pii/S0964830510001198?via%3Dihub

**Lab experiments**
**Ref 42**

W. D. GUENZI, W. E. BEARD/Anaerobic Biodegradation of DDT to DDD in Soil/SCIENCE, VOLUME 156, ISSUE 3778/26.MAY 1967/Pages 1116-1117

ABSTRACT

DDT labeled with carbon-14 was added to soil, and the mixture was incubated anaerobically for 2 weeks and 4 weeks. DDT and seven possible decomposition products were separated by thin-layer chromatography, and the radioactivity of material from individual spots was determined by liquid scintillation. The DDT was dechlorinated by soil microorganisms to DDD, and only traces of other degradation products were detected. No degradation of DDT was detected in sterile soil.

https://www.science.org/doi/abs/10.1126/science.156.3778.1116

**Lab experiment**
**Ref 43**

Huashou Li, Weifeng Ling, Chuxia Lin, Effects of Different Fertilizers on Soil-borne DDTs Dynamics and Its Impacts on DDTs Uptake by Ipomoea aquatica, 2010 19th World Congress of Soil Science, Soil Solutions for a Changing World 89 1 – 6 August 2010, Brisbane, Australia. Published on DVD.

Abstract

A pot experiment was conducted to examine the effects of.various fertilizers on the dynamics of soil-borne DDTs and their subsequent impacts on DDTs uptake by a test plant. The results show that there was a significantly lower soil residual DDTs concentration in the iron-rich fertilizer-treated soil than in other fertilizer-treated soils. However, all the non-iron-rich fertilizers showed no significant effect on the reduction of soil DDTs on the last day of the experiment, as compared to the control. There was a close relationship between the soil residual DDTs and plant tissue DDTs. This suggests that the uptake rate of DDTs by the plant was dependent on the concentration of soil-borne DDTs. Application of iron-rich fertilizer enhanced the degradation of the soil DDTs and subsequently reduced the uptake of DDTs by the test plant. The findings obtained from this study have implications for remediation of DDTs-polluted soils.

Link: https://www.iuss.org/19th WCSS/Symposium/pdf/0303.pdf

**Lab experiments**
**Ref 44**

J. P. E. Anderson and E. P. Lichtenstein, Effect of nutritional factors on DDT-degradation by Mucor alternans, Canadian Journal of Microbiology, October 1971

Abstract

The effects of nutritional factors on the capacity of Mucor alternans to degrade 14C-DDT in shake cultures were investigated. Fungal spores did not grow in media that contained DDT as the sole carbon source, but they remained viable over a 2-month period and developed into a mycelium, once glucose had been added. The degradation of DDT by the

fungus was not related to the mycelial mass. The quantity of water-soluble metabolites produced from DDT was, to some extent, dependent on the insecticide concentration, but largely on the carbon and nitrogen sources in the culture media. Largest quantities of the metabolites were formed with glucose and ammonium nitrate. The concentration of glucose affected this metabolism quantitatively. With the exception of ribose, growth on other sugars resulted in a decrease in the production of DDT-metabolites, which was most noticeable when maltose was used. No qualitative differences in the metabolites were observed. When ammonium nitrate was replaced with other nitrogen sources, the production of water-soluble metabolites was substantially reduced (35 to 75%) and qualitative differences in the appearance of metabolites were also observed.

Link: https://doi.org/10.1139/m71-208

**Lab experiments**
**Ref 45**
D. Kantachote, I. Singleton, N. McClure, R. Naidu, M. Megharaj & B. D. Harch, DDT Resistance and Transformation by Different Microbial Strains Isolated from DDT-Contaminated Soils and Compost Materials, Compost Science & Utilization Volume 11, 2003 - Issue 4, Pages 300-310, Published online: 23 Jul 2013

Abstract
Bioremediation is a potential option to treat 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) contaminated sites. In areas where suitable microbes are not present, the use of DDT resistant microbial inoculants may be necessary. It is vital that such inoculants do not produce recalcitrant breakdown products e.g. 1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethylene (DDE). Therefore, this work aimed to screen DDT-contaminated soil and compost materials for the presence of DDT-resistant microbes for use as potential inoculants. Four compost amended soils, contaminated with different concentrations of DDT, were used to isolate DDT-resistant microbes in media containing 150 mg l–1 DDT at three temperatures (25, 37 and 55°C). In all soils, bacteria were more sensitive to DDT than actinomycetes and fungi. Bacteria isolated at 55°C from any source were the most DDT sensitive. However DDT-resistant bacterial strains showed more promise in degrading DDT than isolated fungal strains, as 1, 1-dichloro 2, 2-bis (4-chlorophenyl) ethane (DDD) was a major bacterial transformation product, while fungi tended to produce more DDE. Further studies on selected bacterial isolates found that the most promising bacterial strain (Bacillus sp. BHD-4) could remove 51% of DDT from liquid culture after 7 days growth. Of the amount transformed, 6% was found as DDD and 3% as DDE suggesting that further transformation of DDT and its metabolites occurred.

Link: https://doi.org/10.1080/1065657X.2003.10702139

**Lab experiments**
**Ref 46**
Kanoknit Sonkong, Poonsuk Prasertsan, Vorasan Sobhon, Screening and identification of p,p′ -DDT degrading soil isolates, Songklanakarin J. Sci. Technol. 30 (Suppl.1), 103-110, April 2008

Abstract
DDT is an organochlorine pesticide that can persist in the environment resulting in environmental problem with chronic effects on human and animal health. The determination of p,p′ -DDT in soil samples from 23 agricultural areas in Songkhla Province found DDT residue in the ranges of 0.17-9.84 ng/g soil. After repeated culturing in mineral salts-yeast extract medium (MSYM) with an addition of 25 ppm p,p′ -DDT (DDT25), 167 morphologically different bacterial strains were isolated. Out of 167 isolates, only 5 strains showed p,p′ -DDT degrading ability as indicated by clear zone around the colony when grown on nutrient agar supplemented up to 100 ppm p,p′ -DDT. These 5 isolates include SB1A01, SB2A02, SB1A10, SB1A12 and SB1B05. Growth of these isolates in MSYM+DDT25 after 10 days indicated reductions of p,p′ -DDT by 30.5, 20.3, 37.4, 30.4 and 32.2%, respectively. Based on the morphological characteristics and 16S rDNA analysis, iso- late SB1A10 which showed the highest degradation ability was found to be 99% identical (1360/1362) to Staphylococcus haemolyticus.

Link: https://www.researchgate.net/publication/26517245_Screening_and_identification_of_pp-DDT_degrading_soil_isolates

**Lab experiments**
**Ref 47**
Fang Cao, Tong Xu Liu, Chun Yuan Wu, Fang Bai Li, Xiao Min Li, Huan Yun Yu, Hui Tong, and Man Jia Chen, Enhanced Biotransformation of DDTs by an Iron- and Humic-Reducing Bacteria Aeromonas hydrophila HS01 upon Addition of Goethite and Anth-

raquinone-2,6-Disulphonic Disodium Salt (AQDS), Journal of Agricultural and Food Chemistry 2012 60 (45), 11238-11244

Abstract
A fermentative facultative anaerobe, strain HS01 isolated from subterranean sediment, was identified as Aeromonas hydrophila by 16S rRNA sequence analysis. The biotransformation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDD), and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDE) by HS01 was investigated in the presence of goethite and anthraquinone-2,6-disulphonic disodium salt (AQDS). The results demonstrated that HS01 was capable of reducing DDTs, goethite and AQDS. And goethite can significantly enhance the reduction of DDT, DDD and DDE to some extent, while the addition of AQDS can further accelerate the reduction of Fe(III) and DDTs. The products of DDT transformation were identified as a large amount of dominant DDD, and small amounts of 1-chloro-2,2-bis-(p-chlorophenyl)ethane (DDMU), unsym-bis(p-chlorophenyl)-ethylene (DDNU), and 4,4'-dichlorobenzophenone (DBP). The results of cyclic voltammetry suggested that AQDS could increase the amounts of reactive biogenic Fe(II), resulting in the enhanced transformation of DDTs. This investigation gives some new insight in the fate of DDTs related to iron- and humic-reducing bacteria.

