Evaluating the effectiveness of gentle remediation options (GRO) on a DDT-contaminated site

A case study of the Kolleberga plant nursery site

Master thesis in Infrastructure and Environmental Engineering

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Abstract

Due to the extensive use of pesticides in the mid-nineties, DDT (Dichlorodiphenyltrichloroethane) and its metabolites (DDE and DDD) are still present in the soil, posing a risk to different ecosystems. In Sweden, it's estimated that there are about 750 contaminated sites with DDT with a high risk of harming humans or the environment. Kolleberga forest nurseries are one of these contaminated sites. Due to its large area and high soil volume, environmentally friendly options such as Gentle Remediation Options (GRO) were suggested as a good alternative for their low cost and sustainability. This thesis is part of an ongoing research project conducted by Chalmers University to evaluate the effectiveness of different GRO strategies at Kolleberga plant nursery. The specific objectives of this thesis are i) to evaluate the ability different of GRO strategies to manage the risks of DDT using statistical methods, and ii) to carry out a detailed risk assessment study for two potential future land use scenarios for the site to evaluate the level of the risk and potential remediation needed. A literature review was conducted to understand the history of DDT contamination, available sustainable remediation techniques, DDT health risks to both the environment and humans; and source to potential receptors pathways (exposure and spreading). Statistical analysis (ANOVA) was conducted using IBM SPPS software to evaluate the effectiveness of phytostabilization and phytoextraction (GRO techniques) to manage the risks posed by DDT in the soil at the Kolleberga plant nursery. The results from the statistical analysis conclude that biochar had a significant impact (i.e., lower DDT concentrations) on the soil pore water concentration. This reduction was also seen in DDT uptake in earthworms. However, this study observed that there is no significant impact on the uptake of DDT in biomass for both grass and legume (phytostabilization) and the stabilization effect of biochar. Pumpkin roots (among leaves, stems & roots) could extract DDT significantly when biochar is added, however, no significant effect of pumpkin alone was observed, even though uptake is higher than grass and legumes. There is no influence of biochar on DDT to DDD or DDT to DDE transformation. Also the plant treatments alone do not significantly impact the degradation process. The ineffectiveness of GRO strategies might be due to soil and weather conditions at the site, anyway, biochar is found effective. Therefore, aided phytoremediation could be recommended to treat the Kolleberga plant nursery. An ecological risk assessment was carried out for two future land use scenarios (a planned tree nursery and a horse-riding center). For the first scenario, a qualitative method was conducted using the available data for DDT uptake in earthworms and assumptions regarding DDT bioavailability. While for the second scenario, a quantitative method was used using exposure equations developed specifically for horses. The results of this study concluded that the implementation of GRO, specifically aided-phytostabilization using biochar reduced the uptake of DDT in earthworms and so reduced the possibility of secondary poisoning to other mammals and predators. The site can be considered safe for keeping horses as the exposure levels of DDT pose no risk to both humans and horses even when they feed on grass planted on the site. However, more studies and research are still needed to further analyze the risks for other animals (predators and mammals), especially in the case of keeping grazing animals at the site for meat production and to produce other animal products such as meat, eggs, and dairy for human consumption.

Keywords: DDT, Forest/tree nurseries, GRO, Statistical Analysis (ANOVA), Risk Assessment, Biochar, Contaminated soil, Sustainable remediation, Conceptual site model (CSM).

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Acknowledgment

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Working with an actual site has been an intriguing and motivating experience for us, and we anticipate that the findings will contribute to valuable insights for essential considerations for further investigations in assessing the risks and evaluating the effectiveness of gentle remediation options (GRO).

We would like to thank and show our appreciation to our friends and family for their support, guidance, suggestions, and encouragement. Their feedback has been invaluable in shaping our ideas, refining our arguments, and improving our writing skills.

Completing this Master's thesis has been a challenging yet rewarding journey and would not have been possible without the contributions and support of all those mentioned above. Their collective efforts have played an integral role in the successful completion of this research endeavor. We are humbled and grateful for their invaluable contributions.

1 Introduction

1.1 Background

In recent years, soil contamination has emerged as a significant problem due to the growing demand for land resources in urban areas. There is a historical legacy of contamination from industrial activities like improper disposal of harmful chemicals e.g., heavy metals, hydrocarbons, toxic waste, and improper farming activities e.g., intensive use of inorganic fertilizers, pesticides, etc., (Ashraf et al., 2013). Soil is considered polluted when the contaminants adversely affect human health or the environment. Some pollutants degrade over time in soil, while others persist for decades, making soil the final destination/reservoir for many of these pollutants (EEA, 2021). Because of this, today we are left with many sites unsuitable for human use and harmful to existing life. A contaminated site is a defined area where hazardous substances are present and are confirmed to pose a danger to the environment and human health, while the potentially contaminated site is not confirmed for soil contamination but is suspected and needs further investigations (EEA, 2022). There are nearly 250,000 contaminated sites throughout Europe and almost 3 million sites with potentially polluting activities estimated across the European Union, many of them need further investigations for the requirement of remediation (EEA, 2020). There are about 85,000 confirmed contaminated sites in Sweden, of which 26,000 are classified as a potential risk, and approximately 1200 of them are classified as risk class 1 and assessed to pose very high risks to human health and the environment, while 8000 are classified as risk class 2, as they pose a high risk to human health and environment, both risk classes (1 and 2) are prioritized by the Swedish EPA (SEPA) (Naturvardsverket, 2021).

The growing awareness of the environmental deterioration due to soil contamination from DDT and other contaminants has led to increased engagement in soil remediation research with an emphasis on the benefits provided by soils to humans. Additionally, the advantages of soil remediation in improving the soil conditions such as soil microbe growth, erosion control, land loss, root proliferation, and soil hydraulic properties (Ashraf et al., 2010) as intrigued the research community, especially remediation using biochar (Zhu et al., 2017). Soil provides many important ecosystem services: it regulates the majority of the ecosystem processes, provides a home to vast biodiversity, and provides the physical foundation for many human activities like supporting buildings, road infrastructure, and urban agriculture (Pereira et al, 2017; FAO, 2020; Morel et al, 2015). The World Soil Charter by the Food and Agriculture Organization of the United Nations (FAO) has incorporated the principles of soil management to ensure the improvement of soil functions, and soil ecosystem services by adopting sustainable techniques of soil remediation and soil governance (WSC, 2015; FAO, 2020). It is essential to conserve soil and one way it can be done is by remediating the contaminated sites to such an extent that the contaminants pose no threat to human health or the environment. The EU's new soil strategy (Figure 1) aims at achieving good soil health by 2050 and showing significant progress the in remediation of contaminated sites, one of the key aims is investing in the prevention and restoration of soil degradation (EC, 2021).

Figure 1: The connections of the new EU Soil Strategy to existing EU initiatives (Source: EC,2021).

Soil plays a crucial role in shaping policies such as European Green Deal-2019, the Sustainable remediation program, and the above-mentioned new EU soil strategy related to land restoration, biodiversity conservation, and addressing climate change (EC, 2023; EC, 2021; SGI, 2014). One of the Swedish environmental objectives is to have a toxin-free environment by minimizing the presence and impact of harmful chemicals and toxins present in the environment. "*The occurrence of man-made or extracted substances in the environment must not represent a threat to human health or biological diversity. Concentrations of non-naturally occurring substances will be close to zero and their impacts on human health and ecosystems will be negligible. Concentrations of naturally occurring substances will be close to background levels*" (SGI, 2014). Stated goals of different environmental agencies and bodies; can complement each other but can also conflict, such as achieving the non-toxic environment goal while also minimizing environmental impacts and promoting urbanization while aiding green space preservation. The above-mentioned goals and strategies are important to take into consideration while evaluating the technologies available for the remediation of soil contaminated with persistent pollutants such as DDT.

Between the 1950s and 1960s, intensive use of fertilizers and pesticides was encouraged to improve crop quality and meet the global food demand. DDT was used to control malaria and typhus at first, but later in 1960, 70-80% of about 400,000 tons of DDT produced was used for pest control in agriculture and forests (Turusov et al., 2002). Even after its ban in the 1970s, the residues are being detected in soil and other environmental media in Asia, South America, and Europe in the last decade. DDT is more persistent in temperate climates compared to the tropics, rapid dissipation is attributed to volatilization and photo-oxidation processes in the tropical regions (Samuel & Pillai, 1989). In a study conducted in 2004, high levels of DDT/DDE were recorded in Russia and Italy (Jaward et al., 2004) see Figure 2 (left). DDT and its metabolites (DDD-dichloro bischlorophenyl ethylene and DDE- dichloro bischrolophenyl ethane) can negatively impact living organisms as they accumulate in the fatty tissues of humans and animals with the body being unable to eliminate them from their bodies. Figure 2 (right) shows the main reservoirs of DDT and DDE stored in human fatty tissues across the world (Mansouri, et al., 2017).

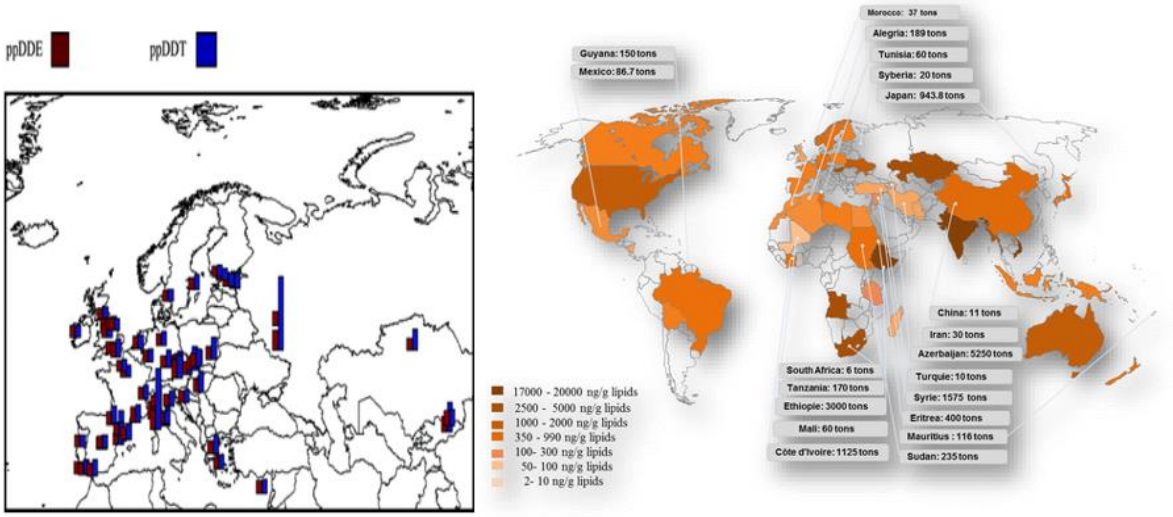

Figure 2: (Left) Map showing the spatial distribution of ppDDE and ppDDT in Europe for the samples taken in July 2002, largest bar ppDDE= 4.2 & ppDDT =32 ng/ sample (Jaward et al., 2004). (Right) Map showing the location of main reservoirs of DDT and DDE in human fatty tissues around the world (Mansouri et al, 2017).

The issue of soil contamination by DDT in Sweden is due to the extensive use of pesticides, predominantly DDT, which was common in plant nurseries and garden centers in the 1940s, to fight bark beetle (Anderson, 2014) (SGI, 2017). Some of these plant nurseries were state-owned and were allowed to use DDT even after the general ban was imposed in 1969 (up to 1974) (SGU, 2021). As a consequence, approximately 750 plant nurseries in Sweden are designated as highly contaminated with DDT (Casey, 2022; SGU, 2021). According to Swedish Geotechnical Institute (SGI, 2018) DDT, and its metabolites and (Dicofol- trichloro bischlorophenyl ethanol) are the most commonly detected pesticides in the soil (SGI, 2017). This can be attributed to the main factors: 1) the application of DDT by spraying using tractors resulted in the homogenous distribution of DDT concentrations in the cultivation fields, 2) the spillage during the treatment process, where plants were dipped in DDT, led to localized "hot-spot pollution" in the cultivation fields (SGU, 2023). In-depth investigations are being conducted to manage the risks associated due to DDT on the soil environment and for future land use planned for the nursery sites. Many of these nurseries are very similar in terms of pollution situations, geology, and land use. These sites generally consist of sandy and well-drained soil suitable for cultivation and can be closer to the groundwater bodies which may pose a risk of leakage to groundwater, but the groundwater is usually deep, several meters below the soil surface, therefore the possibility of DDT-contaminated precipitation is very rare and not often a risk. The spread of pollution by surface water outside the former cultivation fields is also rare due to the large dilution from the precipitation and the lack of ditches around the former fields (SGU, 2023). In this case, the risks are expected to be primarily to the soil ecosystem and land animals that are associated with these sites and do not pose a direct danger to humans (SGU, 2023).

It is the role of the Swedish Environmental Protection Agency (SEPA) to coordinate, prioritize, guide, and follow up on the remediation of contaminated sites in Sweden. There are many remediation techniques often used to treat contaminated sites, where the most used technique

in Sweden is excavation combined with landfilling (dig and dump) (Anderson, 2017). There are many plant nurseries with DDT concentration levels above generic soil guideline values and the volume of soil being dealt with can range between 50 to 500 thousand tons of soil per nursery. It is not considered practical to use conventional methods on such large areas because it would not be economically sustainable and difficult to restore the soil to its natural state with valuable soil functions being lost (SGI, 2018;Structor, 2021; SGU,2023b). For example, Deje forest nursery situated in Sweden's Forshaga municipality, has about 100 hectares of land in total that is investigated to have DDT residues in the ground (SGU, 2023c). Most of these sites have great natural value, so using excavation and dumping will not be relevant (Börjesson, et al, 2022). The excavation method could still be used for remediating the hotspot pollution (SGU, 2023c). Remediation methods such as mechanochemical degradation and chemical/biological decomposition along with phytoremediation have been explored to study the possible remedial techniques to reduce DDT and its metabolites at forest nurseries. But biological methods such as stabilization with biochar and phytoremediation techniques were deemed best to handle large surface areas like forest nurseries (Structor AB, 2021). As the geology of many of these sites is the same, it would be beneficial if a non-intrusive remediation solution could be used repeatedly on a large percentage of the fields. Application of GRO-Gentle Remediation Options, which uses certain plants, soil amendments, and micro-organisms is considered to best suit the plant nurseries with large areas of low-moderately contaminated soil (SGU, 2023b) (Structor, 2021).

Several of these former forest nurseries are investigated for the risks associated with future land use scenarios such as golf operation, livestock farming, horse farming, and growing crops (SGU, 2023a; SGU, 2023b; Börjesson, et al., 2022). According to the Swedish Geological Survey (SGU), plant nurseries that have a low to moderate contamination degree of DDT, could be used as a cultivation area for fodder, for animals such as cows, chickens, and horses. The fields could also potentially accommodate horse activities, small herds of sheep or cows for meat production, and farm dairy (SGU, 2022).

Kolleberga is one of these former forest nurseries, situated in Southern Sweden and owned by Sveaskog AB, contaminated by historical use of DDT. Investigations have shown that the concentrations in soil exceed the generic soil guideline values for less sensitive land use and potentially pose ecological risks. In 2021 a field experiment was set up at the Kolleberga site to test various gentle remediation options (GRO) to manage the risks at the site within the projects PILOT-GRO[1] and GRO[2]. The experiment was set up by Chalmers University of Technology in collaboration with the Swedish Agricultural University (SLU), the Swedish Geotechnical Institute (SGI), Nordvästra Skånes Renhållningsbolag AB (NSR AB) and the Geological Survey of Sweden (SGU). This master's thesis builds upon the results collected in the field experiment.

[1] PILOT-GRO: Evaluation of innovative and gentle in situ remediation strategies to manage risks and improve ecosystem services. Funded by TUFFO, Dnr 1.1-2014-0303.
[2] GRO – Managing risks and improving soil functions by Gentle Remediation Options. Funded by Formas, project-ID 2021-01428

1.2 Aim and Objectives

The overall aim of this master's thesis is to better understand the feasibility of gentle remediation options (GRO) for managing the risks at the DDT-contaminated forest nursery Kolleberga with the following specific objectives:

- evaluate the effectiveness of different GRO strategies to manage the contamination of DDT at the Kolleberga plant nursery site and evaluate their ability to reduce the risks to the biological receptors and the ecosystems using statistical methods.

- Conducting a detailed risk assessment study at the site to evaluate the ecological risk level for two possible future scenarios.

1.3 Limitations

During the study, there have been some limitations and uncertainties that may have affected the results, these include:

- Lack of time in analyzing the data. This was because of the delay in receiving the second-year data from the laboratories which resulted in analyzing the data in a very short time which resulted in difficulties in interpreting the data.
- Some data was lost or unavailable, which made us unable to compare them over time to see if there are any trends over time (e.g., if certain GRO strategies are more effective in the second year). The missing data were pore water concentration for the year 2021, pumpkins/ biomass for the year 2022 due to slug infestation, and the start year data of DDT uptake in earthworms.
- The available data for grass and legume are not sufficient to conclude if phytostabilization is occurring or not. To be able to measure this, data for both the plant's roots and shoots need to be examined. In the experiment, only shoots data were collected.
- The sampling techniques for the second year were different than the first-year techniques. Also, the chemical analyses for the parameters were done by two different labs, which may have resulted in differences between the labs for the measurements such as the total concentration of DDT.

2 Theoretical Background

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874, but it was later on in 1939 that it was discovered that this component had an insecticide effect, and it was commercially released in 1945 as a pesticide/insecticide to combat insect-borne diseases (Jarman et al., 2012). Due to its effectiveness in controlling different types of pests and diseases at a low cost, it was extensively used in agriculture, commercial, residential, and public health applications during the 50s and 60s. Houses were sprayed twice a year with DDT powder to kill resting *Anopheles* mosquitoes (Pérez-Maldonado et al., 2010). As a result of this extensive use, DDT began to be found in a wide variety of organisms. Marine creatures were especially vulnerable as they can accumulate DDT in their flesh above the ambient environmental concentration (Beard, 2006).

2.1 DDT risks

Exposure of DDT can occur from different routes (exposure pathways). The first is the direct consumption of contaminated water, fruits, vegetables, plants, and seeds, which is known as primary poisoning. While secondary poisoning occurs when a predator feeds on contaminated prey (insects and worms) (Sánchez-Bayo et al., 2011). Both these routes can cause a toxicological effect depending on the received dose and whether the animal was exposed once or through several episodes, which can cause DDT to accumulate in their lipid tissues leading to biomagnification (Deribe et al., 2013). The term *biomagnification* means that the concentration of DDT in the fat tissues of the organisms is higher than the concentration in the source (Gray, 2002).

