Soil Remediation and Sustainable Development

- Creating Appropriate Solutions for Marginalized Regions

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Para vos, Puchunguita, te amo

"Now I am terrified at the Earth, it is that calm and patient,
It grows such sweet things out of such corruptions,
It turns harmless and stainless on its axis, with such endless successions of diseas'd corpses,
It distills such exquisite winds out of such infused fetor"

— Walt Whitman (1819 – 1892)

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Abstract

The scope of this doctoral thesis is appropriate soil remediation methods for marginalized regions that go beyond pollution reduction targets and include strategies to support sustainable development. Contaminated soil from industrial or agricultural activities poses potential health threats to animals and humans and also threatens economic systems by making land unsuitable for agriculture and other economic purposes. Remediation of contaminated sites in marginalized regions such as rural areas in developing countries or sparsely populated regions in industrialized countries, need strategies that meet a different set of criteria compared to urban industrial sites in order to be appropriate. A pilot-scale experimental station was built to assess the feasibility of using organic by-products to enhance the degradation of diesel fuel. It was demonstrated that bioremediation based on the use of readily available organic by-products as amendments or phytoremediation based on locally present plants can be appropriate choices of technology in marginalized regions. Systematic sustainability assessments of the regions local environmental and social context of the contaminated site are necessary to design appropriate bioremediation projects. The inclusion of ecological engineering into the framework for strategic sustainable development, as an integrated planning guide, was demonstrated by two case studies to give valuable input to the strategic process when bioremediation is used as a tool to reach sustainability goals. Results from pilot-scale experiments confirm that whey can significantly increase the degradation rate of diesel fuel, but the slow overall degradation rates due to the high clay content in ultisol (a common tropical soil) could be a considerable constraint for efficient pollutant removal in full scale applications. Results from an experiment in soil cylinders show that the vertical migration of added microorganisms was limited in dense soils. Three species of amaranth tested in the field experiment effectively bioaccumulated toxaphene and other persistent organic pollutants which make them potentially interesting candidates for phytoremediation in the region.

Summary in Swedish

Denna doktorsavhandling handlar om tillämplig marksanering i marginaliserade områden där målet går utöver saneringsmål och inkluderar strategier för främjande av hållbar utveckling. Förorenad jord från industri och jordbruksverksamheter innebär potentiella hälsorisker för djur och människor samtidigt som ekonomiska system hotas på grund av mark som blir obrukbar för jordbruk och andra ekonomiska syften. Återställande av förorenade platser i marginaliserade regioner som landsbygd i utvecklingsländer eller glesbefolkade delar av industrialiserade länder kräver strategier som möter andra kriterier än vad som krävs för urbana industriella miljöer för att kunna betecknas som tillämpliga. En försöksstation i pilotskala byggdes för att utvärdera användbarheten av att utnyttja organiska restprodukter för att öka nedbrytningshastigheten av dieselolja. Det påvisades att bioremediering baserad på användning av lättillgängliga organiska restprodukter och fytoremediering baserad på lokala växter kan vara tillämpliga teknikval i marginalisade områden. Systematiska hållbarhetsutvärderingar av den lokala situationen beträffande miljö och sociala frågor är nödvändiga för att kunna utforma tillämpliga bioremedieringsprojekt. Tillämpning av ekologisk ingenjörskonst inom ramverket för strategisk hållbar utveckling, som en integrerad planeringsguide, visade sig genom två fallstudier ge värdefulla riktlinjer för den strategiska processen då bioremediering används som ett redskap för att nå hållbarhetsmål. Resultat från ett experiment i pilotskala bekräftar att tillsatser av vassle signifikant kan öka nedbrytningen av dieselolja men den låga nedbryningshastigheten som tillskrivs den höga lerhalten i ultisol (en vanligt förekommande tropisk jord) kan vara en betydande begränsning för effektiv reduktion av föroreningar i fullskaliga tillämpningar. Resultat från ett experiment i jordcylindrar visar att den vertikala transporten av tillsatta mikroorganismer var begränsad i kompakta jordar. Tre arter av amarant som testades i ett fältexperiment visade sig effektivt kunna bioackumulera toxafen och andra långlivade organiska föroreningar vilket gör dem till potentiellt intressanta kandidater för fytoremediering i regionen.