Link: https://pubs.acs.org/doi/full/10.1021/jf303610w

**Lab experiments**
**Ref 48**
Yuechun Zhao, Xiaoyun Yi, Effects of Soil Oxygen Conditions and Soil pH on Remediation of DDT-contaminated Soil by Laccase from White Rot Fungi, nt. J. Environ. Res. Public Health 2010, 7(4), 1612-1621, Published: 7 April 2010

Abstract
High residues of DDT in agricultural soils are of concern because they present serious threats to food security and human health. This article focuses on remediation of DDT-contaminated soil using laccase under different soil oxygen and soil pH conditions. The laboratory experiment results showed significant effects of soil oxygen conditions and soil pH on remediation of DDT-contaminated soil by laccase at the end of a 25-d incubation period. This study found the positive correlation between the concentration of oxygen in soil and the degradation of DDT by laccase. The residue of DDTs in soil under the atmosphere of oxygen decreased by 28.1% compared with the atmosphere of nitrogen at the end of the incubation with laccase. A similar pattern was observed in the remediation of DDT-contaminated soil by laccase under different flooding conditions, the higher the concentrations of oxygen in soil, the lower the residues of four DDT components and DDTs in soils. The residue of DDTs in the nonflooding soil declined by 16.7% compared to the flooded soil at the end of the incubation. The residues of DDTs in soils treated with laccase were lower in the pH range 2.5–4.5.

Link: https://doi.org/10.3390/ijerph7041612

**Lab experiment**
**Ref 49**
Huashou Li, Weifeng Ling, Chuxia Lin, Effects of different treatments on soil-borne DDT and HCH dynamics and plant uptake, Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes Volume 46, 2011 - Issue 7

Abstract
Pot experiments were conducted to examine the effects of various fertilizers, as well as soil dilution treatments on the dynamics of soil-borne DDTs [sum of dichlorodiphenyltrichloroethane (DDT), chlorodiphenyldichloroethylene (DDE) and di- chlorodiphenyldichloroethane (DDD)] and hexachlorocyclohexanes (HCHs, sum of α-HCH, β-HCH, γ-HCH and δ-HCH) and their subsequent impacts on the uptake of DDTs and HCHs by a test plant. The results show that the soil residual DDTs and HCHs concentrations in the iron-rich fertilizer-treated soil were significantly lower than those in other fertilizer-treated soils. There was a close relationship between the soil residual DDTs and the plant tissue DDTs. This suggests that the uptake rate of DDTs by the plant was dependent on the concentration of soil-borne DDTs. A less close relationship between soil residual HCHs and plant tissue HCHs was also observed. Dilution of pesticide-contaminated soil with the non-contaminated soil not only physically reduced the concentration of pesticides in the soil but also enhanced the loss of soil-borne pesticides, possibly through the improvement of soil conditions for microbial degradation. Soil dilution had a better

effect on promoting the loss of soil-borne HCHs, relative to soil-borne-DDTs. The research findings obtained from this study have implications for management of heavily contaminated soils with DDTs and HCHs. Remediation of DDTs and HCHs-contaminated soils in a cost-effective way can be achieved by incorporating treatment techniques into conventional agricultural practices. Applications of iron-rich fertilizer and soil dilution treatments could cost-effectively reduce soil-borne DDTs and HCHs, and subsequently the uptake of these organochlorine pesticides by vegetables.

Link: https://doi.org/10.1080/03601234.2011.589313

**Lab experiments**
**Ref 50**
A. L. Juhasz, R. Naidu/Apparent degradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) by a Cladosporium sp./Biotechnology Letters 21/November 1999/Pages 991–995

ABSTRACT
Cladosporium sp. strain AJR318,501 was isolated from DDT-contaminated soil by its ability to decolourise the polymeric dye, Poly R-478. When inoculated into potato/dextrose broth containing 100 mg of DDT l−1, a 21% decrease in DDT concentration was observed 12 days after its addition, however, no transformation products were detected by gas chromatography. TLC of culture medium and mycelia extracts revealed 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane and five unknown transformation products associated with the mycelia.

https://link.springer.com/article/10.1023/A:1005694416926

**Lab experiment**
**Ref 51**
F.B. Li, X.M. Li, S.G. Zhou, L. Zhuang, F. Cao, D.Y. Huang, W. Xu, T.X. Liu, C.H. Feng/Enhanced reductive dechlorination of DDT in an anaerobic system of dissimilatory iron-reducing bacteria and iron oxide/Environmental Pollution, Volume 158, Issue 5/May 2010/Pages 1733-1740

ABSTRACT
The transformation of DDT was studied in an anaerobic system of dissimilatory iron-reducing bacteria (Shewanella decolorationis S12) and iron oxide (α-FeOOH). The results showed that S. decolorationis could reduce DDT into DDD, and DDT transformation rate was accelerated by the presence of α-FeOOH. DDD was observed as the primary transformation product, which was demonstrated to be transformed in the abiotic system of $Fe^{2+}$ + α-FeOOH and the system of DIRB + α-FeOOH. The intermediates of DDMS and DBP were detected after 9 months, likely suggesting that reductive dechlorination was the main dechlorination pathway of DDT in the iron-reducing system. The enhanced reductive dechlorination of DDT was mainly due to biogenic Fe(II) sorbed on the surface of α-FeOOH, which can serve as a mediator for the transformation of DDT. This study demonstrated the important role of DIRB and iron oxide on DDT and DDD transformation under anaerobic iron-reducing environments.

https://www.sciencedirect.com/science/article/abs/pii/S0269749109005806

**Lab experiments**
**Ref 52**
Rajkumar Bidlan, H.K. Manonmani/Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by Serratia marcescens DT-1P/Process Biochemistry, Volume 38, Issue 1/September 2002/Pages 49-56

ABSTRACT
Microbial degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)-residues is one of the mechanisms for the removal of this compound from the environment. A DDT-degrading consortium was isolated by long term enrichment of soil samples collected from DDT-contaminated fields. This consortium was acclimated by repeated passages through a mineral salt medium containing increasing concentrations of DDT. This acclimated consortium could degrade 25 ppm of DDT in 144 h. The consortium consisted of four bacteria. Of these, Serratia marcescens DT-1P was used for further studies. Various factors such as inoculum size, concentration of DDT, pH, temperature, presence of co-substrates, the type of carbon source used influenced the degradation of DDT in shake flasks. Complete degradation was observed up to 15 ppm DDT, followed by inhibitory effects at higher concentrations showing a total loss of degradative ability at 50 ppm DDT. Effective degradation of DDT was obtained with the inoculum pre-exposed to DDT for 72 h. Degradation was inhibited in the presence of auxiliary carbon sources such as citrate, rice straw hydrolysate. However, the presence of

yeast extract, peptone, glycerol and tryptone soya broth (TSB) showed complete disappearance of DDT. Mesophilic temperatures (26–30 °C) and near neutral pH (6.0–8.0) were most favourable for degradation. This microbial culture holds the potential for use in bioremediation of DDT-contaminated soils, waste deposits and water bodies.

https://www.sciencedirect.com/science/article/abs/pii/S0032959202000663

**Lab experiments**
**Ref 53**
Abhay Bajaj, Shanmugam Mayilraj, Mohana Krishna Reddy Mudiam, Devendra Kumar Patel, Natesan Manickam/Isolation and functional analysis of a glycolipid producing Rhodococcus sp. strain IITR03 with potential for degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)/Bioresource Technology, Volume 167/September 2014/Pages 398-406

ABSTRACT
A 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degrading bacterium strain IITR03 producing trehalolipid was isolated and characterized from a pesticides contaminated soil. The strain IITR03 was identified as a member of the genus Rhodococcus based on polyphasic studies. Under aqueous culture conditions, the strain IITR03 degraded 282 μM of DDT and could also utilize 10 mM concentration each of 4-chlorobenzoic acid, 3-chlorobenzoic acid and benzoic acid as sole carbon and energy source. The catechol 1,2-dioxygenase enzyme activity resulted in conversion of catechol to form cis,cis-muconic acid. Cloning and sequencing of partial nucleotide sequence of catechol 1,2-dioxygenase gene (cat) from strain IITR03 revealed its similarity to catA gene present in Rhodococcus sp. strain Lin-2 (97% identity) and Rhodococcus strain AN22 (96% identity) degrading benzoate and aniline, respectively. The results suggest that the strain IITR03 could be useful for field bioremediation studies of DDT-residues and chlorinated aromatic compounds present in contaminated sites.
https://www.sciencedirect.com/science/article/abs/pii/S0960852414008517

**Lab experiments**
**Ref 54**
L J Nadeau, F M Menn, A Breen, G S Sayler, Aerobic degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by Alcaligenes eutrophus A5, ASM Journals - Applied and Environmental Microbiology - Vol. 60, No. 1, 01 February 2021
Abstract
Biotransformation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by Alcaligenes eutrophus A5 was demonstrated by analysis of ethyl acetate-extracted products from resting cell cultures. Gas chromatography-mass spectrometry characterization of the neutral extracts revealed two hydroxy-DDT intermediates (m/z = 370) with retention times at 19.55 and 19.80 min that shared identical mass spectra. This result suggested that the hydroxylations occurred at the ortho and meta positions on the aromatic ring. UV-visible spectrum spectrophotometric analysis of a yellow metabolite in the culture supernatant showed a maximum A402 with, under acidic and basic conditions, spectrophotometric characteristics similar to those of the aromatic ring meta-cleavage products. 4-Chlorobenzoic acid was detected by thin-layer chromatography radiochemical scanning in samples from mineralization experiments by comparison of Rf values of [14C]DDT intermediates with that of an authentic standard. These results were further confirmed by gas chromatography-mass spectrometry analysis. This study indicates that DDT appears to be oxidized by a dioxygenase in A. eutrophus A5 and that the products of this oxidation are subsequently subjected to ring fission to eventually yield 4-chlorobenzoic acid as a major stable intermediate.