Based on a study conducted by Marciniak and Witczak, there is a higher level of DDT and its metabolites in fat tissues (adipose tissues) of fish, bivalves, and crayfish found in the Oder River in Poland, indicating bioaccumulation (Marciniak & Witczak, 2009). In another study conducted by Kesic and collaborators, bioaccumulation and biomagnification have been found in Okanagan Valley in fruits, orchards, earthworms, and eggshells of American robins in Canada (Kesic, et al, 2021). This can cause severe consequences to the ecosystem inhabitants leading to an increase in their mortality rate or inhibit their reproduction and growth which reduces their population and imbalances the system. However, this disturbance can be temporary as populations can recover once the toxicant levels drop or disappear (Sánchez-Bayo et al., 2011).

These adverse effects of DDT began to show after the second world war by the 1960s, when scientists found that DDT concentrations in gulls, merganser, and cormorants were thousand times higher than what it was already found in the mud where birds usually fed (Cox, 1991). Another study done by D.A. Ratcliffe found that the thickness of falcon eggshells was decreasing due to the effect of these pesticides on the calcium metabolisms which causes them to be very weak and eventually break more often (Ratcliffe, 1970). Such information has led the way to more discoveries on the negative impacts of such chemicals as it can accumulate in the fatty tissues of mammalians causing serious effects on their reproduction, growth, and immunocompetence (Lunney et al., 2004). As a result, the use of DDT was banned in most countries after 1970 (Beard, 2006), except Mexico and Nicaragua which continued the use of DDT for disease vector control until 2000 (Pérez-Maldonado et al., 2010).

In the past 25 years, more epidemiological studies began to focus on humans and found an association between DDT and several diseases, such as leukemia, prostate cancer, brain cancer, liver cancer, diabetes, pancreatic cancer, lymphopoietic cancer, Hodgkin's lymphoma, multiple myeloma and a great risk of developing fertility and reproductive issues (Beard, 2006). (Rylander et al, 2005) & (Rignell-Hydbom et al, 2007) found that the prevalence of diabetes was higher in Swedish people with elevated serum DDE levels. Exposure to DDT can pose a great health risk, especially for children as they can be easily exposed to DDT by ingestion of soil or contaminated food and dust inhalation (Pérez-Maldonado et al., 2010).

DDT is included within the group of Persistent organic pollutants (POPs), meaning it is slow to degrade in the environment, can bioaccumulate in organisms, is prone to long-range atmospheric transboundary migration and deposition, and imposes danger to humans and the environment in the source and distant regions of the contamination (Ashraf et al., 2013). The reason for its persistence is due to its low vapor pressure, lipophilicity, and resistance to degradation and photo-oxidation. It can degrade via abiotic and/ or biotic processes in the environment into DDD (dichlorodiphenyldichloroethane) and DDE (dichlorodiphenyldichloroethylene) by certain types of plants and micro-organisms under certain conditions. Based on (Aislabie et al., 1997) '*DDT is formed through reductive dichlorination, either microbially mediated or as the result of a chemical reaction. While DDE is formed through photochemical reactions in the presence of sunlight and through dehydrochlorination in bacteria and animals*. The complete mineralization of DDT requires two processes, de-chlorination, and ring cleavage. However, the dechlorinated degradation products (DDE and DDD) are still toxic, can be easily bioaccumulated, and might be more resistant to degradation, especially DDE (Xu et al., 2021). However, due to their similar chemical structure and properties, these compounds are often studied together, i.e., total ΣDDT is the sum of DDT, DDD and DDE including their isomers (Juhasz et al., 2016). The chemical structure of DDT and its metabolites can be seen in (Figure 3) below.

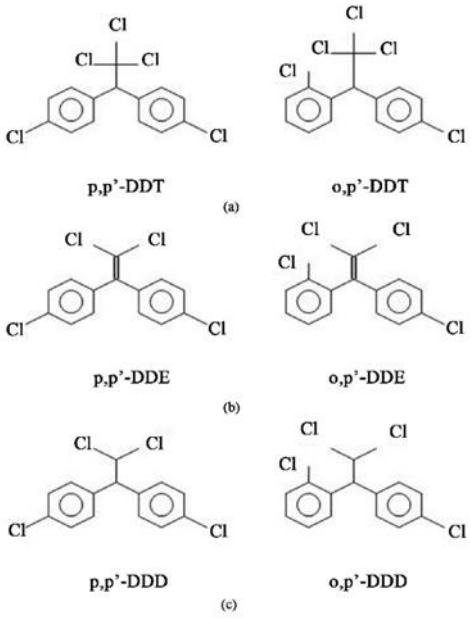

Figure 3: Chemical Structure of DDT's isomers: (a) DDT, (b) DDE, and (c) DDD (Source: Ramirez-Elias, et al., 2016)

2.2 Gentle Remediation Options (GRO)

Gentle remediation options (GRO) are nature-based solutions (NBS) that can be applied for managing contamination while maintaining the ecosystem and ecological soil function or improving it. This in return provides ecosystem services that are essential for humans, e.g., biomass production, flood mitigation, decrease in urban heat islands, recreation areas, habitat for animals, and carbon (Drenning et al., 2022). According to (Cundy et al., 2016 & 2015), GRO can be identified as "r*isk management strategies or technologies involving plant (phyto-), fungi (myco-), and/or bacteria-based methods that result in a net gain (or at least no gross reduction) in soil function as well as effective risk management*". Below are the most common GRO technologies and their definitions.

Table 1: Most common GRO technologies (Conesa et al., 2012), (Drenning, 2021), (Padmavathiamma & Li, 2007), (Limmer & Burken, 2016), (Arthur et al., 2005b), (Martínez-Alcalá et al., 2012), (Vidali, 2001) (Wang et al., 2021).

GRO	Definition
Phytoextraction (Phytoaccumulation)	the process of plants' uptake and translocation of inorganic contaminants from the soil into other parts of the plant.
Phytodegradation (Phytotransformation)	it's the extraction and breakdown of organic contaminants into less harmful components by the plant itself or by their enzymes.
Rhizodegradation	is the same mechanism of *Phytodegradation* but with the intervention of microorganisms as fungi or bacteria that can further degrade the contaminants in the root zone
Phytovolatilization	the process where plants absorb some chemical elements from metals and convert them into gases which can be released into the atmosphere by diffusion.
Phytostabilization (Phytoimmobilization)	the process where plants absorb and accumulate contaminants into their roots, which immobilizes the contaminants and reduces their bioavailability for uptake and migration.
Phytoexclusion	the process of using excluder plants (non-or low accumulators) to mitigate the risk of contaminants of agriculture products grown in polluted soil.
Phytofiltration & Rhizofiltration	the process where plants use their roots or seedlings to absorb, concentrate, and precipitate pollutants from water, waste streams, and constructed wetlands.
Phytohydraulics	is the process by which plants and their microorganisms take up water and then evaporate it into the atmosphere (evapotranspiration), which influences the groundwater level, direction, and velocity.
Bioremediation	is the process in which microorganisms (bacteria, fungi, or plants) are used to degrade organic contaminants into less toxic forms.
Mycoremediation	it's a form of bioremediation, in which fungi are used to degrade, stabilize, or reduce the bioavailability of contaminants.
Vermiremidiation	it's the process of using earthworms to remove or stabilize contaminants.

2.4 Phytoremediation techniques for DDT

Phytoremediation is one of the *in-situ* GRO methods of removing or stabilizing contaminants from soil and water by certain types of plants, bacteria and fungi. It's an umbrella term that includes different mechanisms where plants can extract, degrade or stabilize contaminants based on the level of removal, site conditions, and which type of plants are being used (Padmavathiamma & Li, 2007). Plants use different mechanisms to remove contaminants from water, soil and air. Some of the most common techniques that are used and studied for remediating DDT are phytostabilization, phyto/rhizodegradation and phytoextraction that are illustrated in (Figure 4) below, more detailed about these techniques are presented in the following sections.

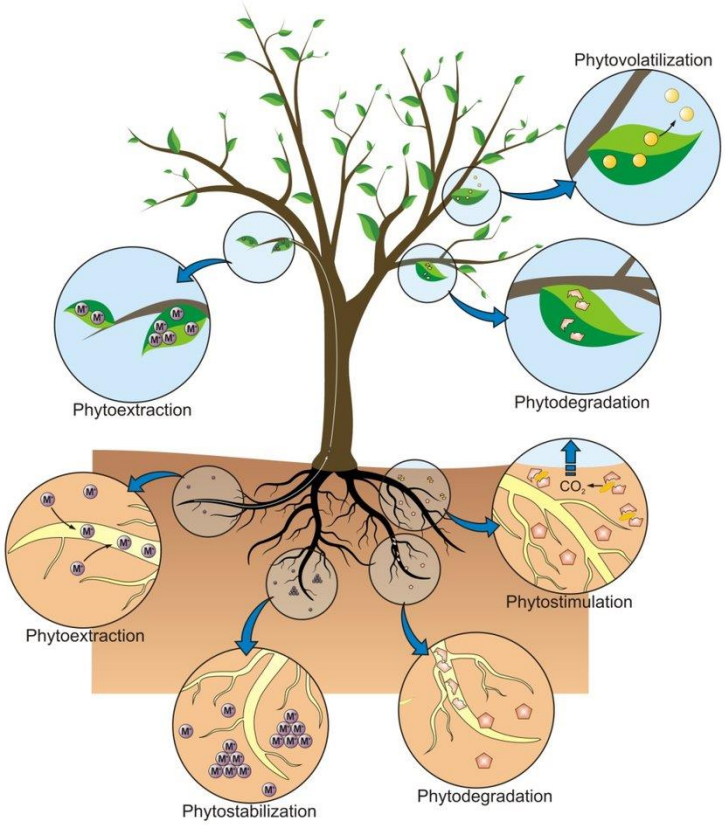

Figure 4: Representation of different phytoremediation processes (Favas et al., 2014)

2.4.1 Phytostabilization

Phytostabilization describes the process where plants absorb and accumulate contaminants in their roots or within the vadose zone (rhizosphere). This will immobilize the contaminants and prevent further migration or leaching to ground water and soil (Padmavathiamma & Li, 2007) (Bolan et al., 2011). This technique is mostly studied for immobilizing metals, therefore, there are very few studies in the literature about stabilizing organic contaminants. According to (Moklyachuk et al., 2012) different types of plants have been studied to see if they are able to stabilize or accumulate high concentrations of DDT in their rhizosphere based on measuring their translocation factor. It was found that DDT levels were decreased up to 30% in the soil with some plants having low translocation factor, which indicates that phytostabilization was occurring. However, because the contaminants are not being removed rather just immobilized, this process requires regular monitoring to ensure its effectiveness (Ghosh & Singh, 2005).

2.4.2 Phytoextraction

Phytoextraction, also known as phytoaccumulation, is the process of plants uptake and translocation contaminants from the soil into other components of the plant where the level of contamination is low and relatively superficial. Based on a promising greenhouse-study in the western Canadian Arctic that was done to evaluate the potential of different plant species such as; rye grass (*Lolium multiflorum*), tall fescue (*Festuca arundinacea* Schreb.), alfalfa (*Medicago sativa*), zucchini (*Cucurbita pepo* L. cv. Senator), and pumpkin (*Cucurbita pepo* cv. Howden) to extract and degrade high and low concentrations of DDT. The results showed that all of these five plants were able to accumulate DDT, especially zucchini and pumpkin (both members of the genus *Cucurbita*) as they were able to accumulate a high amount of ΣDDT in their roots and translocate them into their shoots, while alfalfa was able to degrade (phytodegradation/rhizodegradation) some of the DDT to a less harmful component either by the plant's roots itself or by bacteria (Lunney et al., 2004).

2.4.3 Phyto/Rhizodegradation

Some plants have the ability to extract and then break down the organic contaminants into less harmful components, either by the plant itself or by their enzymes release into the rhizosphere without the intervention of microorganisms. This is called phytodegradation or phytotransformation (Conesa et al., 2012). Plants such as *Elodeacanadensis* (Elodea) and *Pueraria thunbergiana (*kudzu) were both tested by (Garrison et al., 2000) and found to be successful in degrading DDT into DDE and DDD from water and soil samples.

Rhizodegradation is the same mechanism but with the intervention of microorganisms such as fungi or bacteria that can further degrade the contaminants in the plant's root zone (Arthur et al., 2005b). Bioremdeiation including the use of fungi (mycoremdeiation) and bacteria has been getting successful results in the recent years, various types of fungi have been studied to degrade DDT into its metabolites (DDE and DDD) by undergoing dichlorination and hydrogenation processes. Fungi such as *Trichoderma hamatum*, *Rhizopus arrhizus*, *Fomitopsis pinicola*, and *Pleurotus ostreatus* were reported to be successful (Bokade et al., 2021).

There are numerous variables that can affect both phyto-degradation and rhizo-degradation processes such as soil temperature, pH, moisture and organic matter content, and aeration of the soil (Wolińska & Bennicelli, 2010). A study was conducted to assess the potential of (Tomato) *S. lycopersicum*, (sunflower) *H. annuus*, (alfalfa) *M. sativa*, and (soybeans) *G.max* to degrade and accumulate DDTs residuals in soil. It was found that DDT concentrations were diminished after 60 days, due to the combined effect of roots uptake and rhizospheric degradation (Mitton et al., 2014).

2.5 Soil Amendments to aid phytoremediation

The application of Phytoremediation can be divided into "standard" and "enhanced" techniques. The standard refers to the use of the natural process of plants and their naturally occurring microbes without the intervention of any human enhancements. While "enhanced", is the aiding process through the use of microbes by bioaugmentation (the use of external species) or bio stimulation (the use of soil amendments) (OVAM, 2019). Carbon-rich materials as charcoal, biochar, and activated carbon have been used to stabilize organic pollutants and reduce their bioavailability, while surface-active compounds such as organic acids, rhamnolipids, and nanoparticles have been used to aid the process of phytoremediation by increasing the desorption of DDT in contaminated soil and improve nutrient uptake in the plant's roots (Mamirova et al., 2021).

2.5.1 Carbon rich materials

Biochar is a carbonaceous substance created as a result of pyrolysis of organic materials such as feedstock, plants, and sludges (Paz-Ferreiro et al., 2014). It has the ability to immobilize some types of contaminants due to its large surface area, which reduces the contaminants bioavailability to both microorganisms and plants. It can also enhance the ability of plants to produce more biomass and growth by 10%, due to biochar's high nutrient and water holding capacity which improve the plants nutrient turnover and increases its effectiveness of extract or degrade certain contaminants (Sarwar et al., 2017). It can also shift the microbial community structure which improves the soil quality and soil functioning (Maqbool et al., 2012). Denyes et al. (2016) studied two types of biochar (Burt's and Blue-Leaf) by adding them to soil samples with invertebrates (*Eisenia fetida)* and (*Cucurbita pepo)* plant while using polyoxymethylene (POM) passive sampler method. The study intended to show the ability of biochar to immobilize DDT by measuring its bioavailability in *E. fetida*, as the site was heavily contaminated with DDT with values exceeding the agriculture guidelines. The results showed that biochar has a great potential of reducing the DDT accumulation in the invertebrate by 49% with no adverse effects on their health (Denyes et al., 2016).

Biochar can also enhance the degradation process of DDT. According to a study done in New Zealand, where two types of *Salix sp.* (willow biochar) were used in co-contaminated soil. The soil was contaminated with a high concentration of heterogenous arsenic (As) and organochlorines such as HCH, aldrin, and DDT (Gregory et al., 2015). The study has resulted in a significant decrease of both HCH and DDT while only a short-term reduction of water-soluble (As). It's believed that this occurred due to the increase in microbial activity due to the addition of biochar, which resulted in higher volatile fraction, redox potential, water holding capacity and soil pH.

2.3.2 Surface active compounds

Soil amendments can also be beneficial at sites that are highly contaminated with DDT which may require different remediation strategies, such as the Yen Dung site in Vietnam. For example, a pilot scale application conducted by (Dang et al., 2022) used Phyto-Fenton remediation that includes the use of surface-active compounds as ferrous iron ($FeSO_4$) and endogenous hydrogen peroxide (H_2O_2) in aquatic plants. This is a recent approach to mitigate organic pollutants by using the Fenton reaction, it requires the use of Fe^{2+} and H_2O_2 to form a

strong oxidized species which can potentially oxidize pollutants such as POPs (Tran et al., 2020). The use of nano-sized iron particles is highly important for the detoxification of organic pesticides, but the fate of metal oxide that lingers in the soil and its effect on the bacterial communities are concerning. However, the results of the trial showed a great success of degrading DDT into DDE and DDD by dichlorination under anaerobic and aerobic conditions after 120 days by 82.5-98.4% with improvement in soil quality, TOC concentration, and soil microbial community due to the use of (Fe) which acted as a fertilizer and promoted plant growth (Dang et al., 2022).

2.6 GRO Disadvantages

Even though phytoremediation has proven to be a very effective remediation technique by putting into consideration the sustainability and cost aspects, it does not perform well in every site. There are several limitations that have been mentioned by (Pivetz, 2001) which could be summarized as below:

- The process is depth limited due to the shallow distribution of plats roots. This means that if the contaminants are below the roots, the technique might be ineffective.
- It's a slow remediation technique as it depends on the plants grow seasons, some studies have found that it could take up to 10-20 years for the plants to be fully grown to reach its maximum extraction capacity. (Figure 5) below represents the 'relative remediation time' it would take for a full source removal by extraction, degradation, and volatilization.
- Some phytoremediation techniques, such as phytoextraction requires a proper disposal of the biomass, as the contaminants may accumulate in the edible parts of the plant which poses a risk to both humans and animals.
- Not very effective during winter when the growth is limited. The plants may also be subjected to different damage from the weather, pests or diseases that will slow the process and require continuous monitoring.
- Plants can't grow in high toxicity sites, which makes it applicable only at sites where contaminants concentration is low over a large area.