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List of papers

This thesis is based on the following four papers, herein referred to by their Roman numerals:

Paper I Henrik Haller, Anders Jonsson, Morgan Fröling (2017)
Soil Pollution and Sustainable Development in Marginalized Regions – A Study on the application of Ecological Engineering within the Framework for Strategic Sustainable Development for Design of Appropriate Soil Bioremediation Technologies
Submitted for publication in *Journal of Cleaner Production*

Paper II Henrik Haller, Anders Jonsson, Joel Ljunggren & Erik Hedenström (2017)
Organic By-Products for Sustainable Soil Remediation -The Effect of Three Different Amendments on the Degradation of Diesel Fuel in a Tropical Ultisol
Submitted for publication in *International Journal of Biodeterioration & Biodegradation*

Paper III Henrik Haller, Anders Jonsson, Katia Montenegro Rayo & Anielka Dávila López (2016)
Microbial Transport of Aerated Compost Tea Organisms in Sandy Loam and Clay Loam – a Soil Column Study.
International Biodeterioration & Biodegradation 106 p. 10-15.

Paper IV Henrik Haller, Martha Lacayo, Martha Jarquín & Anders Jonsson (2017)
Bioaccumulation and Translocation of Field-Weathered Toxaphene and Other Persistent Organic Pollutants in Three Cultivars of Amaranth (A. cruentus 'R127 México', A. cruentus 'Don León' y A. caudatus 'CAC 48 Perú') - a Field Study from Former Cotton Fields in Chinandega, Nicaragua
Accepted for publication in *Ecological Engineering (Special Issue Ecosystem Restoration: Innovative Engineering tools and New Approaches)*

Author's contribution

Paper I I was the lead author of the paper.

Paper II I was the lead author of the paper. I designed and constructed the pilot-scale experimental station. I also planned the experiment and carried out all the experimental work. I conducted sampling, extraction and GC-FID analysis.

Paper III I was the lead author of the paper. I designed the experimental setup and coordinated the work with the team at Laboratorio de Biotecnología, UNAN-Managua, Nicaragua.

Paper IV I was the lead author of the paper and I had the main responsibility for analysing and structuring the raw data together with my colleagues at Laboratorio de Biotecnología, UNAN-Managua, Nicaragua.

Abbreviations

ACT	Aerated Compost Tea
BAF	Bioaccumulation Factor
CEC	Cation Exchange Capacity
CIPP	Centro Integral para la Propagación de la Permacultura (Nicaraguan NGO)
CT	Compost Tea
GC	Gas Chromatography
DHA	Dehydrogenase activity
DRO	Diesel Range Organics
DPSIR	Driver–Pressure–State–Impact–Response
EE	Ecological Engineering
FID	Flame Ionization Detection
GHG	Greenhouse Gases
FSSD	Framework for Strategic Sustainable Development
MS	Mass Spectrometry
MIUN	Mid Sweden University
NCT	Non-aerated Compost Tea
NGO	Non-Governmental Organization
IEA	Integrated Environmental Assessment
PA	Pyroligneous Acid
PAH	Polycyclic Aromatic Hydrocarbon
POP	Persistent Organic Pollutant
SOM	Soil Organic Matter
UNAN	Universidad Nacional Autónoma de Nicaragua (National Autonomous University of Nicaragua)

1 Introduction

The scope of this doctoral thesis is appropriate soil remediation methods for marginalized regions that go beyond pollution reduction targets and include strategies to support sustainable development.

1.1 Soil pollution

Human societies and globally interconnected economies rely on services provided by the ecosystems that constitute the foundation upon which our civilization is based. The resilience of social-ecological systems depends on a sustainable management of these ecosystems. Environmental degradation causes multimillion losses to society annually and threatens ecosystem functions, human health, and food security (Berkes et al., 2008, Rockström et al., 2009, Steffen et al., 2015, Nellemann and Corcoran, 2010). More than 60% of the world´s ecosystems are considered degraded by either: habitat loss and fragmentation; unsustainable harvest; pollution; climate change; and/or introduction of exotic invasive species (Nellemann and Corcoran, 2010). Numerous xenobiotic and toxic substances from industrial activities are dispersed into the biosphere. Ecosystems typically lack the ability to break down such pollutants and consequently they are accumulated in the biosphere. Among the most common soil pollutants are halogenated hydrocarbons, heavy metals and petroleum products (Singh and Ward, 2004, Molina-Barahona et al., 2005, Nagabhushanam, 2005). Contaminated soil from industrial or agricultural activities poses a potential health threat to animals and humans and can have a detrimental effect on economic systems by making land unsuitable for agriculture and other economic purposes (Wesseling et al., 2001, Molina-Barahona et al., 2005, Ortiz-Hernández et al., 2014).