Link: https://doi.org/10.1128/aem.60.1.51-55.1994

**Lab experiments**
**Ref 55**
Pan X, Lin D, Zheng Y, Zhang Q, Yin Y, Cai L, Fang H, Yu Y., Biodegradation of DDT by Stenotrophomonas sp. DDT-1: Characterization and genome functional analysis, Sci Rep 6, 21332. 2016 Feb

Abstract
A novel bacterium capable of utilizing 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) as the sole carbon and energy source was isolated from a contaminated soil which was identified as Stenotrophomonas sp. DDT-1 based on morphological characteristics, BIOLOG GN2 microplate profile and 16S rDNA phylogeny. Genome sequencing and functional annotation of the isolate DDT-1 showed a 4,514,569 bp genome size, 66.92% GC content, 4,033 protein-coding genes and 76 RNA genes including 8 rRNA genes. Totally, 2,807 protein-coding genes were assigned to Clusters of Orthologous

Groups (COGs) and 1,601 protein-coding genes were mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The degradation half-lives of DDT increased with substrate concentration from 0.1 to 10.0 mg/l, whereas decreased with temperature from 15 °C to 35 °C. Neutral condition was the most favorable for DDT biodegradation. Based on genome annotation of DDT degradation genes and the metabolites detected by GC-MS, a mineralization pathway was proposed for DDT biodegradation in which it was orderly converted into DDE/DDD, DDMU, DDOH and DDA via dechlorination, hydroxylation and carboxylation and ultimately mineralized to carbon dioxide. The results indicate that the isolate DDT-1 is a promising bacterial resource for the removal or detoxification of DDT residues in the environment.

Link: https://doi.org/10.1038/srep21332

**Lab experiments**
**Ref 56**
Yim, YJ., Seo, J., Kang, SI. et al., Reductive Dechlorination of Methoxychlor and DDT by Human Intestinal Bacterium Eubacterium limosumUnder Anaerobic Conditions, Arch Environ Contam Toxicol 54, 406–411 (2008)

Abstract
Methoxychlor [1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane], a substitute for 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), is a compound of environmental concern because of potential long-term health risks related to its endocrine-disrupting and carcinogenic potency. In order to determine the metabolic fate of methoxychlor and DDT in the human intestinal gut, Eubacterium limosum (ATCC 8486), a strict anaerobe isolated from the human intestine that is capable of O-demethylation toward O-methylated isoflavones, was used as a model intestinal microbial organism. Under anaerobic incubation conditions, E. limosum completely transformed methoxychlor and DDT in 16 days. Based on gas chromatography–mass chromatography analyses, the metabolites produced from methoxychlor and DDT by E. limosum were confirmed to be 1,1-dichloro-2,2-bis(p-methoxyphenyl)ethane (methoxydichlor) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), respectively. This study suggests that E. limosum in the human intestinal gut might be a participant in the reductive dechlorination of methoxychlor to the more antiandrogenic active methoxydichlor.

Link: https://doi.org/10.1007/s00244-007-9044-y

**Lab experiments**
**Ref 57**
Gohil, H., Ogram, A. & Thomas, J. , Stimulation of anaerobic biodegradation of DDT and its metabolites in a muck soil: laboratory microcosm and mesocosm studies, Biodegradation 25, 633–642 (2014).

Abstract
The aim of this study was to evaluate the impact of selected electron donors and electron acceptors on the anaerobic biodegradation of DDT and its major metabolites in a muck soil with a long history of exposure to the pesticide. Loss of DDT was measured in anaerobic microcosms supplemented with H2, lactate, and acetate. The greatest loss of DDT (approximately 87 %) was observed in microcosms amended with lactate and no additional electron acceptor compared to the no additional electron donor or acceptor sets. An increase in measureable concentrations of DDx was observed in un-amended microcosms. In larger scale mesocosms, significant increases in dissolved organic carbon (DOC) corresponded with low redox potentials. Increases in DOC corresponded with sharp increases in measured concentrations of DDx, followed by a decrease in measured DDT concentrations in lactate-amended mesocosms. Our studies indicate that sorbed DDx is released upon anaerobic incubation, and that indigenous microorganisms capable of DDx degradation respond to lactate additions. Both the potential for release of sorbed DDx and the potential for biodegradation of DDx should be considered during remediation of DDx-contaminated organic soils at low redox potentials.

Link: https://doi.org/10.1007/s10532-014-9687-0

**Lab experiment**
**Ref 58**
Baczynski, Tomasz. (2012). Influence of process parameters on anaerobic biodegradation of ddt in contaminated soil preliminary lab-scale study. part i. surfactant and initial contamination level. Environment Protection Engineering. 38. 113-125. 10.5277/EPE120410.

Anaerobic biodegradation of DDT in field-polluted soil was investigated in relation to Tween 80 surfactant dose and initial pollution level. Experiments were carried out as lab-scale tests with flooded soil, inoculated with granular sludge. Higher surfactant doses decreased DDT residual concentration and also reduced DDD metabolite accumulation. However, doses higher than optimum caused DDD production to increase again. Results were also better for lower initial contamination levels – DDD accumulation was smaller whereas formation of terminal metabolite DBP was higher, indicating an enhancement of DDT transformation. Tests with pure compounds spiked in clean soil demonstrated that DDD is degraded slowly. Results point to three possible parallel pathways of anaerobic DDT transformation, not, as commonly reported, only one starting with dechlorination to DDD.

Link: https://www.researchgate.net/publication/235991925_Influence_of_process_parameters_on_anaerobic_biodegradation_of_ddt_in_contaminated_soil_preliminary_lab-scale_study_part_i_surfactant_and_initial_contamination_level

**Lab experiments**
**Ref 59**
Wang, G., Zhang, J., Wang, L., Liang, B., Chen, K., Li, S., & Jiang, J. (2010). Co-metabolism of DDT by the newly isolated bacterium, Pseudoxanthomonas sp. wax. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology], 41(2), 431–438.

Abstract
Microbial degradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) is the most promising way to clean up DDT residues found in the environment. In this paper, a bacterium designated as wax, which was capable of co-metabolizing DDT with other carbon sources, was isolated from a long-term DDT-contaminated soil sample by an enrichment culture technique. The new isolate was identified as a member of the Pseudoxanthomonas sp., based on its morphological, physiological and biochemical properties, as well as by 16S rRNA gene analysis. In the presence of 100 mg l-1 glucose, the wax strain could degrade over 95% of the total DDT, at a concentration of 20 mg l-1, in 72 hours, and could degrade over 60% of the total DDT, at a concentration of 100 mg l-1, in 144 hours. The wax strain had the highest degradation efficiency among all of the documented DDT-degrading bacteria. The wax strain could efficiently degrade DDT at temperatures ranging from 20 to 37°C, and with initial pH values ranging from 7 to 9. The bacterium could also simultaneously co-metabolize 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD), 2,2-bis(p-chlorophenyl)-1,1-dichlorethylene (DDE), and other organochlorine compounds. The wax strain could also completely remove 20 mg kg-1 of DDT from both sterile and non-sterile soils in 20 days. This study demonstrates the significant potential use of Pseudoxanthomonas sp. wax for the bioremediation of DDT in the environment.

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3768685/

**Lab experiment**
**Ref 60**
Hua Fang, Bin Dong, Hu Yan, Feifan Tang, Yunlong Yu, Characterization of a bacterial strain capable of degrading DDT congeners and its use in bioremediation of contaminated soil, Journal of Hazardous Materials Volume 184, Issues 1–3, 15 December 2010, Pages 281-289

Abstract
A bacterial strain DDT-6 (D6) capable of utilizing dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE) (DDTs) as its sole carbon and energy source was isolated and identified as Sphingobacteriumsp. The degradation of DDTs by strain D6 in mineral salt medium and in field soil was investigated. The half-lives of the degradation of DDTs increased with increasing concentration ranging from 1 to 50 mg L−1. Favorable degradation conditions for DDTs by strain D6 were found to be pH 7.0 and 30 °C. The degradation of DDTs by strain D6 was found to be statistically significantly enhanced ($p \leq 0.05$) by the addition of glucose. Based on the metabolites detected, a pathway was proposed for DDT degradation in which it undergoes dechlorination, hydrogenation, dioxygenation, decarboxylation, hydroxylation, and phenyl ring-cleavage reactions to complete the mineralization process. The addition of strain D6 into the contaminated soils was found to statistically significantly enhance ($p \leq 0.05$) the degradation of DDTs. The results indicate that the isolate D6 can be used successfully for the removal or detoxification of residues of DDTs in contaminated soil.