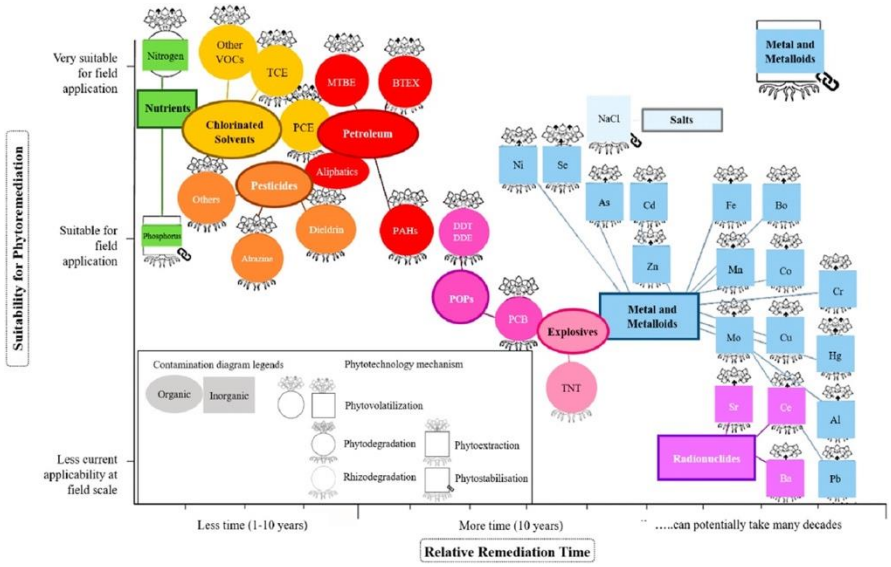

Figure 5: Relative remediation time for phytoremediation technologies (Chowdhury et al., 2020), modified from (OVAM, 2019)

2.7 Risk assessment at contaminated sites

Risk assessment of contaminated sites can be identified as 'a formalized framework, used to identify whether or not contaminated sites present an unacceptable risk to human health, controlled waters, property, or ecological receptors. It's also considered a part of the decision basis regarding remediation of the site. According to Swartjes, "*Risk is a concept that denotes a potential negative impact to an asset*", so there must be a (source) for this impact, which is generally the *Hazard* that causes an adverse effect on the receptors (Swartjes, 2011). The risk assessment framework can be illustrated by several steps as can be seen in (Figure 6) below.

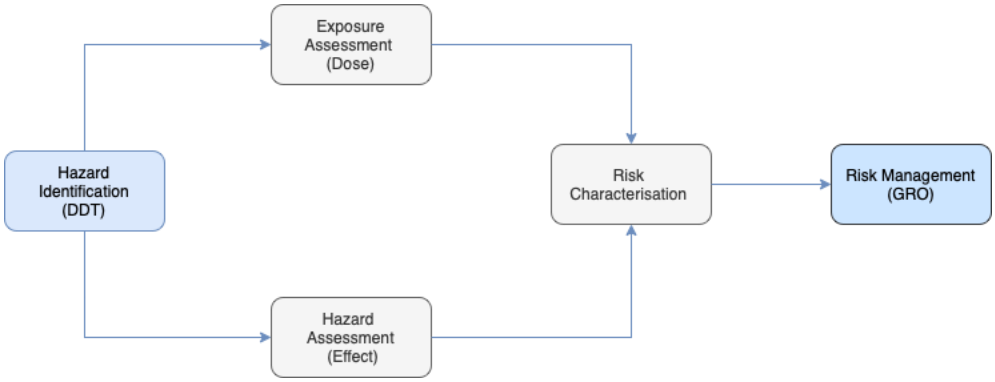

Figure 6: Risk assessment framework, based on (Swartjes, 2011).

The first step in the risk assessment framework is the *hazard identification* or problem identification, which also includes identifying the protection targets (the receptors), the level of protection, and the boundary conditions. The second step is the risk assessment part, which includes two activities, *Exposure assessment* which refers to the exposure dose, or the amount of a contaminant that enters the human body or organisms, more specifically, the amount that can be absorbed into a specific organ. While *hazard assessment* represents the effect of the contaminant on the human body. Both these activities are mainly used in the Human Health Risk Assessment. The combination of these two activities is what is called *Risk Characterization*, which refer to the risk management (mitigations) or the remediation strategies that need to be implemented to reduce or eliminate the risk if the risk levels are unacceptable (Swartjes, 2011).

2.8 Soil guideline values

The soil guidelines values are generic values that are used for evaluating the risk level of a contaminated area in order to see if the data falls under/above an acceptable risk level for both humans and the environment. For this reason, the Swedish Environmental Protection Agency (SEPA) has developed general guidelines values for two types of land uses, sensitive land use (KM) where the levels of DDT should not exceed (0.1 mg/kg TS) and less sensitive land use (MKM) where the levels should be below (1 mg/kg TS) (SGU, 2018). Both of these land uses and their protection objects can be seen in table 2 below with their acceptable concentrations of DDT in (mg/kg TS). The table indicates that for both land uses, sensitive land use and less sensitive land use, the soil ecosystem is the most sensitive receptor. Looking at humans as receptors, it can be noted that the most important exposure pathways are via intake of plants and direct intake of soil. Exposure to humans via inhalation of dust and vapors, as well as dermal contact are less important.

Table 2: summary of the guideline values for individual protection targets for individual exposure routes. All values are given in mg/kg dry weight (TS). These values were extracted from the SEPA model of 2016. This table was adapted from (SGU, 2018).

protection target	**exposure pathway**	**DDT, DDD, DDE (SUM DDT)**	
		KM	**MKM**
Human health	ingestion of soil	31	290
	dermal contact	380	1900
	inhalation of dust	35000	340000
	inhalation of vapor	4500	440000
	exposure via drinking water	170	-
	ingestion of plants	4	-
	health reference value	3,4	250
Soil ecosystem		**0,1**	**1**
Spreading	ground water protection	2,3	7,4
	surface water protection	150	150
Integrated guideline value		**0,1**	**1**

3. The GRO pilot study at Kolleberga forest nursery

3.1 Site description

The study site Kolleberga is situated in the Ljungbyhed locality of Skåne County, south of Sweden and owned by Sveaskog AB (Figure 7). Previously this site was a forest nursery, and trees like pine, spruce, and fir were cultivated for both commercial and research purposes. During the 1950s, the nursery was active, and DDT was used as a pesticide and sprayed all over the field to control weeds and pests, and also the seedlings were dipped before planting. Following the awareness of health and environmental implications, when DDT was banned in Sweden in 1969, the authorities were ordered to give more information on the contamination levels and risks associated, therefore an inventory and survey were carried.

Figure 7: Kolleberga Plantskola location (Rundegren, 2019)

3.2 Previous risk assessment studies at Kolleberga

Based on a detailed risk assessment report done by Tyréns AB in 2020 (Miljöteknisk markundersökning kompletterande utredning, Kolleberga plantskola) (Tyréns, 2020). DDT was found in the entire field in low concentrations near the building areas. They concluded that it poses no risk to humans, nor the ground and surface water based on their results. However, in some points where there were a dipping area and a plant dump (where discarded plants have been thrown away and other parts were used to fill the area with pesticides sprayers), the concentration of DDT exceeded the MKM (less sensitive land use) values by 30 - 60 times. The plant dump is estimated to cover an area of 50x20 m2 with a volume of 8,000-10,000 m3. This area is not included in our study as the concentrations are very high and cannot be remediated with GRO. Additional investigations are currently ongoing to evaluate other remediation options to manage these areas (Tyréns, 2020). (Figure 8) below, shows the study area (red rectangular), the dipping area (orange triangle) and the plant dump (the yellow shape).

Figure 8: overview of Kolleberga school nursery showing the study area in red, previous dipping area in orange, and the previous plant dump in yellow. Photo taken from Google Maps and was highlighted based on the information found in (Tyréns, 2020) report.

Based on Tyréns report, the average concentrations of ΣDDT found in the entire site have been summarized in table 3 below.

Table 3: Average concentrations of DDT in different locations (Tyréns, 2020)

Location	Ave. of ΣDDT concentration (mg/kgTS)	KM threshold	MKM threshold
Field (the study area)	7.25	0.1	1
Dipping area	35		
Plant dump (landfill)	3.8		

The soil types present in the site are majorly glaciofluvial sediments and till, silt and sand are occasionally found. The surface soil is present up to 0.35m depth where DDT was found in the field area. There is a river called Rönne å located at a distance of 200m from the site and it flows in the north direction. There is also a groundwater body (Ängelholm- Ljungbyhed) at the depth of 3-5m in some parts of the nursery and it flows towards the direction of the river. Currently, no water is being used from the river or groundwater for drinking purposes in the locality. The land use in the area is dominated by forestry with few permanent residential buildings close to the urban area, and also agricultural lands.

3.3 Protection targets and exposure routes at Kolleberga

3.3.1 Protection targets

There are several protection targets that could be recognized at Kolleberga, the first and probably the most important is the soil ecosystem which represents the soil biota and its different organisms. Small animals such as earthworms and plants are in direct contact with DDT as they live and grow on the shallow surface of the soil. However, when conducting a risk assessment study, ecological receptors such as animals and humans might be more relevant to consider than the soil. According to Tyréns AB, a high concentration of DDT was found in the fatty tissues of earthworms. Other field animals such as mice (Apodemus *sylvaticus*), badgers (Meles *meles*), and weasels (Mustela *nivalis*) are subjected to secondary poising via the ingestion of plants and earthworms. Humans and grazing animals are also subjected to direct or secondary poisoning if crops are planted on site (Tyréns, 2020).

Based on a risk assessment study done by (Rundegren, 2019) the following animals are the 'species of concern' which may be most sensitive for bioaccumulation and biomagnification of DDT:

Predatory birds:

- Eurasian sparrowhawk *(Accipiter nisus)*
- Kestrel *(Falco tinnunculus)*
- Red kites *(Milvus milvus)*

Field animals:

- European badger *(Meles meles)*

- Common weasel *(Mustela nivalis)*

An important note is that Rundegren (2019) based their calculations on the conservative assumption that these animals are only feeding on earthworms and small animals found at Kolleberga, meaning that 100% of their food intake is from the contaminated site. In reality, however, predatory birds tend to hunt (feed) in a larger area, i.e. they will likely feed on areas that are not contaminated as well.

There is also a river near the site (Rönne å) and groundwater body (Ängelholm-Ljungbyhed). The studies showed that the levels of DDT in the surface and the ground water were very low and can be considered negligible as the levels of DDT are in the shallow surface of the soil and it's unlikely to leach into water bodies. Also, there is no current or likely future abstraction of drinking water from the river, so transfer of DDT through drinking water is not of primary concern (Tyréns, 2020).

3.3.2 Exposure pathways

The exposure pathways of DDT can occur through different routes such as, ingestion of soil, ingestion of plants, inhalation of dust, inhalation of vapors, dermal contact with soil, and/or through the food chain see Figure 9. While inhalation of dust and vapor are the dominant exposure pathways through the air for humans and animals, Kolleberga has DDT levels lower than the MKM (less sensitive land use). This implies that transport of DDT from the soil to the air through volatilization is very negligible and does not pose a major threat to the environment or humans.

Based on a risk assessment study done by (SGU, 2022) at a similar site to Kolleberga (Kårehogen skogsplantskola), the main exposure pathways of DDT to animals and humans are the ingestion of soil and the ingestion of plants, other routes had very little DDT exposure doses that pose no risk to the receptors. That's why in this study, only these two exposure routes will be studied. Figure 9 below illustrates all of the possible exposure pathways and their ecological protection targets.

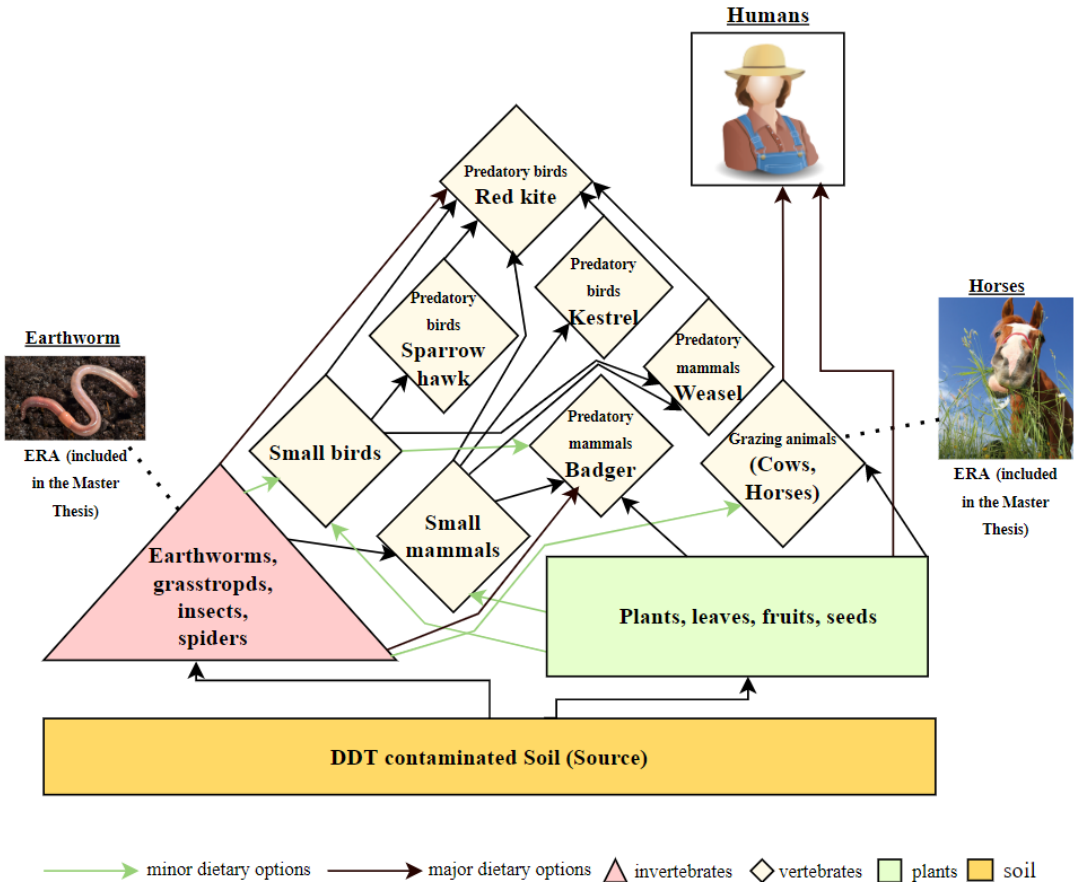

Figure 9: The generalized food web showing connections between the source and potential ecological targets at Kolleberga plant nursery based on Rundegren (2019). ERA in the figure indicates Ecological Risk Assessment.

3.4 The Kolleberga field experiment

3.4.1 Field experiment set-up

The field covers an area of approximately 22,000 m2 with an estimated contaminated soil layer of 0.3 m deep, the agricultural soil activities are assumed to be taking place within this depth. This means that the contaminated soil volume would be about 66,000 m^3. The cost of remediation with conventional options such as excavation would be very high, besides its impact on the environment by the emissions emitted through transportation (Tyréns, 2020). For this reason, GRO was suggested as an environmentally friendly method with lower cost for the site. Selection of potentially viable GRO was based on a risk management framework that was developed to find suitable GRO strategies to manage the risks at contaminated sites in Sweden, as shown in (

) below. This framework was a part of an ongoing study done by Chalmers for identifying suitable remediation options for contaminated sites, and then it was developed specifically for Kolleberga (Drenning, 2021).

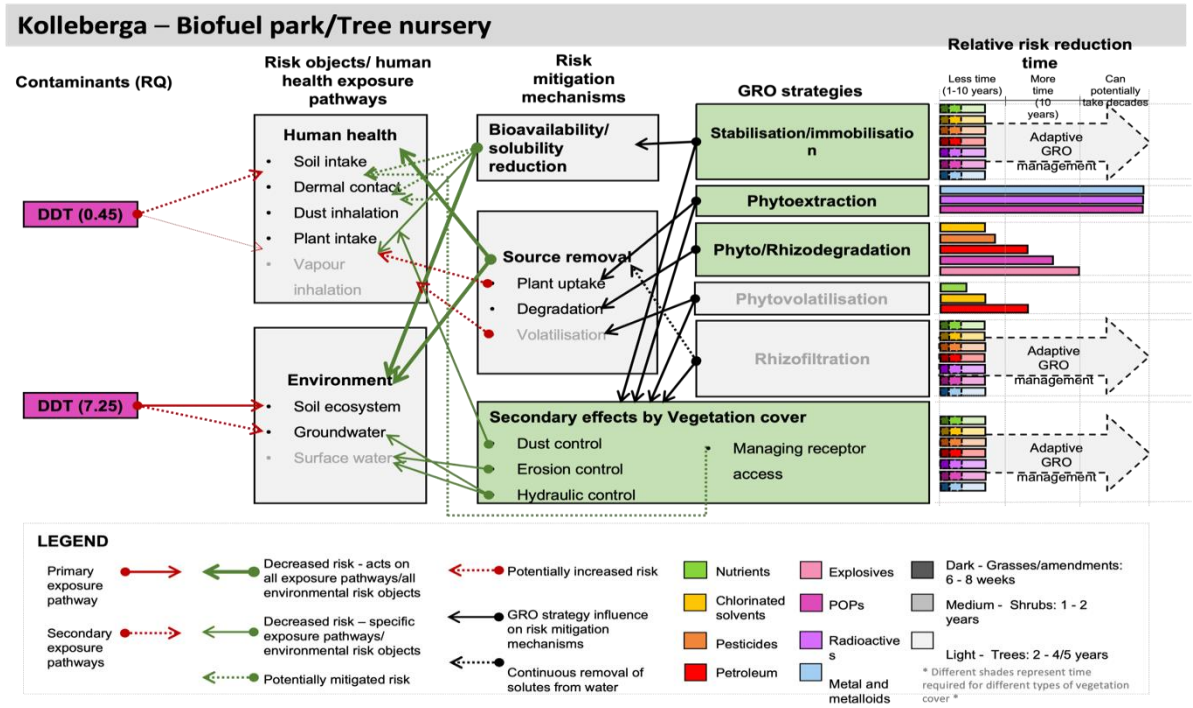

Figure 10: Site specific application of the GRO risk management framework for Kolleberga. The green boxes represent the GRO strategies and their expected risk mitigation mechanisms with their expected time (to the right). Each color represents a different contaminant with DDT being represented in the orange color as (pesticides) (Drenning, 2021).

As it can be seen from the figure, the GRO strategies of phytoextraction and phyto/rhizodegradation have the best risk mitigation mechanisms of source removal, and therefore have a direct effect of reducing the risk exposure pathway to both humans and the environment. While phytostabilization/immobilization can prevent further leaching or movement into soil and water. Phytovolatilization and Rhizofiltration have the lowest impact and therefore were excluded. Biochar was chosen as a soil amendment to be used separately or in combination with GRO for the quickest risk mitigation process, by immobilizing DDT particles and thus reducing its bioavailability (Drenning et al., 2022).

Below, are the steps that were carried out by the research team at Kolleberga school nursery for the experiment during the past three years:

- A transect of 50x5m was excavated to ca. 35cm depth below ground level (depth of contamination/plough depth) and moved to a soil pile (Figure 11).
- The soil pile was mixed to homogenize the soil and half was mixed with biochar at a 3%w/w ratio.
- 24 experimental plots of 2x2m and 35cm depth were dug in the trial area and a fiber cloth was put int the bottom to contain the soil and roots within the soil volume (Figure 12).
- The soil was randomly distributed into the plots – half with and half without biochar – corresponding to a randomized block design, i.e., in triplicate but separated into 3 blocks that contained each of the 8 treatments.
- The remaining soil in the pile and from digging the experimental plots was put back into the excavation area to restore the excavation.
- six different types of plants aimed for different phytoremediation strategies were established in the 24 plots. These include pumpkin, grass mix, legume mix, and salix.

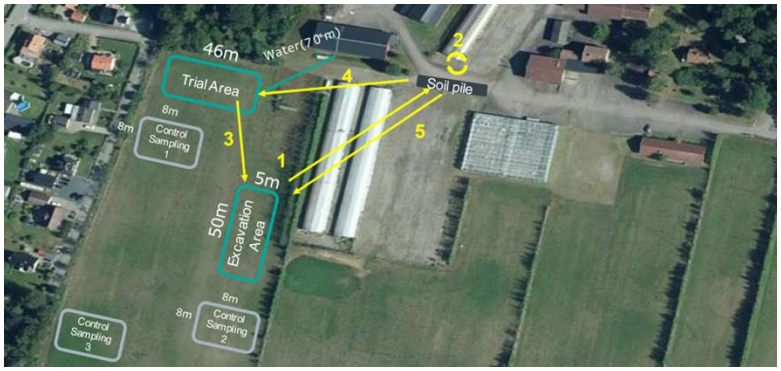

Figure 11: Layout of the pilot study in Kolleberga, photo credit to Paul Drenning.

Treatment	
1	Pumpkin
2	Pumpkin + biochar
3	Grass mix
4	Grass mix + biochar
5	Legume mix
6	Legume mix + biochar
7	Salix
8	Salix + biochar

Figure 12: Top view of the blocks design with different plants with/without biochar, top photo was taken in 2023 during the site visit by us and the bottom photo made by Paul Drenning.

3.4.2 Field experiment parameters

For the above-mentioned experimental plots, samples were collected at the beginning of the experiment (Start) and after the 2021 (Y1) and 2022 (Y2) growth season. Soil and plant analysis was performed by the labs of Orebro University and Eurofins. The soil and plant parameters which are considered in this study are presented in Table 4. The number of samples for each GRO strategy, soil parameters and years for which samples were taken are presented in Table 9 under Appendix A. Soil samples were also collected in the field as a control and a reference for soil quality to compare with the data measured in the experimental plots to assess the effects of GRO compared to field conditions. For Grass and Legume mix biomass was measured for stems or shoots of the plants from the plots, and for Pumpkin stems, roots, and leaves were measured. For Earthworms wet weights are measured. The term ΣDDT in this thesis means the summation of isomers of DDT and its metabolites- o,p-DDE, p,p-DDE, o,p-DDD, p,p-DDD, o,p-DDT & p,p-DDT, which indicates ΣDDT concentrations present in soil and plants used for the analysis to measure the effectiveness of GRO strategies. The data for these parameters are presented in Table 10, Table 11, Table 12, Table 13, and Table 14

Table 4: Sampled parameters and units that are used in the analysis.

Sampled parameters	Unit
Concentration of ΣDDT in the soil	µg DDT/kg DS
Concentration of ΣDDT in the pore water of the soil (POM)	ng DDT/L
Concentration of ΣDDT in earthworms' fatty tissue	ng DDT/kg WW
Concentration of ΣDDT in grass biomass	µg DDT /kg DS
Concentration of ΣDDT in legume biomass	µg DDT /kg DS
Concentration of ΣDDT in pumpkin biomass (roots, stems, leaves)	µg DDT /kg DS

3.4.3 Measurement of ΣDDT

The measurements that are analyzed to evaluate the effectiveness of different GRO strategies are discussed below.

Bioavailability of ΣDDT

The risks posed by hydrophobic organic contaminants (HOCs) such as DDT to biota in contaminated soils is best evaluated by measuring bioavailability (Wang et al., 2018). Hence assess the effectiveness of remediation treatments- phytostabilization and stabilization.

Bioavailability can be measured by two different parameters: accessible quantity and chemical activity (Cui, et al., 2013). For accurate prediction of changes in the bioavailability of DDT in the soil, the freely dissolved contaminant (by chemical activity) in the pore water could be measured using passive samplers such as POM (polyoxymethylene equilibrium biomimetic method) (Wang et al., 2018; Beckingham & Ghosh, 2013). POM aims at "mimicking" the uptake into various organisms and hence termed as "biomimetic" (Cui, et al., 2013). POM-

based samplers are also used for measuring the effect of activated carbon such as biochar on contaminant bioavailability (Denyes, et al., 2016). Another method to assess bioavailability for measuring the risks of DDT contamination is by measuring the bioaccumulation into earthworms (Wang et al., 2018, Kelsey & White, 2005, Peters et al., 2007).

Immobilization of ΣDDT

The phytostabilization or the immobilization effect of certain plants like grass and legumes can be measured by computing and comparing the uptake of ΣDDT concentrations in the biomass (Li et al., 2002; Hussain et al., 2009).

Bioaccumulation of ΣDDT

The phytoextraction capability of pumpkin can be measured by analyzing the uptake of ΣDDT concentrations by pumpkin plants. The transportation of lipophilic ΣDDT is slow within the plant, this reduces the transportation efficacy to the upper plant segments (Neitsch, et al., 2018). Due to this difference in the DDT uptake and translocation within the pumpkin plant segments varies (Whitfield Åslund et al, 2010), therefore extraction in different parts of pumpkins should be analyzed. Bioaccumulation Factors (BAF) indicates the degree to which a chemical substance accumulates or concentrates in the tissues of organisms, while translocation factor (TF) measures the ability of a substance to move from the roots to other plants tissues (Lunney, et al., 2010).

Degradation of DDT

The primary degradation products of DDT are formed by reductive dichlorination into DDD under anaerobic conditions and to DDE by dehydrochlorination under aerobic conditions (Ricking & Schwarzbauer, 2012; Garrison et al., 2000). Comparison between the amounts of DDT transformation products can give an insight into the degradation pathways and end products. Higher DDE: ΣDDT ratios through time indicate that the degradation is taking place, which is also similar for DDD: ΣDDT (Mendez et al., 2016) (Saldanha et al., 2010; Francesca et al., 2012: Kesic et al., 2021).

4 Methodology

This chapter explains the work that has been carried out throughout this master's thesis. It consisted of several steps that can be illustrated in Figure 13 below, these steps are described in more detail in the following sections.

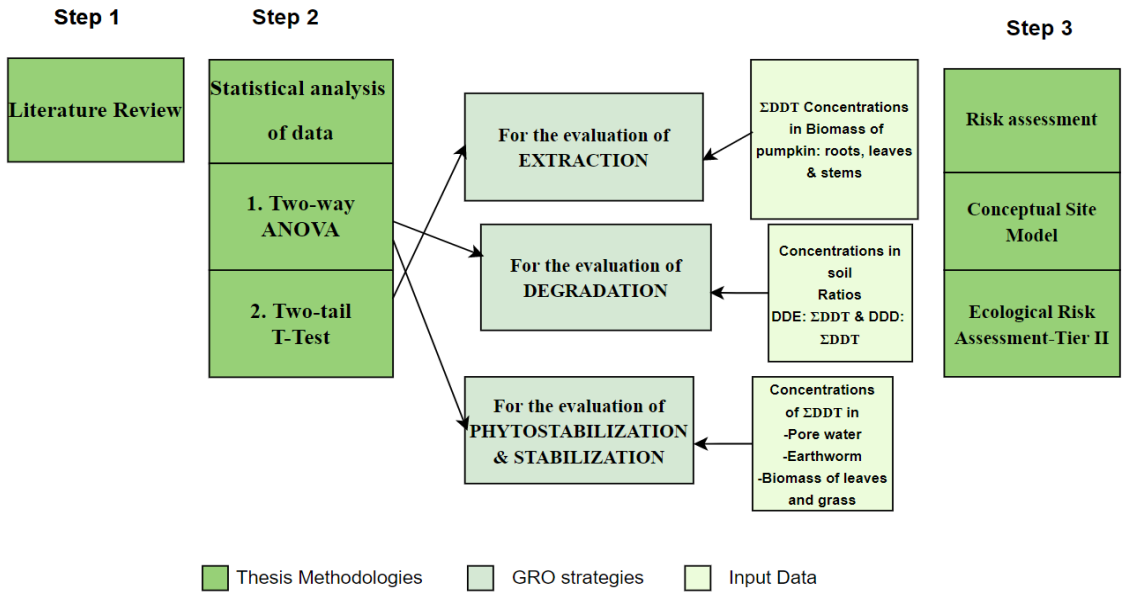

Figure 13: Representation of the workflow throughout the master thesis. Here in the figure, ΣDDT indicate sum of isomers of DDT concentration and tier II is the method used for the risk assessment.

In addition to the theoretical work, a field visit was carried out during May 2023. Photos from the field visit is included in Appendix C.

4.1 Literature Review

A detailed literature search using Chalmers Library, Google Scholar, and Scopus database was carried out as the first step of this master thesis. This was to understand the history of DDT, its potential health risks on both humans and the environment, its exposure pathway, and some GRO remediation that can reduce its risks. Afterwards, a desk study for Kolleberga site was carried out, this was to find any previous studies on the level of contamination on the site and to better understand the topography and the ecological receptors in order to be able to create a conceptual site model.

Some of the search strings that were used are:

- Phytoremediation AND DDT
- GRO AND DDT AND removal.
- Soil amendments AND DDT
- Phytoextraction AND DDT
- Phytostabilization AND DDT
- Phytodegradation AND DDT

- Biochar AND DDT
- Degradation AND DDT

4.2 Conceptual Site Model -CSM

After the literature search for Kolleberga, a conceptual site model for two future scenarios was developed. The first future scenario was developed to illustrate a (planned tree nursery) where different animals and humans are present on the site. While the second future scenario represents a horse-riding center where people and horses are planned to be living and grazing on the site. The purpose of creating these models was to represent the different transportation pathways of DDT and how the ecological receptors are exposed to them. CSMs are helpful in framing the questions required for further investigation, therefore, while creating the model, interpretations, assumptions, and hypotheses are made for the site conditions in order to lead the way for the planning of an investigation, risk assessment, remediation, and after remediation (ISO, 2019).

4.3 Statistical analysis

SPSS statistical software (SPSS Inc., Chicago, USA, version 29) was utilized for conducting statistical analysis to identify if there is significant impact of GRO on measured parameters in the field study. The study involves examining the effects of two independent variables- (a) amendment (with or without biochar) and (b) plant used for the treatment (pumpkin, grass mix, legumes, and willow/salix), across three distinct blocks. The aim was to investigate the impacts of these variables on the dependent variables. The dependent variables affected by the treatments include ΣDDT concentrations present in the soil, porewater, earthworms and in the biomass (pumpkin, grass, and legumes). The differences between treatments are assessed by using the analysis of variance (ANOVA) statistical test, which is where the variances are compared across the means (or average) of different groups.

An ANOVA requires 3 assumptions:

- Independent observations.
- Normality: the dependent variable must follow a normal distribution within each subpopulation.
- Homogeneity: the variance of the dependent variable must be equal over all subpopulations.

The data is tested for the above-mentioned assumptions. The normality assumption by applying Shapiro Wilk test. Levene's test was used to assess the homogeneity of variance. A significance level of $\alpha = 0.05$ for mean was used for all tests, and results were recorded. To compare the independent and dependent variables two-way ANOVA and two-tail t-test are used. The data used for the ANOVA through IBM SPSS and other statistical analysis input data are presented in Appendix A.

4.3.1 Two-way ANOVA (parametric test)

A two-way ANOVA is a statistical method used to estimate how the mean of a quantitative variable, i.e., dependent variable varies based on the levels of two categorical variables i.e., independent variables. In this study two-way ANOVA is conducted to analyze data for phytostabilization, stabilization and degradation. The stepwise procedure showing both test for normality and two-way ANOVA are presented in Figure 14.

To check the effect of one of the independent variables on the dependent variable and effect of interaction between the independent variables "the tests of between the Subjects effects" are conducted. The effect is considered significant if $p<0.05$.

To measure the effect of each plant type treatments on the uptake of ΣDDT in different dependent variables, a Tukey- post hoc test is conducted. But the results are only considered when the uptake in a GRO parameter for ΣDDT is significant for plant treatment (plant type) variable in this study, because it has more than 3 groups for comparison and hence eligible for Tukey test.

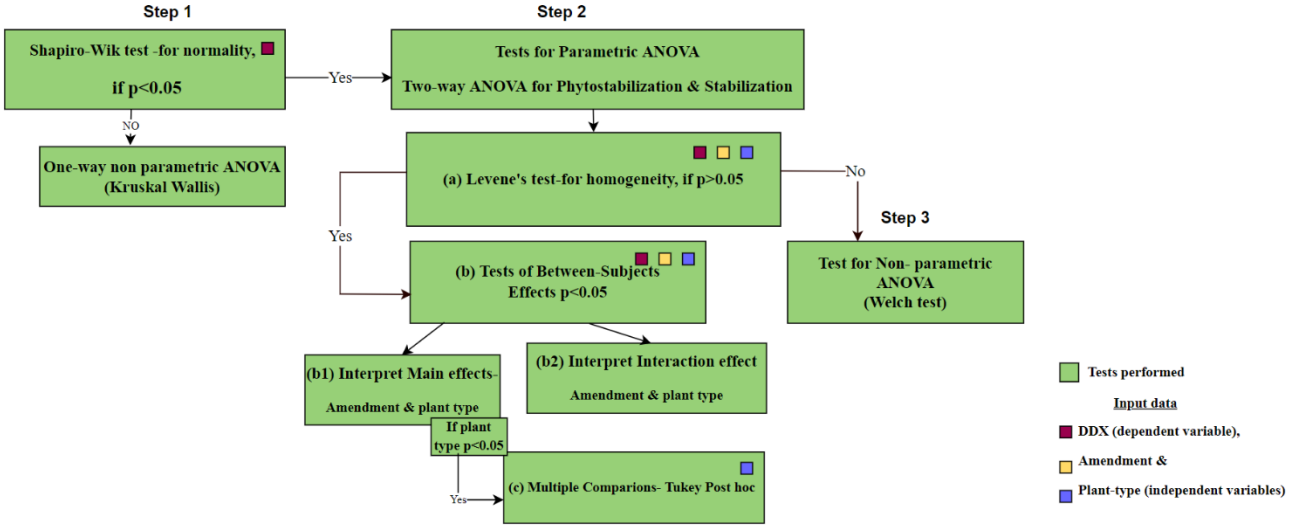

Figure 14: Stepwise procedure for Two-way ANOVA.

4.3.2 One-way ANOVA (non-parametric test)

If the data fails the test for normality (Shapiro Wilk test), then the data is tested for non-parametric one-way ANOVA, which does not assume that the data is normally distributed. non-parametric ANOVA tests used in this study are the Kruskal-Wallis test, Welch test and Mann-Whitney U test. "Yes" in the Figure 14, indicates that the data is normally distributed. Similarly, "Yes" for (a)- Levene's test indicates that the data is homogenous.

When the data fails for normality test, Kruskal-Wallis test is used to analyze the data for phytostabilization, stabilization and degradation. Likewise, if the data fails for homogeneity, then Welch test is conducted. The usage and application of these tests is presented in Figure 14 and Figure 15.

4.3.3 Independent Samples T-test (parametric test)

An independent samples t-test examines if two populations have equal means on some quantitative variable. In this study to measure the effectiveness of phytoextraction, independent samples t-test is conducted. The stepwise procedure followed to conduct the tests is presented in Figure 15.

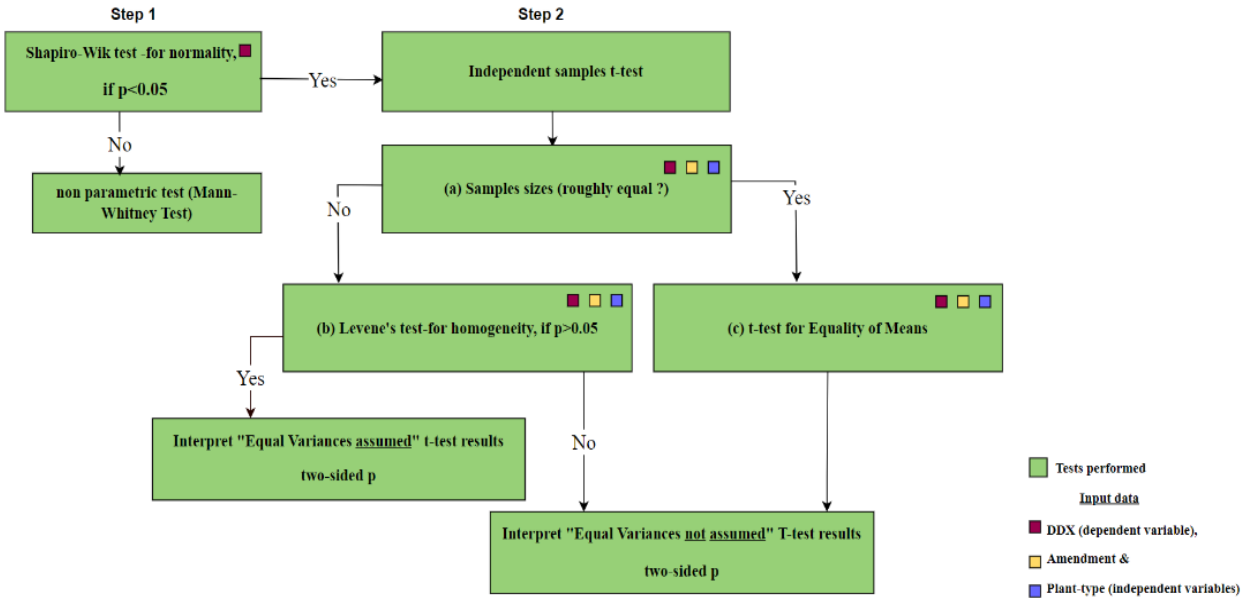

Figure 15: Stepwise procedure for conducting Independent Samples T-test.

When the data fails for the normality test, then the data is tested using a non-parametric test, a test alternative for the independent samples t-test called Mann-Whitney U test to analyze phytoextraction data, that is not normally distributed.

The Levene's test is conducted if the samples sizes for both of the dependent variables is roughly equal, then the adjusted results from the t-test for equality of means is reported as shown in Figure 15.

To complement the statistical analysis, bioaccumulation factor (BAF) and translocation factors (TF) that measures the efficiency and effectiveness of pumpkin plants to absorb and accumulate DDT, are calculated using the following formulas:

$$BAF = (\text{Concentration of } \Sigma DDT \text{ in plant part}) / (\text{DDT concentration in soil}) \quad \text{Equation 1}$$

$$TF = BAF_{shoot} / BAF_{root} \quad \text{Equation 2}$$

These factors are measured to understand the relative uptake in the pumpkin parts- roots, stem & leaves.