Link: https://doi.org/10.1016/j.jhazmat.2010.08.034

# IMPLEMENTATION

**Ref.61 – implement, bacteria**

Chiraz Abbes, Ahlem Mansouri, Naima Werfelli & Ahmed Landoulsi/Aerobic Biodegradation of DDT by Advenella Kashmirensis and Its Potential Use in Soil Bioremediation/Soil and Sediment Contamination: An International Journal /Volume 27, 2018 - Issue 6

Abstract
The aim of this study was to select a bacterial strain able to degrade 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), and to use it for bioaugmentation in order to decontamination soil. Advenella Kashmirensis MB-PR (A. Kashmirensis MB-PR) was isolated from DDT contaminated soil, and the degradation ability of DDT by this strain in the mineral salt medium was screened by gas chromatography. The efficiency of degradation was 81% after 30 days of bacterial growth. The study of intermediary products during the degradation of DDT showed the appearance and accumulation of DDD and DDE, which emerged from the first days of the experiment. Other metabolites were detected at a lower number of chlorine atoms, such as DBH. DNA samples were isolated and screened for the linA gene, encoding dehydrochlorinase. The bioaugmentation by A. Kashmirensis MB-PR of polluted sterile soil showed that 98% of DDT disappeared after 20 days of experience. This study demonstrates the significant potential use of A. Kashmirensis MB-PR for the bioremediation of DDT in the environment.

https://www.tandfonline.com/doi/abs/10.1080/15320383.2018.1485629

**Ref.62 – implementation, earthworms**

Hui-JuanXu/JingBai/WenyanLi/J. ColinMurrell/YulongZhang/JinjinWang/ ChunlingLuo/ YongtaoLi/ Mechanisms of the enhanced DDT removal from soils by earthworms: Identification of DDT degraders in drilosphere and non-drilosphere matrices/ Journal of Hazardous Materials, Volume 404, Part B/ 15 February 2021/ 124006

Abstract
The remediation of soil contaminated by 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) remains an important issue in environmental research. Although our previous studies demonstrated that earthworms could enhance the degradation of DDT in soils, the underlying mechanisms and microorganisms involved in these transformation processes are still not clear. Here we studied the transformation of DDT in sterilized/non-sterilized drilosphere and non-drilosphere matrices and identified DDT degraders using the technique of DNA-stable isotope probing. The results show that DDT degradation in non-sterilized drilosphere was quicker than that in their non-drilosphere counterparts. Earthworms enhance DDT removal mainly by improving soil properties, thus stimulating indigenous microorganisms rather than abiotic degradation or tissue accumulating. Ten new genera, including Streptomyces, Streptacidiphilus, Dermacoccus, Brevibacterium, Bacillus, Virgibacillus, were identified as DDT ring cleavage degrading bacteria in the five matrices tested. Bacillus and Dermacoccus may also play vital roles in the dechlorination of DDTs as they were highly enriched during the incubations. The results of this study provide robust evidence for the application of earthworms in remediating soils polluted with DDT and highlight the importance of using combinations of cultivation-independent techniques together with process-based measurements to examine the function of microbes degrading organic pollutants in drilosphere matrices.

https://www.sciencedirect.com/science/article/abs/pii/S0304389420319968

**Ref.63 – IMPLEMENTATION**
Huanfang Huang, Yuan Zhang, Wei Chen, Wenwen Chen, Dave A.Yuen, Yang Ding, Yingjie Chen, Yao Mao, Shihua Qi/Sources and transformation pathways for dichlorodiphenyltrichloroethane (DDT) and metabolites in soils from Northwest Fujian, China/Environmental Pollution, Volume 235/ April-2018/Pages560-570

ABSTRACT
Dicofol (2,2,2-trichloro-1,1-bis-(p-chlorophenyl)ethanol) found in the environment is not only a miticide originated from commercial use, but also a metabolite of dichlorodiphenyltrichloroethane (DDT), which is often overlooked. To verify the sources and transformation pathways of DDT and related metabolites in soils, we measured p,p'-(dicofol + DBP) (sum of p,p'-dicofol and 4,4′ -dichlorobenzophe-

none), DDT and six metabolites in soils from Northwest Fujian, China. The ratios of 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane (o,p'-DDT)/1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane (p,p'-DDT) and the mass balance demonstrated that p,p'-(dicofol + DBP) predominantly originated from p,p'-DDT transformation rather than from actual dicofol application. p,p'-(dicofol + DBP) accounted for 45.0% as the primary metabolites of DDT in this study, more than 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethylene (p,p'-DDE) and 1,1-dichloro-2,2-bis-(p-chlorophenyl) ethane (p,p'-DDD), which might lead to large overestimations of the fresh DDT input by using the traditional ratio of (∑2DDD + ∑2DDE)/∑2DDT (with all o,p'- and p,p'- isomers included). In paddy fields where the conditions alternate between aerobic (dry period) and anaerobic (wet period), both p,p'-DDD and p,p'-DDE were likely to degrade to 1-chloro-2,2-bis-(p-chlorophenyl)ethylene (p,p'-DDMU), which further transformed to 2,2-bis(p-chlorophenyl)ethylene (p,p'-DDNU). Degradation of p,p'-DDMU to p,p'-DDNU mainly occurred in waterlogged paddy soils. However, p,p'-DDNU might not transform to other higher-order metabolites in aerobic surface soils. Overall, our study confirmed p,p'-(dicofol + DBP) as metabolites of p,p'-DDT, suggested DDE and DDD were parallel precursors of DDMU, and further verified the transformation pathways of DDT in surface soils.

https://www.sciencedirect.com/science/article/abs/pii/S0269749117341933?fbclid=IwAR0cwfKPt_Yx-An-OtekZu-4nPPVzVJXNO-5sYUzeybpcep-K9uQTXP_w3rGI

**Implementation**

**Ref 64**

Naiying Wu, Shuzhen Zhang, Honglin Huang, Xiaoquan Shan, Peter Christie, Youshan Wang/DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant/Environmental Pollution, Volume 151, Issue 3/February 2008/Pages 569-575

ABSTRACT

A greenhouse pot experiment was conducted to investigate the colonization of alfalfa roots by the arbuscular mycorrhizal (AM) fungus Glomus etunicatum and application of the non-ionic surfactant Triton X-100 on DDT uptake by alfalfa and depletion in soil. Mycorrhizal colonization led to an increase in the accumulation of DDT in roots but a decrease in shoots. The combination of AM inoculation and Triton X-100 application enhanced DDT uptake by both the roots and shoots. Application of Triton X-100 gave much lower residual concentrations of DDT in the bulk soil than in the rhizosphere soil or in the bulk soil without Triton X-100. AM colonization significantly increased bacterial and fungal counts and dehydrogenase activity in the rhizosphere soil. The combined AM inoculation of plants and soil application of surfactant may have potential as a biotechnological approach for the decontamination of soil polluted with DDT.

https://www.sciencedirect.com/science/article/abs/pii/S026974910700190X

Implementation → Mushroom → spent mushroom waste

**Ref 65**

Adi Setyo Purnomo, Toshio Mori, Ichiro Kamei, Takafumi Nishii, Ryuichiro Kondo, Application of mushroom waste medium from Pleurotus ostreatus for bioremediation of DDT-contaminated soil,International Biodeterioration & Biodegradation, Volume 64, Issue 5, August 2010/Pages 397-402

ABSTRACT

The ability of spent mushroom waste (SMW) from Pleurotus ostreatus to degrade 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) was investigated. DDT was degraded by 48% during a 28 d incubation and 5.1% of the DDT was mineralized during a 56 d incubation by SMW from P. ostreatus. The degradation potential in artificial DDT-contaminated soil was also investigated. The SMW from P. ostreatus degraded the DDT by 40% and 80% during a 28 d incubation in sterilized (SL) and un-sterilized (USL) soils, respectively. [U-14C]DDT was mineralized by 5.1% and 8.0% during a 56 d incubation in SL and USL soils, respectively. These results indicate that SMW from P. ostreatus is a medium which can be potentially used for bioremediation in DDT-contaminated environments.