4.4 Ecological Risk Assessment

Ecological Risk assessment is a tool of organizing and analyzing the data with their probabilities (likelihood of occurrence) of an adverse ecological effect as a result of exposure to human activities stressors. These stressors can be chemical, physical, or biological (Hope, 2006). In this thesis, ecological risk assessment study was conducted in order to assess the possibilities of DDT exposure on two risk assessments scenarios (future land uses), one for the current land use where the site is kept as a forest nursery site and a second scenario suggested earlier by the Swedish Geological Survey (SGU, 2022) where the site can be converted into a horse-riding center, where horses and owners are staying on the site with occasional visitors and riders.

A common method to evaluate whether the exposure is higher than the effect is by calculating a *risk ratio*, which is the estimated exposure dose divided by the estimated no-effect level. The estimated no-effect level can be a concentration at which no effects are expected or a tolerable daily intake (TDI) for humans or toxicological reference value (TRV) for animals. The acceptable risk ratio for humans is set to be 0.5 based on the risk assessment report done by (SGU, 2022), this is because they assumed that 50% of DDT can come from other sources than the exposure routes, while for horses the risk ratio is considered 1 as the exposure of DDT for them can occur only from the contaminated area (SGU, 2022).

However, for this thesis, the human health risk assessment and its sensitivity analysis was not carried out as this was already investigated by other stakeholders (Tyréns AB and the SGU). Both of them concluded that the DDT levels pose a negligible risk to humans for both future land use scenarios. Therefore, only ecological risk assessment was carried out for two possible exposure routes which are the ingestion of soil (by horses or grazing animals) and the ingestion of plants (as horses tend to eat a large amount of food growing on the site). Other exposure routes for horses such as dermal contact, ingestion of ground water, and inhalation of dust might not be relevant for this particular study, or it is unlikely to change from the previous studies.

The ecological risk assessment process was based on Tier II of the international standard ISO-19204 for soil quality procedure (ISO 19204, 2017). This is because it gives a more accurate assessment (a refined screening) to the results rather than just depending on Tier I which uses only the concentration of DDT in the soil. Tier II includes the measurements of chemical, toxicological, and ecological endpoints.

4.4.1 Exposure equations

The risk assessment study for the first future scenario (plant tree nursery) was conducted qualitatively rather than quantitively. The risk level was estimated based on the available data obtained from laboratory analysis of the ΣDDT uptake in earthworms. In terms of risk management, it was assumed that the decrease in the DDT uptake would decrease the possibility for secondary poisoning to other field or predatory animals.

For the second future scenario (horse riding center), the exposure doses of DDT for horses were calculated quantitively based on exposure equations obtained from (SGU, 2022). Based on previous studies at Kolleberga and other contaminated sites with DDT with low to moderate contamination, the ingestion of soil and the ingestion of plants have the highest risk levels.

- Exposure equations for ingestion of soil:

$$D\ soil = (C\ soil \times IR\ soil \times BA \times Exp.A \times ET1 \times ET3) \qquad \textit{Equation 31}$$

$$RR\ soil = \frac{D\ soil}{TRV} \qquad \textit{Equation 42}$$

Where:

D_{soil}	*Dose of DDT via ingestion of soil (mg DDT/kg body weight, day)*
C_{soil}	*Average exposure concentration in soil (mg DDT/kg TS soil), site-specific*
IR_{soil}	*Intake rate of soil for horses (kg TS soil/kg body weight, day)*
BA	*Bioavailability of DDT (%)*
Exp A	*Exposure area (m^2 /m^2)*
ET1	*Exposure time daily outdoors (hours/24 hours)*
ET3.	*Annual exposure time (months/12 months)*
TRV	*Toxicological reference value (mg/kg body weight, day)- lifetime integrated*
RR_{soil}	*Risk ratio via ingestion of soil*

- Exposure equations for ingestion of plants:

$$D\ plants = (C\ plants \times\ IR\ plants \times\ BA\ \times\ F\ plants \times\ ET3) \qquad \textit{Equation 53}$$

$$RR\ plants = \frac{D\ plants}{TRV} \qquad \textit{Equation 64}$$

Where:

D_{plant}	*Dose of DDT via ingestion of plants (mg DDT/kg body weight, day)*
C_{plants}	*Average exposure concentration in plants (mg DDT/kg TS plants), site-specific*
IR_{plants}	*Intake rate of plants for horses (kg TS plants/kg body weight, day)*
BA	*Bioavailability of DDT (%)*
F_{plants}	*Percentage of plants coming from DDT-contaminated areas (%)*
ET3	*Annual exposure time (months/12 months)*
RR_{plants}	*Risk ratio via ingestion of plants*

4.4.2 Input data for Ecological Risk Assessment

The input data for the exposure equations (3,4,5, and 6) were retrieved from (SGU 2022), while changing some parameters (as they are site-specific) according to Kolleberga data, these parameters are highlighted in green in Table 5 and Table 6 below. An average concentration of (10 mg DDT/kg TS) was used for DDT concentration in soil *(C_{soil})* to calculate the exposure dose via ingestion of soil and an average exposure concentration of (0.095 mg DDT/kg TS plant) were used to represent (C_{plant}) as an average concentration of DDT in grass for the years 2021 and 2022. Further details can be seen in (table 11 Appendix A).

Table 5: Input data for the exposure equation of ingestion of soil in horses retrieved from (SGU, 2022), the green box represents site-specific Kolleberga data.

Ingestion of soil					
Parameter	unit	value			
		Stable season	Pre-season	high summer	late summer
C soil	mg DDT/kg TS soil	10	10	10	10
IR soil	kg TS soil/kg body weight, day	0,002	0,002	0,002	0,001
BA	%	100%	100%	100%	100%
Exp.A	m2/m2	100%	100%	100%	100%
ET1	hr/24 hr	50%	50%	100%	100%
ET3	month/ 12 month	50%	25%	8%	17%
TRV	mg DDT/kg body weight, day	0,147	0,147	0,147	0,147

Table 6: Input data for the exposure equation of ingestion of plants in horses retrieved from (SGU, 2022), the green boxes represent site-specific Kolleberga data.

Ingestion of plants					
Parameter	unit	value			
		Stable season	Pre-season	high summer	late summer
C plants	mg DDT/kg TS plant	0,095	0,095	0,095	0,095
IR plants	kg TS plants/kg body weight, day	0,03	0,03	0,02	0,02
BA	%	100%	100%	100%	100%
F plants	%	0%	100%	50%	100%
ET3	month/ 12 month	50%	25%	8%	17%
TRV	mg DDT/kg body weight, day	0,147	0,147	0,147	0,147
Exposure dose via ingestion of plants	mg DDT/kg body weight, day	0	0,0007	0,0001	0,0003
Risk ratio via the ingestion of plants		0,000	0,005	0,001	0,002

5 Results and Discussion

5.1 Statistical analysis and Effectiveness of GRO treatments

A summary of the results is presented in Table 7 and Table 8 below. The results are presented and discussed in more detail in the following sections.

*Table 7: Summary of ANOVA results performed on the parameters of phytostabilization. Note: significant parameters are marked in green and highly significant factors are marked with "**".*

		One-Way ANOVA (non-parametric ANOVA)	Two-Way ANOVA (parametric ANOVA)	
GRO Strategy	**Parameter**	**Kruskal Wallis test**	**Tests of Between-Subjects Effects (p<0.05)**	
			Main effect	**Interaction effect**
Stabilization	ΣDDT concentration in pore water of the soil (POM)	Biochar, p<0.01** (2022) plant type, p=0.875 (2022)	Biochar, p=0.029 (start)	p=0.083
	ΣDDT Uptake in Grass		Biochar, p= 0.173	
	ΣDDT Uptake in Legume	Biochar, p= 0.522		
	ΣDDT Uptake in Earthworms fat		Biochar, p=0.043 plant type, p=0.465	p=0.542
Degradation	DDD: DDT	Biochar, p=0.64 plant type, p=0.105		p=0.5
	DDE: DDT		Biochar, p=0.75 plant type, p=0.051	p=0.62

Table 8: Summary of ANOVA performed for uptake of DDT by pumpkin biomass – results of comparisons between groups with/without biochar. Note: Significant parameters are marked in green.

		Non-parametric test	Independent Samples T-test
GRO Strategy	**DDT uptake in pumpkin**	**Mann-Whitney U test**	**T-test for equality of means (p<0.05)**
Extraction	1. Stem 2. Roots 3. Leaves	0.2 0.128	0.01

5.1.1 Stabilization (biochar and plants)

DDT concentrations in the pore water:

A Two-Way ANOVA was performed to evaluate the effectiveness of stabilization (biochar addition) and Phytostabilization (plant treatments) on ΣDDT concentrations in pore water for the start and the 2022 data. The results indicated that the difference in porewater concentrations is significant when adding a biochar amendment ($p=0.029$) for start data and highly significant ($p<0.001$) for 2022 data, not significant for plant treatments ($p=0.371$), and not significant for the interaction between the biochar amendment and plant treatments ($p=0.083$). The results are summarized in Table 7. The output results from the SPSS are presented in Table 18 and Table 19, Appendix A2.

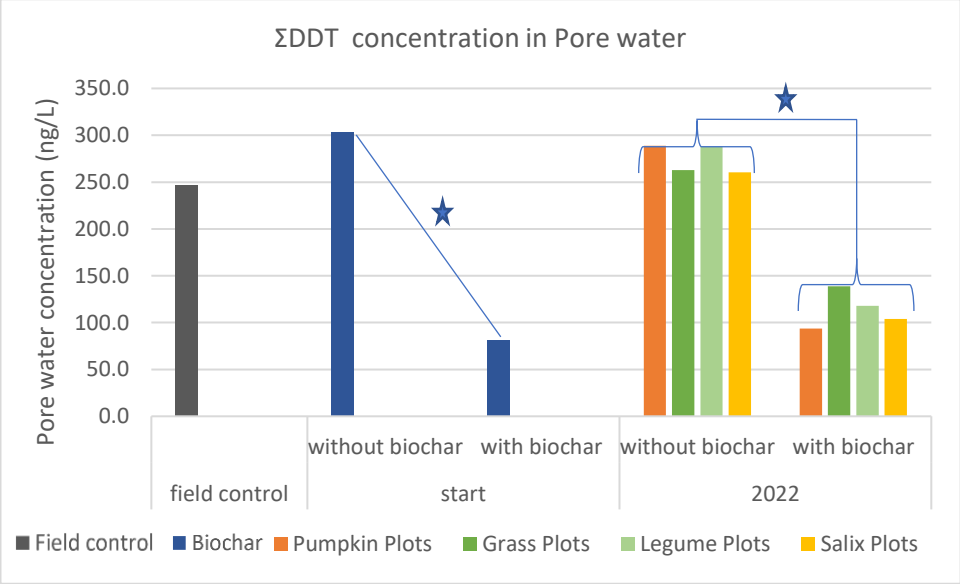

Figure 16: ΣDDT concentration in the pore water, n=3. (Star indicates that the comparison is significant, i.e., for biochar.).

Figure 16 above illustrates the comparison of ΣDDT concentration in porewater from different soil samples taken from the treatment plots and the field control. Porewater concentrations between groups at the start of the experiment (Field, Plots with or without biochar) and after the second year (2022) are compared to evaluate the effectiveness of aided-phytostabilization. As shown in Table 7, there is a statistically significant effect with the addition of biochar ($p=0.029$). At the start, there is an 'instant effect' to reduce porewater concentrations with biochar addition to plots with a reduction by 73.26%. Similarly, there is a reduction in the ΣDDT concentrations observed in the 2022 data (by 47 to 67%). However, there are only minor differences in DDT porewater concentrations due to the plant treatments (0.2 to 9%), which are found to be insignificant. The natural variation in the DDT concentrations in soil samples could be a reason for these minor differences.

From this evaluation it can be concluded that biochar is more effective in reducing the DDT concentrations in the pore water, thus bioavailability of the DDT. In a previous study, it has been observed that biochar does not significantly reduce uptake of DDT by pumpkin and the uptake is not well predicted using POM; however, the porewater concentration as measured using POM is a good proxy for bioavailability and correlates with DDT uptake in earthworms (Denyes et al., 2016).

DDT uptake in the grass and legume mix:

Figure 17 below illustrates DDT uptake in grass and legume during the years 2021 and 2022 with/without the addition of biochar.

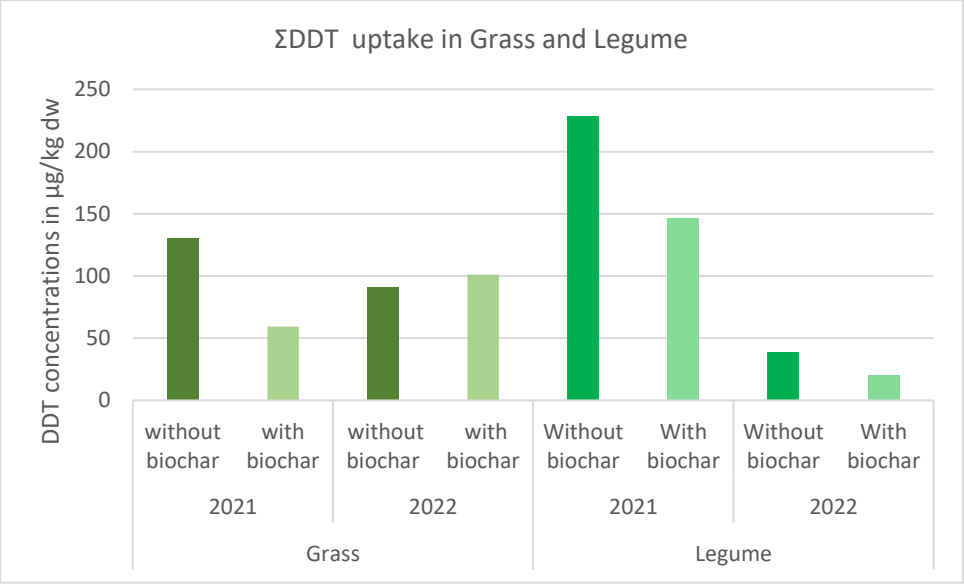

Figure 17: ΣDDT (mean values) uptake in- grass and legume, n=3.

According to two-way ANOVA, the difference in uptake of DDT is not significant between legume (p=0.522) and grass (p=0.173), (

Figure 17, Table 23 and Table 22 in Appendix A2). According to (Lunney et al., 2004), both grass and legume plants are considered stabilizing species due to their ability to extract DDT in their large, fibrous roots while being unable to translocate them into their shoots as pumpkin, which indicates that these plants are stabilizing species rather than accumulators. In the Kolleberga field experiment, DDT uptake was only measured in the plants shoots and there are no data for DDT uptake in the roots, therefore, it is not possible to see if the plants accumulate any DDT in their roots thus providing a phytostabilization effect. However, the uptake of DDT into the aboveground biomass of grass and legumes is very low, which could indicate that they may still be suitable for growing at Kolleberga to mitigate exposure to grazing animals.

DDT uptake in the earthworms:

A Two-Way ANOVA was performed to evaluate the effectiveness of stabilization (biochar addition) and Phytostabilization (plant treatments) on ΣDDT concentrations (uptake) in earthworm fat for the year 2, i.e., 2022 data. The results indicated that the difference in DDT concentrations in earthworms is significant when adding a biochar amendment (p=0.043), not significant for plant treatments (p=0.465), and not significant for the interaction between the biochar amendment and plant treatments (p=0.542). The output results from the SPSS are presented in Table 21 in Appendix A2.

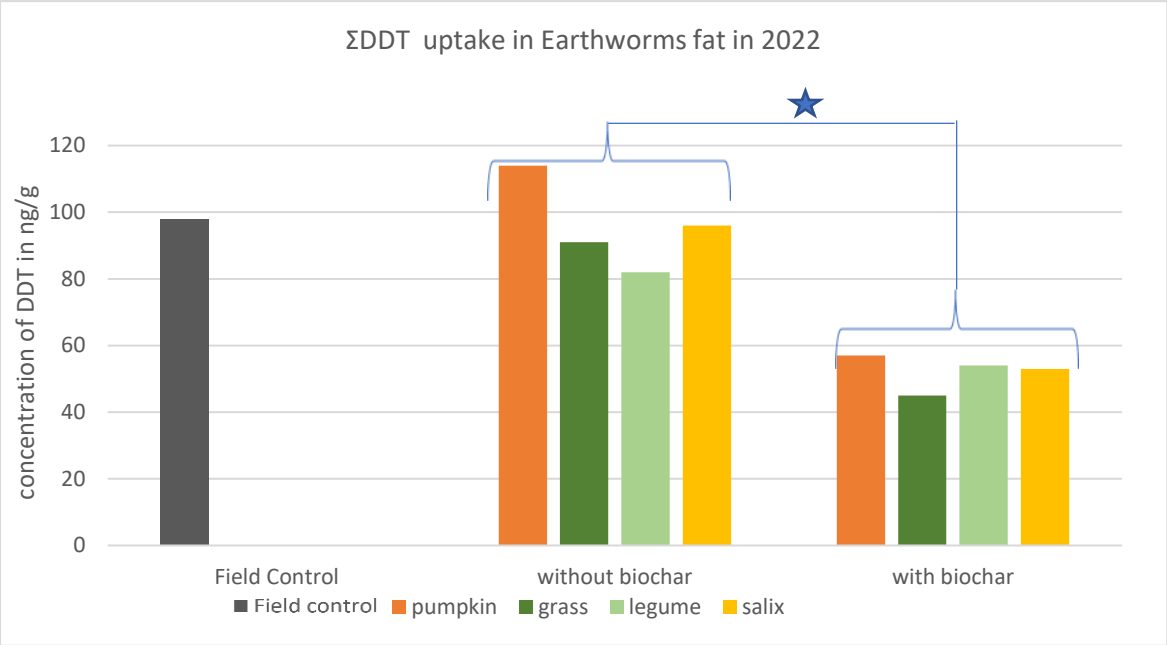

Figure 18: ΣDDT uptake (mean values) in earthworms in 2022, n=3. (Star indicates a significance in SPSS)

Figure 18 above illustrates the comparison of average concentration of ΣDDT in the earthworms taken from different soil samples from the treatment plots and field control. A good reduction can be observed in the concentrations of DDT with the biochar plots compared to the plant's plots (40- 58%). This reduction might be due to the ability of biochar to immobilize ΣDDT particles (as shown in

Figure 18 for the porewater concentrations). This aligns well with previous studies, where it has been observed that addition of biochar at 0.2-2% w/w could reduce the bioavailability of DDT by 83.9-99.4% (Wang et al., 2018) and 49% (Denyes et al., 2016).

5.1.2 Extraction

DDT uptake in pumpkin:

Phytoextraction effectiveness can be demonstrated by calculating the bioaccumulation factors (BAF) and translocation factors (TF), using Equation 1 and 2 (section 4.3.3). As mentioned previously, if the BAF is higher than 1, it indicates that the accumulation in the pumpkin parts is greater than the concentration in the soil (USEPA, 2003). When biochar was added to the soil, the BAF for pumpkin plant parts (roots, leaves and stems) was found to be <1. Conversely, when biochar is not added to the soil the BAF<1 for leaves and stems, but >1 for roots (see Table 15, Appendix A1). This suggests that there is a decrease in the bioaccumulation of DDT as a result of the application of biochar in the roots, while there is no impact on leaves and stems. However, the (TF) were found to be less than 1 with/without biochar, meaning that DDT particles were not easily translocated from roots to the stem. Figure 19 below shows the ΣDDT uptake in different parts of the pumpkin during the two years experiment 2021 and 2022.

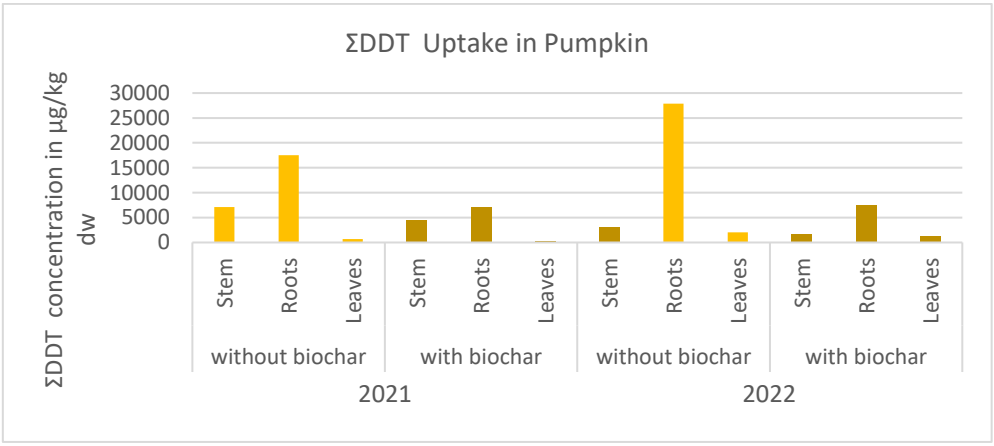

Figure 19: DDT uptake in different parts of the pumpkin, n=3.

The difference in DDT uptake with/without the addition of biochar was found to be insignificant for all the plant parts (stems, p=0.2 & leaves, p=0.128) except for the roots, p=0.01 (Table 29) and (Table 28). Figure 19

Figure 19: DDT uptake in different parts of the pumpkin, n=3. shows that there is a large decrease in the uptake in the roots in the plots where biochar is added in each year (2021 &2022) which is likely due to the biochar's ability of reducing the bioavailability of ΣDDT. The pumpkin harvest for 2022 is not representative of the true phytoextraction potential as indicated in Figure 19 because of the loss of the biomass due to the slug infestation which consumed the leaves, flowers, and stems thereby greatly inhibiting aboveground biomass production. In any case, the measured BAF of pumpkin at Kolleberga is low which indicates that phytoextraction may be not effective to remove DDT.

For comparison of DDT uptake between plants, Figure 20 below represent DDT uptake in pumpkin, grass, and legume. As it could be seen pumpkin have the highest uptake of DDT compared to grass and legume which gives an indication of which crop is safer to plant at the site as animal food for the future scenario of horse-riding center or other grazing animals. In

this case, grass has the lowest DDT uptake in the above ground parts (stems) which makes it a safe option for animals' consumption.

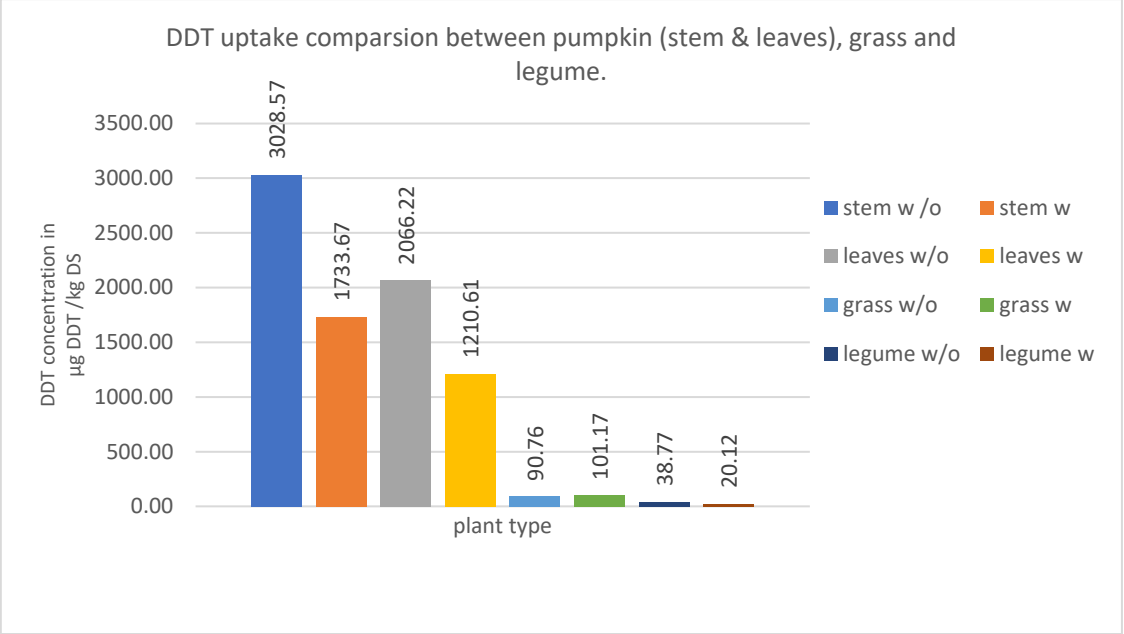

Figure 20: ΣDDT uptake comparison between parts of Pumpkin (stems and leaves), grass, and legume, n=3. Note: w/o and w in the figure represent without biochar and with biochar respectively.

5.1.3 Degradation

As shown in Figure 21 below, there is a slight increase in the degradation ratios (DDD & DDE to ΣDDT) indicating that biological degradation is occurring, and the addition of biochar may positively impact/increase the rate of degradation. However, the degradation process of DDT still takes a long time. DDT is found to be recalcitrant to degradation and has a half-life of 4-30 years (Li, et al., 2010). Where biochar is added, there is an increase of 6 to 16% in the DDD: ΣDDT ratio and 0.2 to 6% increase in the DDE: ΣDDT ratio.

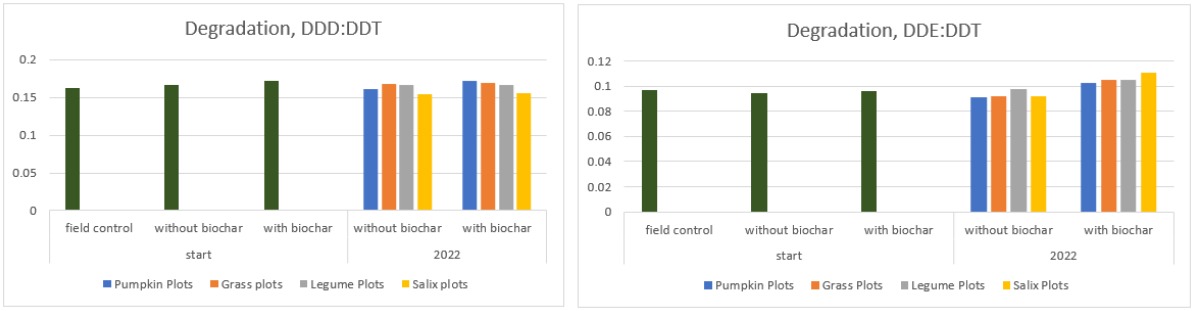

Figure 21: Degradation ratios of DDT metabolites, n=3.

According to a two-way ANOVA, the difference in degradation ratios were found to be insignificant for both DDD: ΣDDT (biochar, p=0.64 and plant treatments, p=0.105) and DDE: ΣDDT (biochar, p=0.75 and plant treatments, p=0.051). Investigating the effects of plants further, no plants were found to be significant although Legume and Salix were just above the significance threshold (p=0.06). According to Elliot et al (1994), a ratio (DDE: DDT) lower than 0.2 indicates that the degradation of DDT in soil may have been inhibited. The average ratios in this study range 0.12- when biochar is not added and 0.13 when biochar is added for DDD: ΣDDT, similarly for DDE: ΣDDT, 0.072 when biochar is not added and 0.081 when biochar is added, thus this again implies that there is no effect of biochar or plant treatments on degradation of ΣDDT and the degradation is very low.

The ability of biochar to stimulate microbial activity to enhance microbial degradation has been reported by Gregory et al; (2015). Therefore, the slight increase in the ratios could be attributed as an effect of increased microbial activity. Many studies have reported that different types of fungi such as *Pleaurotus, Hypholoma* and others can be highly effective to biologically degrade DDT in historically contaminated soil, a technique commonly referred to as mycoremediation (Purnomo et al., 2011). For Kolleberga, considering that the degradation rates are currently very slow and only slightly improved by biochar or plants, the incorporation of DDT degrading fungi is worth exploring.

5.2 Ecological risk assessment

This section presents the conceptual site model developed for the two future land uses at Kolleberga, with an assessment of the ecological risks and the implications for risk management using GRO.

5.2.1 Ecological risk assessment for the planned tree nursery

To further understand this future land use, the following conceptual site model was developed to show the protection targets (ecological receptors) and the possible exposure pathways in Figure 22 below.

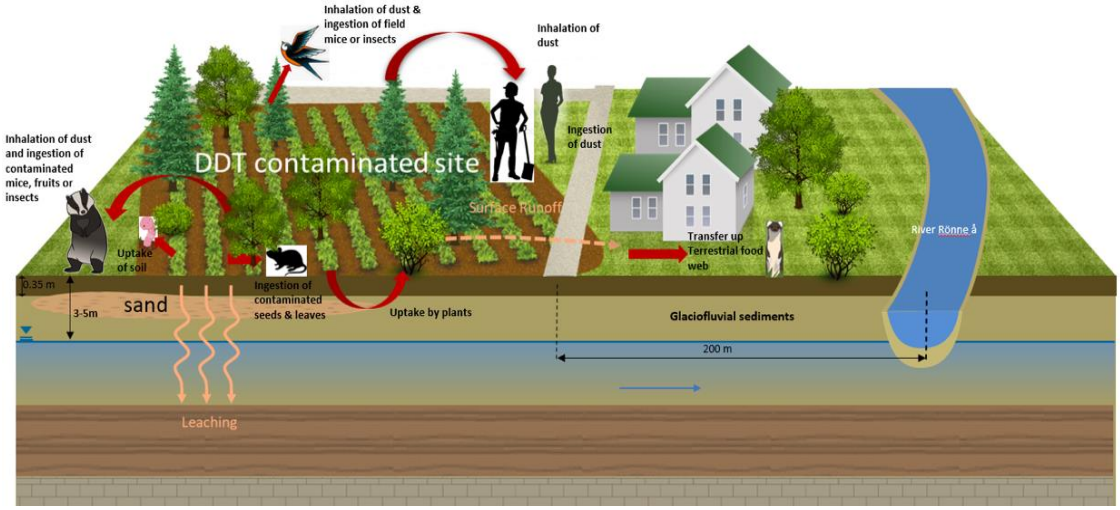

Figure 22: Conceptual model for the site Kolleberga tree nursery, showing the exposure pathways and receptors involved.

As shown in the CSM above and previously discussed, the primary risk in this land use is the uptake of DDT by the earthworms and upwards transfer via the food chain (secondary poisoning) to animals such as filed mice and birds who feed on worms. The results of the statistical analysis presented in Figure 22 above show that the addition of biochar achieved a significant reduction of 30-41% in earthworms by stabilization. This is due to the ability of biochar to stabilize DDT in soil which reduces its bioavailability and bioaccessibility to earthworms. This reduction in pore water concentration and thus uptake in earthworms gives an indication of reducing the possibility of secondary poisoning to both the birds and mammals that have the highest chances of risk and thus mitigate the risks of DDT exposure to other animals, especially if they feed only at Kolleberga. This successful result was also discussed by (Wang et al., 2018), where they found that the addition of (0.2 – 2%) biochar to contaminated soil with DDT can reduce the earthworm's bioaccumulation by (83.9-99.4%) and as mentioned before, (Denyes et al., 2016) showed that the addition of 2.8% of granulated activated carbon was able to reduce the bioaccumulation of DDT in earthworms by 49% using the same passive sampler method (POM) as the method used at Kolleberga. However, the qualitative method used for this scenario for evaluating the risk, might not give accurate results as it only analyses one parameter for a number of different species. Therefore, more future studies are needed such as calculating the exposure doses for each animal and conducting a sensitivity analysis to further analyse the level of exposure.

5.2.2 Ecological risk assessment for the horse operation

Based on the information that was found in previous studies at Kolleberga and other contaminated sites with DDT, the following conceptual site model has been developed to illustrate the ecological receptors (humans and horses) and their exposure routes of DDT in Figure 23 below.

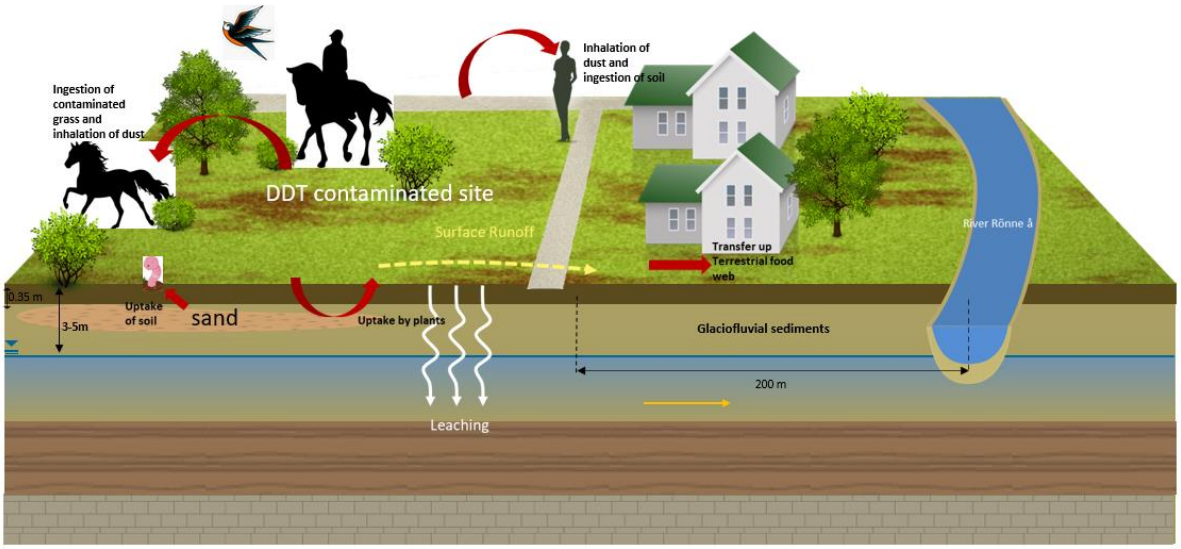

Figure 23: Conceptual model for the site Kolleberga tree nursery, showing the exposure pathways and receptors involved, future land use as a pastureland for horse grazing.

As it can be seen in the above figure, there are many potential receptors present at the site (predators, mammals, and humans). However, due to time limitations only horses were studied. By using the exposure equations (eq. 3 and eq. 5 in section 4.4.1), the calculated exposure doses for horses are illustrated in (

Figure 24) below (for more details see table 30 and 31, Appendix B).

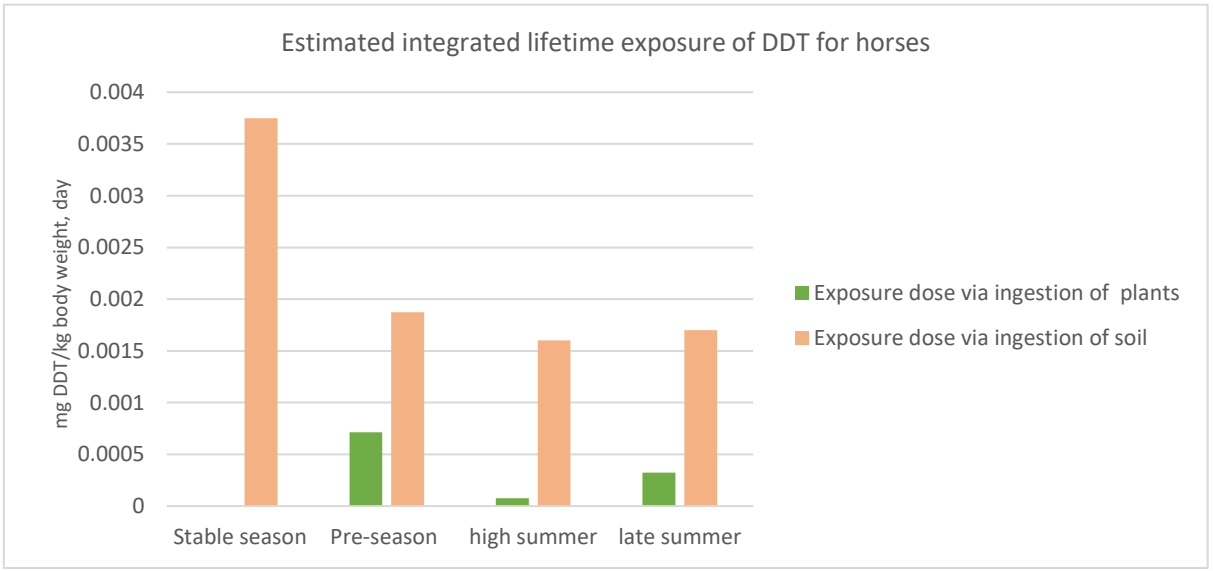

Figure 24: Estimated exposure doses of DDT for horses during different seasons for the year 2022.

As shown in Figure 25, the calculated dose of DDT from the ingestion of soil is much higher than the calculated dose via the ingestion of plants, where a total of 89% of the dose comes from the ingestion of soil and only 11% from the ingestion of plants. However, they are both significantly lower than the toxicological reference value of (0.147 mg DDT/ kg TS) used by (SGU, 2022).

The calculated risk ratios (Equation 4 and 6) for the two exposure pathways, are shown in Figure 25 below. Again, the calculated risk ratio is much lower than the acceptable risk value of 1 and therefore, the risk levels can be considered 'acceptable' for horses for the two exposure pathways.