Link: https://www.sciencedirect.com/science/article/abs/pii/S0964830510000624?via%3Dihub

**Implementation → earthworms**

**Ref 66**

Hui-Juan Xua, Jing Bai, Wen-YanLi, Li-Xia Zhao, Yong-Tao Li, Removal of persistent DDT residues from soils by earthworms: A mechanistic study, Journal of Hazardous Materials Volume 365, 5 March 2019, Pages 622-631

Abstract

Earthworms have been reported to enhance DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) removal from soils, but the mechanism underlying is still poorly understood. This study therefore worked on the links between DDT transformation in drilosphere and non-drilosphere matrices and the properties of these matrices in sterile and non-sterile soil columns with and without earthworms to reveal related mechanisms. The results show that earthworms shortened the half-time of DDT in soils from over 14 weeks to about 8 weeks; DDT residues were lower ($p < 0.05$) and its transformation products were higher ($p < 0.05$) in drilosphere matrixes than those in their non-drilosphere counterparts; DDD and DDMU was higher ($p < 0.05$) in the gut, and DDE was higher ($p < 0.05$) in the burrow; and the bioaccumulation of DDT in earthworm tissues only contributed less than 0.03% to the DDT removal enhanced by earthworms. The results further demonstrate that drilosphere is the hotspot of soil DDT transformation with oxidative degradation dominant in the burrow and reductive dechlorination in the gut, and earthworms enhanced DDT removal mainly by digesting and promoting the microbial degradation of DDT by indigenous microorganisms via improving soil properties. Knowledge of the mechanisms of DDT transformation by earthworms will support the use of earthworms in remediating DDT-contaminated soils.
Keywords: Dechlorination; Dehydrochlorination; Drilosphere; Microbial degradation; T-RFLP.

Link: https://www.sciencedirect.com/science/article/abs/pii/S0304389418310628?via%3Dihub

**Implementation → earthworms**
**Ref 67**

Hui-Juan Xu, Jing Bai, Wenyan Li, J. Colin Murrell, Yulong Zhang, Jinjin Wang, Chunling Luo, Yongtao Li, Mechanisms of the enhanced DDT removal from soils by earthworms: Identification of DDT degraders in drilosphere and non-drilosphere matrices, Journal of Hazardous Materials Volume 404, Part B, 15 February 2021, 124006

Abstract

The remediation of soil contaminated by 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) remains an important issue in environmental research. Although our previous studies demonstrated that earthworms could enhance the degradation of DDT in soils, the underlying mechanisms and microorganisms involved in these transformation processes are still not clear. Here we studied the transformation of DDT in sterilized/non-sterilized drilosphere and non-drilosphere matrices and identified DDT degraders using the technique of DNA-stable isotope probing. The results show that DDT degradation in non-sterilized drilosphere was quicker than that in their non-drilosphere counterparts. Earthworms enhance DDT removal mainly by improving soil properties, thus stimulating indigenous microorganisms rather than abiotic degradation or tissue accumulating. Ten new genera, including Streptomyces, Streptacidiphilus, Dermacoccus, Brevibacterium, Bacillus, Virgibacillus, were identified as DDT ring cleavage degrading bacteria in the five matrices tested. Bacillus and Dermacoccus may also play vital roles in the dechlorination of DDTs as they were highly enriched during the incubations. The results of this study provide robust evidence for the application of earthworms in remediating soils polluted with DDT and highlight the importance of using combinations of cultivation-independent techniques together with process-based measurements to examine the function of microbes degrading organic pollutants in drilosphere matrices.

Link: https://doi.org/10.1016/j.jhazmat.2020.124006

**Implementation → compost**
**Ref: 68**

Adi Setyo Purnomo, Futoshi Koyama, Toshio Mori, Ryuichiro Kondo, DDT degradation potential of cattle manure compost, Chemosphere Volume 80, Issue 6, July 2010, Pages 619-624

Abstract

The purpose of this study was to investigate the ability of cattle manure compost (CMC) to degrade 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT). DDT was degraded during composting and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD) was detected as a metabolic product. Degradation of DDT at 60 °C was the most effective of all the stages of composting. Fourteen strains of fungi were isolated and identified from CMC, and most of them were closely related to Mucor circinelloides and Galactomyces geotrichum. These fungi demonstrated a high ability to degrade DDT both at 30 and 60 °C in potato dextrose broth (PDB) medium. DDD and 4,4-dichlorobenzophenone (DBP) were detected as meta-

bolic products. Degradation of DDT-contaminated soil was also investigated. Composting materials in the mesophilic stage exhibited the highest ability to degrade DDT in un-sterilized (USL) contaminated soil during a 28 d incubation period. The isolated fungi possessed the ability to degrade DDT in sterilized (SL) and un-sterilized (USL) soils. These results indicated that CMC contains fungi that can be potentially used for bioremediation in DDT-contaminated environments.

Link: https://doi.org/10.1016/j.chemosphere.2010.04.059

**Implementation → compost**
**Ref 69**
Roger L. BernierNeil C. C. GrayLori E. Moser, Compost decontamination of ddt contaminated soil, International application published under the Patent Cooperation Treaty (PCT), International Publication Number WO 97/11794, 3 April 1997

Abstract
The present invention provides a process of decontaminating soil and/or sediments containing DDT type contaminants by converting these contaminants into harmless materials thereby decontaminating the soil to whatever extent desired, either partial decontamination or complete remediation. The process comprises treating solid and/or sediment which contains populations of viable anaerobic and aerobic microbes capable of transforming DDT type contaminants into harmless materials and being viable under both anaerobic and aerobic conditions.

Link: https://patents.google.com/patent/WO1997011794A1

**Implementation → compost**
**Ref 70**
IOL News, Cow manure to mop up DDT, IOL News → Technology, Sep 16, 1999

Abstract
”Sheffield, England - Canadian researchers are using chicken droppings, cow manure and waste paper in a new technique to clean up land contaminated with DDT and other dangerous pesticides.
[...]
In pilot studies in the laboratory in Canada, the researchers were able to get 97 percent degradation of the DDT in eight weeks. Further tests on a contaminated site in Tampa, Florida were also promising.
But Gray said the system would have to be customised for each site based on what is in the soil.”

Link: https://www.iol.co.za/technology/cow-manure-to-mop-up-ddt-12597

**Implementation → compost**
**Ref 71**
Xiao Deng, Chunyuan Wu, Yi Li, Jingkun Liu, Qinfen Li, Effects of Chicken Manure Compost on the Production of Dissolved Organic Carbon and the Degradation of p, p’-DDT in Loam Soil, Atlantis Press, Available Online October 2016.

Abstract
Using pot experiment to study the effects of chicken manure compost (CM) on the production of dissolved organic carbon (DOC) and the degradation of p, p’-DDT in loam soil under three different water conditions. The objective of this study was providing theoretical basis for the remediation of pesticide contaminated soil and the clean production of agricultural products. The results showed that CM could effectively accelerate the production of DOC and the degradation of p, p’-DDT in loam soil under three different water conditions. After adding 1%~3% CM in loam soil, the concentrations of DOC were respectively increased by 4.05%~40.8%, 6.84%~45.1% and 8.40%~260% under the conditions of flooded, maximum moisture capacity and 60% relative water content, and the degradation rates of p, p’-DDT were respectively increased by 4.92%~38.0%, 4.92%~34.9% and 0.42%~33.5% under the conditions of flooded, maximum moisture capacity and 60% relative water content. Which indicated that the improving effect of CM on the degradation of p, p’-DDT in loam soil was best under flooded.

Link: https://doi.org/10.2991/iccahe-16.2016.22

**Implementation → coffee grain ?**
**Ref 72**
R. Rodríguez-Vázquez, D.S. Acosta-Ramírez, REGENERATION OF DDT CONTAMINATED SOILS BY CO-COMPOSTING, Acta Horticulturae 1076 II International Symposium on Organic Matter Management and Compost Use in Horticulture, Article number 1076_25, Pages 217-224.