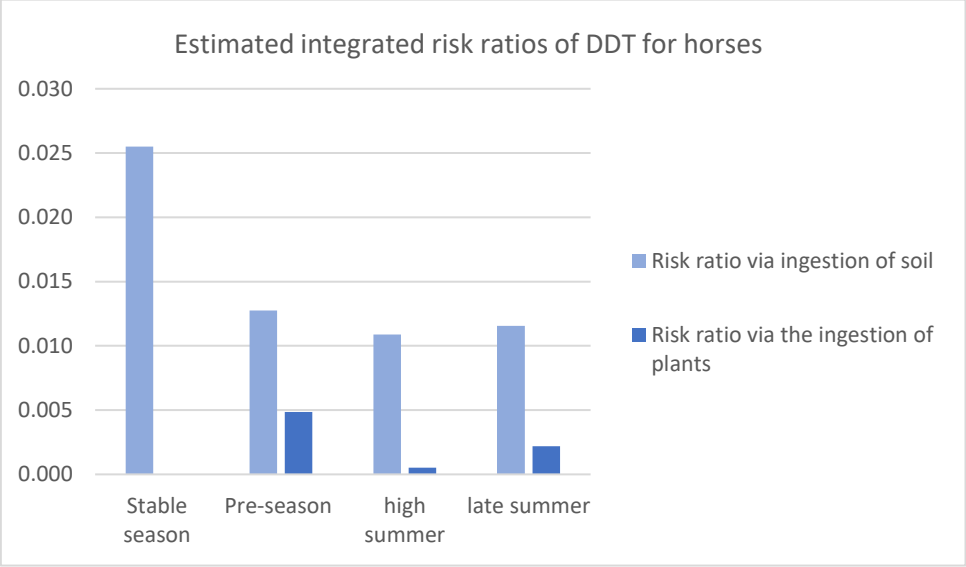

Figure 25: Calculated risk ratios for horses for the two exposure pathways

A comparison between the calculated risk ratios from the Kolleberga experiment with the risk ratios for Kårehogen (SGU, 2022) for the same exposure pathways is shown in Figure 26 below.

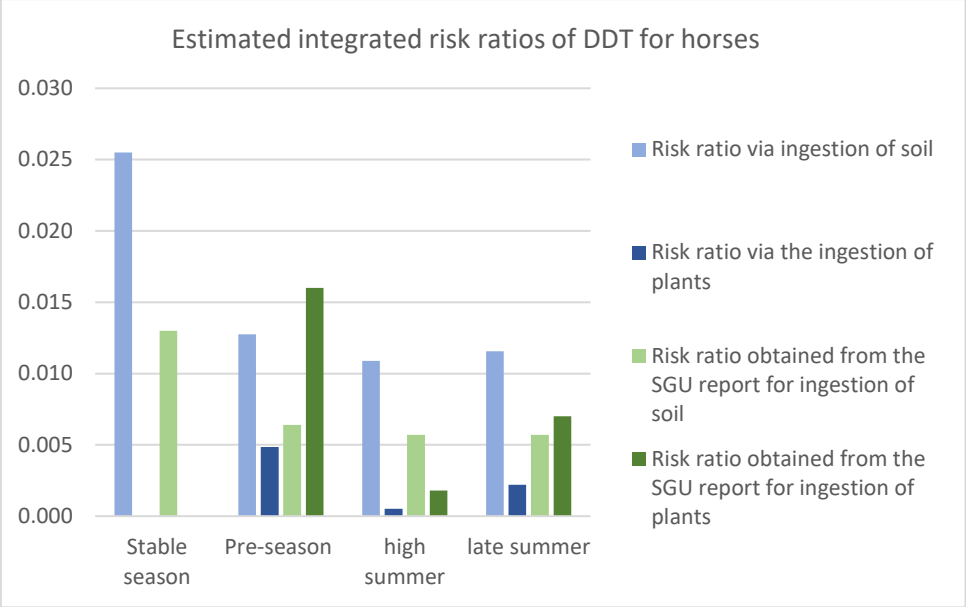

Figure 26: Comparison of the calculated risk ratios between the results from Kolleberga (blue) and SGU's results from Kårehogen (green) (SGU, 2022).

The comparison indicates that Kolleberga has a higher risk ratio from the ingestion of soil but a lower risk ratio from the ingestion of plants than Kårehogen. This difference is due to the higher exposure concentration of soil of (10 mg DDT/kg body weight, day) used in Kolleberga exposure equations compared to the (5 mg DDT/kg body weight, day) used in Kårehogen (SGU, 2022). Also, the average uptake of DDT in the grass (C_{plants}) of (0.095 mg DDT/kg TS plant) is also a lot lower than Kårehogen value of (0.31 mg DDT/kg TS plant) at Kårehogen. These two parameters have the greatest impact on the calculations.

It should also be mentioned that SGU (SGU, 2022) has also assumed the bioavailability of DDT in the ingestion of plants (BA) is 100% due to 'lack of data', which means that the horses will absorb 100% of the exposed DDT. This is a conservative estimate which likely overestimates the amount of DDT that gets absorbed into the bloodstream. The physicochemical and physiological process in the gastrointestinal tract may limit or reduce the amount of DDT that gets absorbed, which means that the oral bioavailability of DDT is less than 100% via direct intake of contaminated soil (Smith et al., 2012). Indicating that the risk levels can be/are likely to be lower than the calculated values at both sites.

6 Conclusion and recommendations

6.1 Conclusions

The results of this master's thesis indicate that of the three GRO strategies tested at Kolleberga (aided-phytostabilization, phytoextraction, and phytodegradation), aided-phytostabilization is the most effective strategy to manage the risks from DDT contamination due to in large part to the addition of biochar. According to the analysis, biochar provides significant reductions to DDT bioavailability which results in decreased concentrations in soil porewater, uptake into earthworms, and uptake into various plants parts, and thus effective risk mitigation. The main conclusions for the three separate GRO strategies at Kolleberga can be summarized as follows:

(Aided-)Phytostabilization or stabilization by soil amendments based on the measurements of ΣDDT concentration in porewater, uptake of ΣDDT in earthworms, and ΣDDT uptake in both grass and legume mix:

- ⇒ There is a considerable reduction of 47 to 67% of ΣDDT in the pore water when biochar was added.
- ⇒ 40-58% reduction in the uptake of DDT into the earthworms in the plots where biochar was added.
- ⇒ There was no significant change in the uptake of both grass and legume even with the addition of biochar. This may indicate that these plants are not the best candidates for remediation purposes of Kolleberga or our data are not sufficient. The available data for these two crops were collected only for shoots and not roots, which are not enough to see if phytostabilization through DDT sequestering in roots is occurring.

Phytoextraction based on the average uptake in different parts of the pumpkin:

- ⇒ The uptake of DDT of pumpkin is low, according to the bioaccumulation factor, which is less than 1, which indicates that it is not viable for phytoextraction. The DDT uptake in the roots was high (BAF =2.62), but when measuring its translocation factor, it was found that its less than 1, meaning the plant was unable to effectively translocate DDT into above ground parts.

Phytodegradation based on the change in ratios in DDD:DDT and DDE: DDT:

- ⇒ There is slight increase in the degradation ratios of DDD: DDT (3%) and DDE: DDT (6 to 15%) with the addition of biochar to the plots but it is not significant, which indicates that degradation is occurring but very slowly and not likely to lead to reduction of DDT in the short term.

The use of only plant based GRO strategies (phytoremediation) might not be suitable for this particular site, however, the biochar has been shown to have a significant effect on the key parameters relating to the actual risks at Kolleberga. In the scientific literature, there are many studies on the same plant species with better results, so the ineffectiveness of these plants at Kolleberga might be due to soil and weather conditions that negatively affected their removal abilities. Therefore, future studies are still needed to reach a concrete conclusion.

Connecting these results to the risk assessment studies, we can conclude that the site could be used as a forest nursery (as planned) or as a horse operation. The risk assessment study for the 'forest nursery' showed that the uptake of DDT by earthworms has been reduced by 40% by adding 3% biochar, which reduces the risk of secondary poisoning and possibility of accumulation through the food chain to predatory animals. However, this conclusion is based on a qualitative approach and simplifying assumptions, thus there might be some uncertainties regarding whether the risks are completely managed. Further studies are needed to determine to what extent the reduction in earthworm DDT concentrations equates to a reduction in the dose of DDT transferred to predators.

The risk assessment for the scenario "horse operation" shows that the risk levels at a concentration of 10 mg/kg TS of DDT and its metabolites (DDE and DDD) is acceptable and there is no potential risk for both humans and horses on the site via the main two exposure pathways (ingestion of soil and ingestion of plants) even if horses were fed mainly from the grass planted on the site, as grass showed very little uptake of DDT compared to pumpkins as could be seen in (Figure 20). However, the calculations were carried out including only horses as the primary ecological receptors, while there are still many animals on the site that need to be further investigated and are prone to secondary poisoning via the food chain (predators and mammals). A human health risk assessment needs to be carried out if grazing animals were kept on the site for the purpose of animal product production such as milk, eggs, and meat.

6.2 Practical recommendations

Based on the results of this work, the stabilization ability of biochar gave the greatest success of reducing and managing the risk of DDT at this specific concentration of 10 mg/kg TS. Neither phytoextraction nor phytodegradation were effective enough to be considered for practical recommendation for Kolleberga. Therefore, only aided-phytostabilization using biochar would be suitable to recommend. However, further studies are needed to evaluate the effect of using biochar and how it may affect the soil microbial activities or other organisms inhabiting the site. Also, future studies combining the use of phytoremediation with mycoremediation (the use of fungi) might be beneficial at such sites as the later has been receiving a great interest in remediating organic contaminants in soil (Bhandari., 2017).

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8 Appendix

8.1 Appendix A1 (Statistical Analysis Data)

Note: The data was obtained from the labs of Orebro University and Eurofins, where B (1 to 3) indicates blocks, T (1 to 8) indicates plant type and start, Y1 & Y2 are years 2020, 2021 and 2022.

Table 9: Summary of the number of samples considered in this study.

GRO Stratagy	GRO parameter	Time	Plant	Amendment		total no. of samples
				Biochar	none	
Phytostabilization	Pore water	Start	Field control	3		3
			Plots control	3	3	6
		Y2	Pumpkin	3	3	6
			Grass	3	3	6
			Legume mix	3	3	6
			Salix	3	3	6
	Earthworm fat	Y2	Field control	3		3
			Pumpkin	3	3	6
			Grass	3	3	6
			Legume mix	3	3	6
			Salix	3	3	6
	Grass		Field control	3		3
		Y1		3	3	6
		Y2		3	3	6
	Legume	Y1		3	3	6
		Y2		3	3	6
Phytoextraction	Pumpkin		Field control	3		3
	a. stems	Y1		3	3	6
		Y2		3	3	6
	b. roots	Y1		3	3	6
		Y2		3	3	6
	c. leaves	Y1		3	3	6
		Y2		3	3	6
Phytodegradation	Degradation in soil	Start	Field control	3		3
			Plots control	3	3	6
		Y2	Pumpkin	3	3	6
			Grass	3	3	6
			Legume mix	3	3	6
			Salix	3	3	6

Table 10: Concentrations of DDT and its metabolites in pore water measured using POM., ΣDDT is the sum of isomers of DDT and metabolites, and SUM DDX includes ΣDDT and other metabolites (includes DDM, DBP, DDMU & dicofol) found in the pore water concentration, here, year 2021 indicate start data and 2022 is Y1.

				Unit-µgDDT/kg DS							
Year	Treatment/ plant type	Block	Amendment	SUM DDX	ΣDDT	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT
2021	Plots	B1	none	206.9	185.6	1	11	6	41	18	108
2021	Plots	B2	none	478.7	382.3	1	24	11	88	38	220
2021	Plots	B3	none	364.7	341.5	1	23	9	72	34	201
2021	Field-control-1	FC	FC	411.4	376.5	1	22	11	89	38	215
2021	Field-control-2	FC	FC	315.1	299.4	1	21	10	64	29	176
2021	Field-control-3	FC	FC	72.5	64.7	0	4	2	15	6	37
2021	Plots	B1	biochar	87.4	80.0	0	3	2	19	7	49
2021	Plots	B2	none	83.0	77.3	0	3	2	19	6	47
2021	Plots	B3	none	90.6	86.2	0	3	2	19	6	56
2022	Pumpkin	B1	none	330.1	312.1	0.63	21.5	5.6	28.3	43.1	212.9
2022	Pumpkin	B1	biochar	102.7	98.6	0.13	4.9	1.9	11.4	13.2	67.0
2022	Grass	B1	none	274.0	259.9	0.71	21.1	4.4	22.5	27.6	183.6
2022	Grass	B1	biochar	133.7	128.3	0.17	5.8	2.1	12.8	18.9	88.5
2022	Legume	B1	none	301.5	275.7	0.70	20.0	4.1	18.4	19.8	212.7
2022	Legume	B1	biochar	139.2	130.5	0.21	6.7	1.6	8.5	20.7	92.7
2022	Salix	B1	none	290.1	266.9	0.73	21.2	3.7	17.4	25.5	198.4
2022	Salix	B1	biochar	117.6	110.4	0.17	5.5	1.5	8.0	12.0	83.3
2022	Pumpkin	B2	none	335.3	319.7	0.62	21.3	4.8	23.8	57.7	211.5
2022	Pumpkin	B2	biochar	112.6	108.6	0.15	5.1	2.0	12.0	10.2	79.1
2022	Grass	B2	none	254.8	241.5	0.57	18.6	3.9	20.6	26.7	171.2
2022	Grass	B2	biochar	147.3	139.5	0.22	6.8	1.7	8.7	17.8	104.3
2022	Legume	B2	none	328.8	306.0	0.72	20.5	4.2	19.4	40.8	220.5
2022	Legume	B2	biochar	114.8	108.0	0.18	5.2	1.4	8.5	2.6	90.1
2022	Salix	B2	none	298.5	267.5	0.69	19.6	3.8	18.3	49.6	175.6
2022	Salix	B2	biochar	123.1	116.3	0.19	5.6	1.7	8.8	11.7	88.4
2022	Pumpkin	B3	none	248.8	234.8	0.52	17.8	3.2	16.4	25.3	171.5
2022	Pumpkin	B3	biochar	77.2	74.2	0.10	3.6	1.3	8.4	5.0	55.8
2022	Grass	B3	none	304.2	286.9	0.68	19.9	4.5	22.9	34.2	204.7
2022	Grass	B3	biochar	156.9	148.8	0.24	7.2	1.8	9.8	24.6	105.1
2022	Legume	B3	none	309.6	282.6	0.69	21.5	3.4	16.0	30.2	210.7
2022	Legume	B3	biochar	121.8	115.2	0.16	5.4	1.5	8.4	10.6	89.1
2022	Salix	B3	none	269.1	246.7	0.63	18.9	3.6	17.0	22.2	184.5
2022	Salix	B3	biochar	88.7	84.6	0.12	3.9	1.0	6.2	13.5	59.9
2022	Field control 1	FC	FC	374.7	334.1	0.7	25.8	4.6	18.7	51.0	233
2022	Field control 2	FC	FC	268.9	251.7	0.6	19.6	3.5	15.9	32.5	180
2022	Field control 3	FC	FC	308.1	279.9	0.9	21.0	3.4	15.2	28.6	211

Table 11: Concentrations of ΣDDT in biomass- grass and legume.Here, Y1- 2021 and Y2- 2022.

Unit-µgDDT/kg DS				STEMS/SHOOTS						
Time	**Block**	**plant type**	**amendment**	**DDD-o,p**	**DDD-p,p**	**DDE-o,p**	**DDE-p,p**	**DDT-o,p**	**DDT-p,p**	**ΣDDT**
Y1	B1	Grasses	None	0.74	4.87	0.98	68.25	20.27	80.78	176.20
Y1	B2	Grasses	None	0.69	3.35	0.63	48.61	14.97	67.40	135.87
Y1	B3	Grasses	None	0.31	1.79	0.42	35.67	8.27	32.48	78.55
Y1	B1	Grasses	Biochar	0.36	1.71	0.34	26.75	7.98	29.40	66.87
Y1	B2	Grasses	Biochar	0.27	1.42	0.29	25.16	6.49	26.54	60.50
Y1	B3	Grasses	Biochar	0.30	1.19	0.37	21.65	6.41	20.70	50.95
Y2	B1	Grasses	None	1.01	4.27	0.87	42.20	16.10	66.40	130.85
Y2	B2	Grasses	None	0.64	2.67	0.57	25.10	10.40	41.40	80.78
Y2	B3	Grasses	None	0.48	1.96	0.40	18.20	8.12	31.50	60.66
Y2	B1	Grasses	Biochar	0.82	3.29	0.54	20.20	13.60	55.40	93.86
Y2	B2	Grasses	Biochar	0.88	3.73	0.57	20.90	15.70	70.10	111.88
Y2	B3	Grasses	Biochar	0.81	3.59	0.58	20.70	13.40	58.70	97.78
Y1	B1	Legume mix	None	2.48	10.18	1.83	58.59	52.54	220.78	346.04
Y1	B2	Legume mix	None	0.73	2.72	0.75	25.58	13.80	43.63	87.04
Y1	B3	Legume mix	None	1.85	7.20	1.74	64.75	39.70	135.87	251.57
Y1	B1	Legume mix	Biochar	1.38	4.27	1.00	32.59	23.56	78.55	141.17
Y1	B2	Legume mix	Biochar	0.89	3.97	0.77	26.54	20.49	85.13	137.99
Y1	B3	Legume mix	Biochar	1.72	5.93	1.10	40.02	29.40	82.58	160.28
Y2	B1	Legume mix	None	0.457	1.44	0.369	11.7	6.16	17.9	38.026
Y2	B2	Legume mix	None	0.229	0.694	0.216	6.45	3.07	7.97	18.629
Y2	B3	Legume mix	None	0.606	2.24	0.511	15.6	9.31	31.4	59.667
Y2	B1	Legume mix	Biochar	0.346	1.71	0.346	7.42	3.91	12.7	26.432
Y2	B2	Legume mix	Biochar	0.192	0.53	0.157	4.53	2.35	5.84	13.599
Y2	B3	Legume mix	Biochar	0.239	0.872	0.178	5.28	3.26	10.5	20.329

Table 12: Concentrations of ΣDDT in biomass- pumpkin-stems, roots, leaves & fruits. Here, Y1- 2021 and Y2- 2022.