Abstract
Application of biotechnology for pesticides elimination through diverse biotransformation, biodegradation or mineralization processes is an important task

in agriculture. Among these processes, bioremediation, using living organisms, such as bacteria, fungi, actinomycetes, worms and plants, amended with agricultural residues has shown a high decrease of pesticides, and their derivatives, among other compounds, that affect soil productivity. Bioremediation technologies rely on physical, chemical, geological and biological soil characteristics, as well as on environmental conditions. Depending on the methodology, bioremediation technologies are divided into: Biostimulation (addition of nutrients or appropriate conditions), Bioaugmentation (addition of native or exogenous organisms), Phytoremediation (plant application), Landfarming (nutrients addition); their applications are classified as: Ex-situ, In-situ and On-site. In Mexico more than 50% of these biological technologies are applied in contaminated soils, due to their sustainability. In this work a biological technology, based on ′ Solid Cultivation On-site′ , (Mexican Patent No. 291975), using coffee grain and straws, low humidity and aeration, is described for the removal of the organo¬chloride pesticide DDT and their metabolites. The technology was previously tested at commercial scale for hydrocarbons removal, creating a suitable soil for recreational use. Other applications of the mentioned biological technology were on soils for bean, pumpkin and corn crops, which resulted in high quality products.

Link: https://doi.org/10.17660/ActaHortic.2015.1076.25

**Implementation**
**Ref 73**
Adi Setyo Purnomo, Futoshi Koyama, Toshio Mori, Ryuichiro Kondo, DDT degradation potential of cattle manure compost, Chemosphere
Volume 80, Issue 6, July 2010, Pages 619-624

Abstract
The purpose of this study was to investigate the ability of cattle manure compost (CMC) to degrade 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT). DDT was degraded during composting and 1,1-dichloro- 2,2-bis (4-chlorophenyl) ethane (DDD) was detected as a metabolic product. Degradation of DDT at 60 °C was the most effective of all the stages of composting. Fourteen strains of fungi were isolated and iden- tified from CMC, and most of them were closely related to Mucor circinelloides and Galactomyces geotri- chum. These fungi demonstrated a high ability to degrade DDT both at 30 and 60 °C in potato dextrose broth (PDB) medium. DDD and 4,4-dichlorobenzophenone (DBP) were detected as metabolic products. Degradation of DDT-contaminated soil was also investigated. Composting materials in the mesophilic stage exhibited the highest ability to degrade DDT in un-sterilized (USL) contaminated soil during a 28 d incubation period. The isolated fungi possessed the ability to degrade DDT in sterilized (SL) and un-sterilized (USL) soils. These results indicated that CMC contains fungi that can be potentially used for bioremediation in DDT-contaminated environments.

Link: https://doi.org/10.1016/j.chemosphere.2010.04.059

**Implementation**
**Ref 74**
Brian S. Anderson, John W. Hunt, Bryn M. Phillips, Matt Stoelting, Jonathon Becker, Russell Fairey, H. Max Puckett, Mark Stephenson, Ronald S. Tjeerdema, Michael Martin, Ecotoxicologic change at a remediated superfund site in San Francisco, California, USA, Environmental Toxicology, Volume19, Issue 4, April 2000, Pages 879-887

Abstract
Lauritzen Channel is an industrial waterway adjacent to the former United Heckathorn facility in the inner Richmond Harbor area of San Francisco Bay, California, USA. Marine sediments at this Superfund site were dredged from late 1996 through early 1997 to remove the primary chemicals of concern: DDT, and dieldrin. This study assessed the Lauritzen Channel marine environment immediately before and approximately one year after the dredging of sediments. The study included chemical analysis of sediments, tissue concentrations of transplanted mussels, toxicity testing of sediment samples, and characterization of benthic community structure. Results indicated that sediment toxicity to bivalve larvae (Mytilus galloprovincialis) decreased in postre-mediation samples, but that toxicity to the amphipod Eohaustorius estuarius increased significantly. Assessment of benthos at this site suggested a transitional benthic community structure. In addition, postremediation sediments remained contaminated by a variety of organic chemical compounds, including DDT, dieldrin, chlordane, polycyclic aromatic hydrocarbons, and polychlorinated bi-phenyls. Tissue concentrations of DDT and dieldrin in mussels (M. galloprovincialis) were lower than those in preremediation samples, indicating that although sediment concentrations of organochlorine pesticides remained high, concentra-

tions of these chemicals in the water column were reduced after dredging. This study demonstrates that the components of the site assessment were useful in determining effectiveness of the remediation activities.

Link: https://doi.org/10.1002/etc.5620190414

**Implementation**
**Ref 75**
Biao Fan, Yuechun Zhao, Ganhui Mo, Weijuan Ma, Junqin Wu, Co-remediation of DDT-contaminated soil using white rot fungi and laccase extract from white rot fungi, J Soils Sediments 13, 1232–1245, 30 April 2013

Abstract
Purpose
2,2-Bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT), one of the most widely used organochlorine pesticides in soil, was banned in the 1970s for agricultural use because of its detrimental impacts on wildlife and harmful effects on human health via the food chain. However, high levels of DDT are frequently detected in agricultural soils in China. Considering this situation, this study investigated the use of white rot fungi and laccase derived from white rot fungi to co-remediate DDT-contaminated soil.
Materials and methods
A culture of white rot fungi was used to inoculate soil samples and also to extract laccase from. Soil was contaminated with four components of DDT (p,p′ -DDE, o,p′ -DDT, p,p′ -DDD, and p,p′ -DDT). Individual DDT components and the sum of the DDT components (p,p′ -DDE, o,p′ -DDT, p,p′ -DDD, and p,p′ -DDT—collectively referred to as DDTs) were both analyzed by GC at various stages during the incubation period. The efficacy of co-remediating DDT-contaminated soil using white rot fungi and laccase was tested by investigating how degradation varied with varying amounts of white rot fungi, sterilizing soil, temperature, soil pH, concentrations of DDT, and concentration of the heavy metal ion Cd2+.
Results and discussion
“”It was concluded that the reduction of DDTs in soil using white rot fungi and laccase was higher than reduction using only white rot fungi or laccase by nearly 14 and 16 %, respectively. Five milliliters fungi per 15 g soil and 6 U laccase per gram soil were the optimal application rates for remediation, as shown by a reduction in DDTs of 66.82 %. The difference in the reduction of individual DDT components and DDTs between natural and sterilized soils was insignificant. The optimal temperature and pH in the study were 28 °C and 4.5, respectively. In addition, reduction of individual DDT components and DDTs increased with increasing concentrations of DDT and decreased with increasing concentrations of Cd2+.
Conclusions
Compared with the remediation of DDT using only white rot fungi or laccase, the co-remediation of DDT using white rot fungi and laccase degraded DDT in soil more rapidly and efficiently; the highest reduction of DDTs was 66.82 %
.
Link: https://doi.org/10.1007/s11368-013-0705-3

**Implementation**
**Ref 76**
Pengfei Xiao, Toshio Mori, Ichiro Kamei, Ryuichiro Kondo, A novel metabolic pathway for biodegradation of DDT by the white rot fungi, Phlebia lindtneri and Phlebia brevispora, Biodegradation 22, 859–867 (2011)

Abstract
1,1,1-trichloro-2,2-bis(4-chlorophenyl)eth- ane (DDT) was used as the substrate for a degradation experiment with the white rot fungi Phlebia lindtneri GB-1027 and Phlebia brevispora TMIC34596, which are capable of degrading polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated biphenyls (PCBs). Pure culture of P. lindtneri and P. brevispora with DDT (25 lmol l-1) showed that 70 and 30% of DDT, respectively, disappeared in a low-nitrogen medium after a 21-day incubation period. The metab- olites were analyzed using gas chromatography/mass spectrometry (GC/MS). Both fungi metabolized DDT to 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (DDD), 2,2-bis(4-chlorophenyl)acetic acid (DDA) and 4,4- dichlorobenzophenone (DBP). Additionally, DDD was converted to DDA and DBP. DDA was converted to DBP and 4,4-dichlorobenzhydrol (DBH). While DBP was treated as substrate, DBH and three hydroxylated metabolites, including one dihydroxylated DBP and two different isomers of monohydroxylated DBH, were produced from fungal cultures, and these hydroxylated metabolites were efficiently inhibited by the addition of a cytochrome P-450 inhibitor, piperonyl butoxide. These results indicate that the white rot fungi P. lindtneri and P. brevispora can degrade DBP/DBH through hydroxylation of the aromatic ring. Moreover, the single-ring aromatic metabolites, such as 4-chloro-

benzaldehyde, 4-chlorobenzyl alcohol and 4-chlorobenzoic acid, were found as metabolic products of all substrate, demon- strating that the cleavage reaction of the aliphatic-aryl carbon bond occurs in the biodegradation process of DDT by white rot fungi.