Unit	Time	Y1	Y1	Y1	Y1	Y1	Y1	Y2	Y2	Y2	Y2	Y2	Y2
µgDDT/kg DS	Block	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
	amendment	None	None	None	Biochar	Biochar	Biochar	None	None	None	Biochar	Biochar	Biochar
STEMS/SHOOTS	DDD-o,p	76.68	63.74	77.65	70.05	36.79	31.55	68.2	34.6	74.8	35.7	29.6	37.1
	DDD-p,p	99.79	119.79	121.93	148.66	102.78	82.14	185	90.9	78	37.6	76	69.4
	DDE-o,p	50.59	39.68	39.89	27.70	17.11	11.44	41.8	26.5	39.9	25.6	20.5	23.5
	DDE-p,p	838.50	483.42	635.29	570.05	225.67	133.69	377	302	350	344	306	345
	DDT-o,p	3285.56	2524.06	2244.92	2119.79	1054.55	732.62	1283	582	971	631	547	545
	DDT-p,p	3565.78	3783.96	3146.52	4081.28	2390.37	1757.22	2775	992	814	521	882	725
	ΣDDT	7914.44	7058.82	6310.16	7058.82	3850.27	2780.75	4730	2028	2327.7	1594.9	1861.1	1745
ROOTS	DDD-o,p	125.1	135.8	108.0	68.8	39.1	32.5	773	320	308	178	74.4	79.2
	DDD-p,p	128.3	132.6	89.3	99.6	55.2	75.7	3263	595	598	264	230	300
	DDE-o,p	121.9	128.3	99.6	44.4	28.2	27.3	267	190	162	77.1	51.8	55.3
	DDE-p,p	3289.8	2550.8	2211.8	1003.2	718.7	655.6	5284	3604	3393	1603	1162	1265
	DDT-o,p	8160.4	10058.8	7068.4	3963.6	2225.7	1712.3	12810	5637	5835	2606	1973	1707
	DDT-p,p	6484.5	7061.0	4572.2	4736.9	2492.0	3206.4	23852	8160	8650	3541	3846	3778
	ΣDDT	18288.8	20107.0	14117.6	9946.5	5561.5	5668.4	46249	18506	18946	8269.1	7337.2	7184.5
LEAVES	DDD-o,p	4.64	7.87	8.75	3.70	3.12	7.26	12.5	17.8	17.9	10.6	10.8	15.6
	DDD-p,p	17.09	33.30	33.63	15.78	9.23	39.18	70.8	94.7	79.9	45.1	45.6	78.3
	DDE-o,p	3.21	6.37	6.37	2.31	2.04	2.22	9.47	13.6	13	6.88	7.76	10.2
	DDE-p,p	49.95	96.31	93.05	57.68	54.30	45.92	164	269	235	117	125	164
	DDT-o,p	93.48	130.92	139.41	59.09	49.62	61.27	248	473	468	185	195	286
	DDT-p,p	279.14	571.99	538.03	202.74	129.50	226.03	849	1628	1535	610	713	1006
	ΣDDT	446.19	848.84	816.19	337.36	250.30	380.89	1354	2496	2348.8	974.58	1097.16	1560.1
FRUITS	DDD-o,p	1.85	6.04	8.15	6.00	4.89	22.31	No harvestable fruit this year :(					
	DDD-p,p	1.35	3.03	4.78	2.31	2.82	16.22						
	DDE-o,p	0.36	0.95	0.65	0.56	0.58	2.26						
	DDE-p,p	4.41	9.37	8.21	3.68	5.17	16.87						
	DDT-o,p	36.57	60.29	51.80	66.06	42.88	180.11						
	DDT-p,p	16.76	43.86	27.42	25.47	33.30	138.21						
	ΣDDT	60.94	119.71	101.21	104.47	89.24	380.89						

Table 13: Concentrations of isomers of DDT and metabolites in soil.

	Unit- µg DDT/kg DS									
Year	**Treatment/ Plant type**	**Block**	**Amendment**	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**	**ΣDDT**
2021	Field-control-1	FC	none	23	702	216	1001	965	4574	7481
2021	Plots-none-control	B2	none	26	829	254	1249	1269	5111	8739
2021	Plots-biochar-control	B2	biochar	20	608	233	1018	1067	4315	7260
2021	Plots-biochar-control	B3	biochar	17	558	177	845	834	3547	5977
2021	Plots-none-control	B1	none	33	836	257	1222	1196	5578	9123
2021	Plots-none-control	B3	none	28	820	256	1183	1283	5523	9092
2021	Plots-biochar-control	B1	biochar	31	864	274	1282	1269	5348	9067
2021	Plots-biochar-control	B1	biochar	20	492	172	769	748	3199	5400
2021	Plots-none-control	B2	none	35	913	296	1347	1392	5568	9551
2022	Pumpkin	B1	none	18	591	189	917	914	4049	6678
2022	Pumpkin	B1	biochar	24	729	234	1057	1097	4522	7663
2022	Grass	B1	none	23	591	215	1035	907	4296	7067
2022	Grass	B1	biochar	25	729	215	997	1054	4245	7266
2022	Legume	B1	none	22	693	215	935	1069	4130	7063
2022	Legume	B1	biochar	22	749	234	1045	1076	4327	7454
2022	Salix	B1	none	24	590	187	890	1057	3721	6468
2022	Salix	B1	biochar	21	675	196	878	955	4549	7273
2022	Pumpkin	B2	none	26	617	196	907	968	4385	7099
2022	Pumpkin	B2	biochar	20	652	225	989	861	3910	6658
2022	Grass	B2	none	21	633	219	969	927	4170	6939
2022	Grass	B2	biochar	26	747	219	971	989	4314	7265
2022	Legume	B2	none	23	663	231	1029	1030	4159	7135
2022	Legume	B2	biochar	29	746	223	1022	1150	4338	7508
2022	Salix	B2	none	25	622	191	864	1057	4429	7188
2022	Salix	B2	biochar	30	831	191	850	788	4137	6827
2022	Pumpkin	B3	none	25	674	220	1034	1067	4657	7676
2022	Pumpkin	B3	biochar	23	650	184	845	876	3595	6172
2022	Grass	B3	none	21	576	177	796	858	3869	6297
2022	Grass	B3	biochar	24	707	216	1011	952	4088	6997
2022	Legume	B3	none	24	678	217	955	1036	4362	7272
2022	Legume	B3	biochar	21	894	236	1103	1163	4890	8306
2022	Salix	B3	none	22	578	174	827	973	4034	6607
2022	Salix	B3	biochar	25	775	216	988	1089	4153	7246
2022	Field control 1	FC	none	30	662	185	874	947	4263	6960
2022	Field control 2	FC	none	20	415	117	673	533	2823	4581
2022	Field control 3	FC	none	28	548	115	908	780	3710	6087

Table 14: Concentrations of isomers of DDT & metabolites in the fat tissues of earthworms for the year 2022.

		measured in unit-ng/g (wet weight)						
Treatmeant	**Fat %**	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**o,p-DDT**	**p,p-DDD**	**p,p-DDT**	**total DDT**
Pumpkin w/out biochar (T1)	2.79	34.18	847.83	166.53	787.35	728.55	3347.77	5912.21
	1.96	22.16	730.51	119.42	736.39	451.20	2791.05	4850.73
	2.09	14.82	613.59	89.07	574.01	359.18	2273.97	3924.64
Pumpkin with biochar (T2)	3.56	11.69	316.24	102.16	465.06	420.39	1959.26	3274.81
	1.81	4.37	148.11	33.56	203.27	139.48	958.57	1487.37
	2.04	3.57	119.34	31.09	179.98	141.60	822.36	1297.93
Grass w/out biochar (T3)	2.68	21.52	709.90	123.02	654.17	451.96	2539.88	4500.45
	1.82	16.00	584.69	82.42	573.69	318.31	2323.36	3898.47
	2.23	14.75	589.10	78.01	481.65	346.22	2199.60	3709.34
Grass with biochar (T4)	2.87	7.02	229.67	72.22	330.42	290.03	1375.43	2304.80
	1.97	3.69	185.98	47.26	258.01	195.72	1148.71	1839.37
	2.01	2.79	149.68	33.20	221.91	147.83	1051.04	1606.46
Clover & Lucerne w/out biochar (T5)	2.36	18.14	721.60	137.97	665.21	533.22	2594.41	4670.55
	2.01	15.27	586.00	109.16	557.21	381.03	2374.09	4022.76
	1.69	11.82	470.54	90.11	496.12	277.52	1855.81	3201.92
Clover & Lucerne with biochar (T6)	2.39	6.15	227.60	63.33	329.07	261.70	1333.36	2221.20
	2.15	5.56	223.28	51.99	329.75	191.11	1332.12	2133.81
	2.40	5.96	270.07	60.08	346.71	294.91	1698.97	2676.70
Salix w/out biochar (T7)	2.84	18.49	661.51	102.64	645.80	391.13	2511.26	4330.84
	1.94	12.64	534.79	91.35	531.59	323.79	2172.38	3666.54
	2.10	18.12	669.02	112.49	673.82	442.45	2622.88	4538.78
Salix with biochar (T8)	3.00	6.98	259.16	66.01	382.36	251.60	1696.72	2662.83
	2.16	6.14	222.26	61.47	348.76	241.50	1438.30	2318.42
	1.92	3.09	136.66	28.73	223.01	141.00	909.32	1441.81
Field-control-1	3.4	2.0	45.9	49.76	1442.49	17.14	253.09	4736.50
Field-control-2	2.2	1.8	10.3	19.36	748.50	8.99	121.97	2410.43
Field-control-3	2.6	1.6	12.5	24.49	764.44	4.01	115.28	2944.88

Table 15: Bioaccumulation and translocation factors for ΣDDT uptake in pumpkin parts. Note values >1 are highlighted.

Part of the plant	**Bio-accumulation factor (BAF)**	**Y1- 2021**	**Y2-2022**
Stems/shoots	Pumpkin without biochar	0.69	0.28
	Pumpkin with biochar	0.43	0.21
Roots	Pumpkin without biochar	1.69	2.62
	Pumpkin with biochar	0.66	0.92
Leaves	Pumpkin without biochar	0.06	0.20
	Pumpkin with biochar	0.03	0.11
	Translocation factor (TF)		
Roots --> Stems	Pumpkin without biochar	0.41	0.11
	Pumpkin with biochar	0.65	0.23

8.2 Appendix A2 (Statistical Analysis Results)

The results from the IBM SPSS for ΣDDT uptake in pore water concentration, earthworms, grass, legume, pumpkin and degradation in soil are presented below, the blue marking indicate the comparison is significant for that particular test and red mean insignificant.

Table 16: Results presenting the Shapiro-Wilk test for normality. The results marked red mean that the data rejected the assumption for normality and need for Kruskal- Wallis test.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
POM_22	.202	24	.013	.864	24	.004
POM	.239	12	.057	.862	12	.052
DDT_legume	.183	12	.200*	.850	12	.037
DDT_grass	.152	12	.200*	.925	12	.331
total_DDT_in_EW_fat	.131	24	.200*	.942	24	.179
DDXS	.209	12	.153	.859	12	.048
DDXR	.243	12	.049	.757	12	.003
DDXL	.155	12	.200*	.892	12	.125
DDD_DDTxSOIL	.103	24	.200*	.967	24	.598
DDE_DDTxSOIL	.127	24	.200*	.914	24	.042

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table 17: Levene's Test for ΣDDT in pore-water Concentration, start data.

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
POM	Based on Mean	.284	1	10	.606
	Based on Median	.298	1	10	.597
	Based on Median and with adjusted df	.298	1	9.758	.597
	Based on trimmed mean	.238	1	10	.636

Table 18: Tests of Between-Subjects Effects results for ΣDDT in pore water concentration, start data.

Tests of Between-Subjects Effects

Dependent Variable: POM

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	91811.868[a]	1	91811.868	15.337	.003
Intercept	618766.004	1	618766.004	103.367	<.001
amendmentstart	91811.868	1	91811.868	15.337	.003

Table 19: Results from the Kruskal Wallis test for porewater concentration, data 2022.

Test Statistics[a,b]

	POM_22
Kruskal-Wallis H	17.280
df	1
Asymp. Sig.	<.001
Exact Sig.	<.001
Point Probability	.000

a. Kruskal Wallis Test

b. Grouping Variable: with_or_without_biochar

Table 20: Levene's Test for ΣDDT concentration in fat of earthworms.

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
total_DDT_in_EW_fat	Based on Mean	2.573	7	16	.05
	Based on Median	.337	7	16	.92
	Based on Median and with adjusted df	.337	7	7.768	.91
	Based on trimmed mean	2.254	7	16	.08

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Dependent variable: total_DDT_in_EW_fat

b. Design: Intercept + plant_type + Amendment + plant_type * Amendment

Table 21: Tests of Between-Subjects Effects results for ΣDDT concentration in earthworms' fat.

Tests of Between-Subjects Effects

Dependent Variable: total_DDT_in_EW_fat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	12937.167[a]	7	1848.167	1.390	.275	.378
Intercept	152004.167	1	152004.167	114.307	<.001	.877
amendment_22	6402.667	1	6402.667	4.815	.043	.231
plant_type	3570.833	3	1190.278	.895	.465	.144
amendment_22 * plant_type	2963.667	3	987.889	.743	.542	.122
Error	21276.667	16	1329.792			
Total	186218.000	24				
Corrected Total	34213.833	23				

a. R Squared = .378 (Adjusted R Squared = .106)

Table 22: Kruskal Wallis test results for ΣDDT in biomass of Legume.

Test Statistics[a,b]

	DDT_legume
Kruskal-Wallis H	.410
df	1
Asymp. Sig.	.522
Exact Sig.	.589
Point Probability	.104

a. Kruskal Wallis Test

b. Grouping Variable: Amen_b_n

Table 23: Levene's Test for ΣDDT concentration in biomass of Grass.

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
DDT_grass	Based on Mean	4.453	1	10	.061
	Based on Median	4.149	1	10	.069
	Based on Median and with adjusted df	4.149	1	6.846	.082
	Based on trimmed mean	4.442	1	10	.061

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Dependent variable: DDT_grass

b. Design: Intercept + Amen_b

Table 24: Tests of Between-Subjects Effect for Grass

Tests of Between-Subjects Effects

Dependent Variable: DDT_grass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2731.914[a]	1	2731.914	2.154	.173
Intercept	109201.848	1	109201.848	86.101	<.001
Amen_b	2731.914	1	2731.914	2.154	.173
Error	12682.954	10	1268.295		
Total	124616.716	12			
Corrected Total	15414.868	11			

a. R Squared = .177 (Adjusted R Squared = .095)

Table 25: Kruskal Wallis test results for DDD: ΣDDT degradation ratios, for plant type (left) and biochar (right).

Test Statistics[a,b]

	DDD_DDTxSOIL
Kruskal-Wallis H	6.140
df	3
Asymp. Sig.	.105

a. Kruskal Wallis Test

b. Grouping Variable: plan

Test Statistics[a,b]

	DDD_DDTxSOIL
Kruskal-Wallis H	.213
df	1
Asymp. Sig.	.644
Exact Sig.	.671
Point Probability	.041

a. Kruskal Wallis Test

b. Grouping Variable: amend_ratio

Table 26: Levene's test for DDE: DDT degradation ratio.

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
DDE_DDTxSOIL	Based on Mean	.890	7	16	.536
	Based on Median	.151	7	16	.991
	Based on Median and with adjusted df	.151	7	12.185	.991
	Based on trimmed mean	.793	7	16	.604

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Dependent variable: DDE_DDTxSOIL

b. Design: Intercept + amendment + plant_type + amendment * plant_type

Table 27: Tests of Between-subjects effects, for DDE: DDT degradation ratio.

Tests of Between-Subjects Effects

Dependent Variable: DDE_DDTxSOIL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.000[a]	7	5.756E-5	1.649	.192	.419
Intercept	.405	1	.405	11600.507	<.001	.999
amendment	3.663E-6	1	3.663E-6	.105	.750	.007
plant_type	.000	3	.000	3.206	.051	.375
amendment * plant_type	6.363E-5	3	2.121E-5	.608	.620	.102
Error	.001	16	3.490E-5			
Total	.406	24				
Corrected Total	.001	23				

a. R Squared = .419 (Adjusted R Squared = .165)

Table 28: Independent Samples Test for Pumpkin

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance		Mean	Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Difference	Difference	Lower	Upper
Sum_DDT_stem	Equal variances assumed	.548	.476	1.447	10	.089	.178	1913.04715	1322.02650	-1032.61145	4858.70575
	Equal variances not assumed			1.447	9.743	.090	.179	1913.04715	1322.02650	-1043.17375	4869.26805
Sum_DDT_roots	Equal variances assumed	4.137	.069	3.183	10	.005	.010	15374.51640	4829.47479	4613.77597	26135.25682
	Equal variances not assumed			3.183	5.199	.012	.023	15374.51640	4829.47479	3101.32511	27647.70769
Sum_DDT_leaves	Equal variances assumed	1.868	.202	1.509	10	.081	.162	618.25010	409.81956	-294.88479	1531.38499
	Equal variances not assumed			1.509	8.306	.084	.168	618.25010	409.81956	-320.76022	1557.26042

Table 29: Results from the Mann- Whitney U test for uptake of ΣDDT in pumpkin parts- leaves and stems.

Test Statistics[a]

	Sum_DDT_leaves	Sum_DDT_stem
Mann-Whitney U	10.000	8.500
Wilcoxon W	31.000	29.500
Z	-1.281	-1.524
Asymp. Sig. (2-tailed)	.200	.128
Exact Sig. [2*(1-tailed Sig.)]	.240[b]	.132[b]

a. Grouping Variable: Amen_b_n

8.3 Appendix B (Risk Assessment Data)

Table 30: input data and the estimated exposure dose via ingestion of soil for horses with its risk ratio. The orange color represents the site-specific values for Kolleberga, white color represents SGU's values, while the yellow color represents the calculated exposure doses and the calculated risk ratio.

Ingestion of soil					
Parameter	unit	value			
		Stable season	Pre-season	high summer	late summer
C soil	mg DDT/kg TS soil	10	10	10	10
IR soil	kg TS soil/kg body weight, day	0,002	0,002	0,002	0,001
BA	%	100%	100%	100%	100%
Exp.A	m2/m2	100%	100%	100%	100%
ET1	hr/24 hr	50%	50%	100%	100%
ET3	month/ 12 month	50%	25%	8%	17%
TRV	mg DDT/kg body weight, day	0,147	0,147	0,147	0,147
D soil	mg DDT/kg body weight, day	0,0038	0,0019	0,0016	0,0017
RR soil		0,026	0,013	0,011	0,012

Table 31: input data and the estimated exposure dose via ingestion of plants for horses with its risk ratio. The orange color represents the site-specific values for Kolleberga, white color represents SGU's values, while the yellow color represents the calculated exposure doses via ingestion of plants and the calculated risk ratio.

Ingestion of plants					
Parameter	unit	value			
		Stable season	Pre-season	high summer	late summer
C plants	mg DDT/kg TS plant	0,095	0,095	0,095	0,095
IR plants	kg TS plants/kg body weight, day	0,03	0,03	0,02	0,02
BA	%	100%	100%	100%	100%
F plants	%	0%	100%	50%	100%
ET3	month/ 12 month	50%	25%	8%	17%
TRV	mg DDT/kg body weight, day	0,147	0,147	0,147	0,147
D plants	mg DDT/kg body weight, day	0	0,0007	0,0001	0,0003
RR plants		0,000	0,005	0,001	0,002

8.4 Appendix C. Photos from the field visit

The following photos were taken from the site visit on May 16th, 2023, showing different parts of the experiment.