Link: https://doi.org/10.1007/s10532-010-9443-z

**Implementation → Slurry system?**
**Ref 77**
Ricardo Dalla Villa, Raquel F. Pupo Nogueira, Oxidation of p,p′ -DDT and p,p′ -DDE in highly and long-term contaminated soil using Fenton reaction in a slurry system, Science of The Total Environment Volume 371, Issues 1–3, 1 December 2006, Pages 11-18

Abstract
The degradation of DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane] and DDE [2,2-bis(4-chlorophenyl)-1,1-dichloroethylene] in highly and long-term contaminated soil using Fenton reaction in a slurry system is studied in this work. The influence of the amount of soluble iron added to the slurry versus the mineral iron originally present in the soil, and the influence of H2O2 concentration on the degradation process are evaluated. The main iron mineral species encountered in the soil, hematite (Fe2O3), did not show catalytic activity in the decomposition of H2O2, resulting in low degradation of DDT (24%) and DDE (4%) after 6 h. The addition of soluble iron (3.0 mmol L– 1) improves the reaction reaching 53% degradation of DDT and 46% of DDE. The increase in iron concentration from 3.0 to 24 mmol L– 1 improves slightly the degradation rate of the contaminants. However, similar degradation percentages were obtained after 24 h of reaction. It was observed that low concentrations of H2O2 were sufficient to degrade around 50% of the DDT and DDE present in the soil, while higher degradation percentages were achieved only with high amounts of this reagent (1.1 mol L– 1).

Link: https://doi.org/10.1016/j.scitotenv.2006.05.010

**Implementation → fungus → white rot**
**Ref 78**
JOHNA.BUMPUS, STEVEN D. AUST, Biodegradation of DDT [1,1,1-Trichloro-2,2-Bis(4-Chlorophenyl)Ethane] by the White Rot Fungus Phanerochaete chrysosporium, Applied and environmental microbiology, Sept.1987,p.2001-2008

Abstract
Extensive biodegradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by the white rot fungus Phanerochaete chrysosporium was demonstrated by disappearance and mineralization of [14C]DDT in nutrient nitrogen-deficient cultures. Mass balance studies demonstrated the formation of polar and water-soluble metabolites during degradation. Hexane-extractable metabolites identified by gas chromatography-mass spectrometry included 1,1,-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (dicofol), 2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol (FW-152), and 4,4'-dichlorobenzophenone (DBP). DDD was the first metabolite observed; it appeared after 3 days of incubation and disappeared from culture upon continued incubation. This, as well as the fact that [14C]dicofol was mineralized, demonstrates that intermediates formed during DDT degradation are also metabolized. These results demonstrate that the pathway for DDT degradation in P. chrysosporium is clearly different from the major pathway proposed for microbial or environmental degradation of DDT. Like P. chrysosporium ME-446 and BKM-F-1767, the white rot fungi Pleurotus ostreatus, Phellinus weirii, and Polyporus versicolor also mineralized DDT.

Link: https://doi.org/10.1128/aem.53.9.2001-2008.1987

**Implementation → bacteria**
**Ref 79**
Jie Qu, Yang Xu, Guo-MinAi, Ying Liu, Zhi-Pei Liu, Novel Chryseobacterium sp. PYR2 degrades various organochlorine pesticides (OCPs) and achieves enhancing removal and complete degradation of DDT in highly contaminated soil, Journal of Environmental Management Volume 161, 15 September 2015, Pages 350-357

Abstract
Long term residues of organochlorine pesticides (OCPs) in soils are of great concerning because they seriously threaten food security and human health. This article focuses on isolation of OCP-degrading strains and their performance in bioremediation of contaminated soil under ex situ conditions. A bacterium, Chryseobacterium sp. PYR2, capable of degrading various OCPs and utilizing them as a sole carbon and energy source for growth, was isolated from OCP-contaminated soil. In culture experi-

ments, PYR2 degraded 80–98% of hexachlorocyclohexane (HCH) or 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) isomers (50 mg L−1) in 30 days. A pilot-scale ex situ bioremediation study of highly OCP-contaminated soil augmented with PYR2 was performed. During the 45-day experimental period, DDT concentration was reduced by 80.3% in PYR2-augmented soils (35.37 mg kg−1 to 6.97 mg kg−1) but by only 57.6% in control soils. Seven DDT degradation intermediates (metabolites) were detected and identified in PYR2-augmented soils: five by GC/MS: 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE), 1-chloro-2,2-bis (4-chlorophenyl) ethylene (DDMU), 1-chloro-2,2-bis (4-chlorophenyl) ethane (DDMS), and dichlorobenzophenone (DBP); and two by LC/MS: 4-chlorobenzoic acid (PCBA) and 4-chlorophenylacetic acid (PCPA). Levels of metabolites were fairly stable in control soils but varied greatly with time in PYR2-augmented soils. Levels of DDD, DDMU, and DDE in PYR2-augmented soils increased from day 0 to day 30 and then decreased by day 45. A DDT biodegradation pathway is proposed based on our identification of DDT metabolites in PYR2-augmented systems. PYR2 will be useful in future studies of OCP biodegradation and in bioremediation of OCP-contaminated soils.

Link: https://doi.org/10.1016/j.jenvman.2015.07.025

**Implementation → Fungi**
**Ref 80**
Yi Huang, Jie Wang, Degradation and mineralization of DDT by the ectomycorrhizal fungi, Xerocomus chrysenteron, Chemosphere Volume 92, Issue 7, August 2013, Pages 760-764

Abstract
One strain of ectomycorrhizal fungi, Xerocomus chrysenteron, had been investigated for its ability to degrade 1,1,1-trichloro-2,2-bis(4-chlorophe-nyl) ethane (DDT) by measuring unlabeled DDT and identifying its metabolites, and determining the mineralization of [13C]DDT in pure cultures. After 45 d incubation, about 55% of the added DDT disappeared from the culture system, less than 5% remained in the nutrient solution, and about 44% was retained in the mycelium. Inoculation with mycelium enhanced the degradation of DDT in soil, and alleviated enrichment of DDT in plants. The metabolites identified by gas chromatography–mass spectrometry were 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE), and 4,4′ -dichlorobenzophenone (DBP). There were significant differences in the δ13C of released CO2 between [13C]DDT and DDT cultures, which indicated X. chrysenteron was able to mineralize DDT to CO2.

Link: https://doi.org/10.1016/j.chemosphere.2013.04.002

**Implementation → fungi → white rot**
**Ref 81**
Guanyu Zheng, Ammaiyappan Selvam, Jonathan W.C.Wong, Oil-in-water microemulsions enhance the biodegradation of DDT by Phanerochaete chrysosporium, Bioresource Technology Volume 126, December 2012, Pages 397-403

Abstract
A novel approach was developed using oil-in-water (O/W) microemulsions formed with non-ionic surfactant, cosurfactant (1-pentanol) and linseed oil, at the cosurfactant to surfactant ratio (C/S ratio, w/w) of 1:3 and oil to surfactant ratio (O/S ratio, w/w) of 1:10, to enhance the biodegradation of DDT by the white rot fungus Phanerochaete chrysosporium. Results showed that microemulsions formed with Tween 80 effectively enhanced the biodegradation of DDT by P. chrysosporium and the enhancement was about two times that of Tween 80 solution, while microemulsion formed with Triton X-100 exhibited negative effect. Further studies revealed that microemulsion formed with Tween 80 enhanced the biodegradation of DDT through transporting DDT from crystalline phase to mycelium as well as their positive effect on the growth of P. chrysosporium; of these, the former is likely the most important and pre-requisite for the biodegradation of DDT by P. chrysosporium.

Link: https://doi.org/10.1016/j.biortech.2012.02.141

**Implementation → washing (cosolvents and surfactants)**
**Ref 82**
Smith, E., Smith, J., Naidu, R. et al. Desorption of DDT from a Contaminated Soil using Cosolvent and Surfactant Washing in Batch Experiments, Water, Air, & Soil Pollution 151, 71–86 (2004)

Abstract
1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p′ -DDT) is a recalcitrant organic compound that is dif-

ficult to remove from contaminated soil due to its low solubility. In this study we investigated the effectiveness of both cosolvents and surfactants in enhancing the solubility of p,p′ -DDT from a soil that has been contaminated with DDT for nearly 40 yr. The presence of selected surfactants removed less than 1 to 11% of p,p′ -DDT compared to cosolvents, which removed less than 1 to 77% of p,p′ -DDT from the same soil. The low solubility of p,p′ -DDT in the presence of surfactants was attributed to the decreased surfactant concentration to below critical micelle concentrationfollowing sorption by soil surfaces. Enhanced solubility of p,p′ -DDT was achieved with the use of cosolvents that releasedup to 77% of p,p′ -DDT from a contaminated soil. Increasing the solution concentration and hydrophobicity of the cosolvent increased the amount of p,p′ -DDT desorbed. For example, the amount of p,p′ -DDT desorbed increased in the order 5% 1-propanol << 50% ethanol << 50% 1-propanol. Repeated washing of the soil with various cosolvents, in all but two cases, markedly increased the total amount of p,p′ -DDT desorbed from the soil. For example, repeated washing of the soil with 50% ethanol increased the amount of p,p′ -DDT removed by 42% while repeated washings of the soil with 50% 1-propanol had little effect on the amount of p,p′ -DDT desorbed. Increasing the soil-solution ratio from 1:2 to 1:10 in the presence of 40% 1-propanol increased the amount of p,p′ -DDT desorbed by 100%; suggesting that the soil-solution ratio was an important parameterin controlling the amount of p,p′ -DDT desorbed.

Link: https://doi.org/10.1023/B:WATE.0000009899.03630.78

**<u>Implementation → Bacteria</u>**
**<u>Ref 83</u>**

Beunink, J., Rehm, HJ. Synchronous anaerobic and aerobic degradation of DDT by an immobilized mixed culture system, Appl Microbiol Biotechnol 29, 72–80 (1988).

Abstract
For the investigation of a mixed anaerobic and aerobic degradation of xenobiotics the reductive dechlorination of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) to 1,1-dichloro-2,2-bis (4-chlorophenyl)ethane (DDD) and the oxidative degradation of the DDT-conversion product 4,4′ -dichlorodiphenylmethane (DDM) were studied. Enrichments from digested sewage sludge led to the isolation of an Enterobacter cloacae-strain which is able to reductive dechlorination of DDT during the fermentation of lactose. From fresh sewage sludge 11 bacterial strains were isolated in batch-culture and in continuous culture utilizing diphenylmethane, a non chlorinated structural analogon of DDM, as sole source of carbon and energy. One of these isolates, Alcaliaenes sp. cometabolizes DDM during the aerobic growth with diphenylmethane. By coimmobilization of Alcaligenes sp. and Enterobacter cloacae in Ca-alginate a system could be established, in which the reductive dechlorination of DDT and the oxidative degradation of DDM and diphenylmethane proceeds simultaneously in one reactor vessel.

Link: https://doi.org/10.1007/BF00258354

**<u>Implementation → Bacteria</u>**
**<u>Ref 84</u>**

Sun, G., Zhang, X., Hu, Q. et al. Biodegradation of Dichlorodiphenyltrichloroethanes (DDTs) and Hexachlorocyclohexanes (HCHs) with Plant and Nutrients and Their Effects on the Microbial Ecological Kinetics, Microb Ecol 69, 281–292 (2015).

Abstract
Four pilot-scale test mesocosms were conducted for the remediation of organochlorine pesticides (OCPs)-contaminated aged soil. The results indicate that the effects on degradation of hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) were in the following order: nutrients/plant bioaugmentation (81.18 % for HCHs; 85.4 % for DDTs) > nutrients bioaugmentation > plant bioaugmentation > only adding water > control, and nutrients/plant bioaugmentation greatly enhanced the degradation of HCHs (81.18 %) and DDTs (85.4 %). The bacterial community structure, diversity and composition were assessed by 454-pyrosequencing of 16S recombinant RNA (rRNA), whereas the abundance of linA gene was determined by quantitative polymerase chain reaction. Distinct differences in bacterial community composition, structure, and diversity were a function of remediation procedure. Predictability of HCH/DDT degradation in soils was also investigated. A positive correlation between linA gene abundance and the removal ratio of HCHs was indicated by correlation analyses. A similar relationship was also confirmed between the degradation of HCHs/DDTs and the abundance of some assemblages (Gammaproteobacteria and Flavobacteria). Our results offer microbial ecological insight into the degradation of HCHs and DDTs in aged contaminated soil, which is helpful for

the intensification of bioremediation through modifying plant–microbe patterns, and cessation of costly and time-consuming assays.

Link: https://doi.org/10.1007/s00248-014-0489-z

**Implementation → Biostimulation and surfactant addition**
**Ref 85**
Bibiana Betancur-Corredor, Nancy J.Pino, Santiago Cardona, Gustavo A.Peñuela, Evaluation of biostimulation and Tween 80 addition for the bioremediation of long-term DDT-contaminated soil, Journal of Environmental Sciences Volume 28, 1 February 2015, Pages 101-109

Abstract
The bioremediation of a long-term contaminated soil through biostimulation and surfactant addition was evaluated. The concentrations of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) and its metabolites 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (DDD) and 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDE) were monitored during an 8-week remediation process. Physicochemical characterization of the treated soil was performed before and after the bioremediation process. The isolation and identification of predominant microorganisms during the remediation process were also carried out. The efficiency of detoxification was evaluated after each bioremediation protocol. Humidity and pH and the heterotrophic microorganism count were monitored weekly. The DDT concentration was reduced by 79% after 8 weeks via biostimulation with surfactant addition (B + S) and 94.3% viabiostimulation alone (B). Likewise, the concentrations of the metabolites DDE and DDD were reduced to levels below the quantification limits. The microorganisms isolated during bioremediation were identified as Bacillus thuringiensis, Flavobacterium sp., Cuprivadius sp., Variovorax soli, Phenylobacterium sp. and Lysobacter sp., among others. Analysis with scanning electron microscopy (SEM) allowed visualization of the colonization patterns of soil particles. The toxicity of the soil before and after bioremediation was evaluated using Vibrio fischeri as a bioluminescent sensor. A decrease in the toxic potential of the soil was verified by the increase of the concentration/effect relationship EC50 to 26.9% and 27.2% for B + S and B, respectively, compared to 0.4% obtained for the soil before treatment and 2.5% by natural attenuation after 8 weeks of treatment.

Link: https://doi.org/10.1016/j.jes.2014.06.044

**Implementation → Bacteria → sewage sludge**
**Ref 86**
Qi Liang, Mei Lei, Tongbin Chen, Jun Yang, Xiaoming Wan, Sucai Yang, Application of sewage sludge and intermittent aeration strategy to the bioremediation of DDT- and HCH-contaminated soil, Journal of Environmental Sciences Volume 26, Issue 8, 1 August 2014, Pages 1673-1680

Abstract
Adding organic amendments to stimulate the biodegradation of pesticides is a subject of ongoing interest. The effect of sewage sludge on the bioremediation of dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) contaminated soil was investigated in bench scale experiments, and intermittent aeration strategy was also used in this study to form an anaerobic–aerobic cycle. Bioremediation of DDT and HCH was enhanced with the addition of sewage sludge and the intermittent aeration. The removal rates of HCH and DDT were raised by 16.8%–80.8% in 10 days. Sewage sludge increased the organic carboncontent from 6.2 to 218 g/kg, and it could also introduce efficient degradation microbes to soil, including Pseudomonas sp., Bacillus sp. and Sphingomonas sp. The unaerated phase enhanced the anaerobic dechlorination of DDT and HCH, and anaerobic removal rates of β-HCH, o,p′ -DDT and p,p′ -DDT accounted for more than 50% of the total removal rates, but the content of α-HCH declined more in the aerobic phase.

Link: https://doi.org/10.1016/j.jes.2014.06.007

**Implementation → Stimulation of native microbes**
**Ref 87**
Ortíz, I., Velasco, A., Le Borgne, S. et al. Biodegradation of DDT by stimulation of indigenous microbial populations in soil with cosubstrates, Biodegradation 24, 215–225 (2013).

Abstract
Stimulation of native microbial populations in soil by the addition of small amounts of secondary carbon sources (cosubstrates) and its effect on the degradation and theoretical mineralization of DDT [l,l,l-trichloro-2,2-bis(p-chlorophenyl)ethane] and its main metabolites, DDD and DDE, were evaluated. Microbial activity in soil polluted with DDT, DDE and DDD was increased by the presence of phenol,

hexane and toluene as cosubstrates. The consumption of DDT was increased from 23 % in a control (without cosubstrate) to 67, 59 and 56 % in the presence of phenol, hexane and toluene, respectively. DDE was completely removed in all cases, and DDD removal was enhanced from 67 % in the control to ~86 % with all substrates tested, except for acetic acid and glucose substrates. In the latter cases, DDD removal was either inhibited or unchanged from the control. The optimal amount of added cosubstrate was observed to be between 0.64 and
2.6 mg C g−1dry soil. The CO2 produced was higher than the theoretical amount for complete cosubstrate mineralization indicating possible mineralization of DDT and its metabolites. Bacterial communities were evaluated by denaturing gradient gel electrophoresis, which indicated that native soil and the untreated control presented a low bacterial diversity. The detected bacteria were related to soil microorganisms and microorganisms with known biodegradative potential. In the presence of toluene a bacterium related to Azoarcus, a genus that includes species capable of growing at the expense of aromatic compounds such as toluene and halobenzoates under denitrifying conditions, was detected.

Link: https://doi.org/10.1007/s10532-012-9578-1


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