1.2 Bioremediation

Bioremediation is a technology that uses living organisms and their enzymes to degrade, remove or immobilize toxic compounds (Alexander, 1999, Vidali, 2001). Bioremediation may be applied on a very large scale, such as the clean-up at the beach of Prince William Sound after the Exxon Valdez oil spill, however most applications have been on a more moderate scale (Pritchard et al., 1992, Boopathy, 2000). Soil bioremediation is generally classified as *ex situ* if the contaminated soil is physically removed from the original site or *in situ* if the soil is treated in place without excavation. *Ex situ* bioremediation is typically rather energy-intense and more expensive than *in situ* bioremediation due to the necessary transportation (Mohee and Mudhoo, 2012, Tajam et al., 2010). In many situations bioremediation is an appropriate and cost-effective alternative to traditional physico-chemical remediation techniques (Singh and Ward, 2004, Jonsson and Haller, 2014). In recent years, bioremediation applications have increased and some authors predict a further growth as technological advances surmount the current limitations (Mohee and Mudhoo 2012).

Some of the challenges that bioremediation researchers deal with today include (Alexander, 1999, Boopathy, 2000, Singh and Ward, 2004, Mohee and Mudhoo, 2012):

(i) how to achieve a better understanding about the characteristics of and changes to the soil microbial community during bioremediation processes,

(ii) how to improve the limited bioavailability of hydrophobic and recalcitrant compounds,

(iii) how to map the complex anaerobic/aerobic metabolic pathways of contaminants,

(iv) how to find ways to increase the microbial transport so that microorganisms and pollutants can have contact with each other,

(v) how to map plants that possess the capacity to bioaccumulate heavy metals or organic pollutants.

As new technologies emerge that surmount these limitations, bioremediation may become an increasingly attractive option for soil remediation. In my research these challenges have been borne in mind and I have expressly addressed microbial transport and exploration of plants (amaranth) with the capacity to bioaccumulate POPs.

1.3 Soil remediation and sustainable development in marginalized areas

Soil remediation has been a developing field for several decades and conventional remediation technologies include energy-intensive and soil disrupting technologies such as soil excavation and landfill disposal. Sustainable remediation technology has been defined as:

> *"a remedy or combination of remedies whose net benefit on human health and the environment is maximized through the careful use of limited resources" (Mohee and Mudhoo 2012).*

At present, sustainable remediation technologies are only modestly implemented and in many marginalized areas such as rural areas in developing countries or sparsely populated regions in industrialized countries, soil contamination is largely overlooked because of lack of economic incentives and lack of governance, knowledge and skills (Ongley and Booty, 1999, Westbye et al., 2013). Such regions are often accorded less interest by authorities and investors (Gutberlet, 1999, Macfarlane et al., 2000, Elands and Wiersum, 2001, Saith, 2001, Orcao and Cornago, 2007, Breman et al., 2010).

The geographical scope of this doctoral thesis is marginalized regions where economic incentives are small for soil remediation of any kind to be effectuated. Such locations demand energy- and resource-efficient remediation methods but the time constraints may not be as limiting as in highly developed urban environments. In developing countries the use of toxic substances tends to be less regulated than in industrial countries and the extent of the soil contamination is largely unknown which means that many activities may unwittingly occur on polluted land (Wesseling et al., 2001). In Nicaragua, for instance, where the experimental work presented in this thesis was performed, soil contamination occurs accidentally from spills but also intentionally, as only 56 % of the municipalities have regular waste collection services and 87% of the country's waste dumps are unauthorized (Harding-

Lacayo, 2005). Soil pollution is a serious problem in Nicaragua and in many other developing countries, but due to the economic situation, environmental concerns are typically given low priority (Carvalho et al., 2003, Merkl et al., 2006). Numerous large contaminated sites in developing countries thus need remediation (Ongley and Booty, 1999, Wesseling et al., 2001, Carvalho et al., 2003, E Ite, 2004).

Resource-related problems might also occur in sparsely populated regions in industrial countries. As an example, in the county of Jämtland in northern Sweden, 3,100 potentially polluted sites have been identified out of which 359 are contaminated by fuel handling and 22 are oil depots. The number of smaller spills from forestry machines etc. is an unknown factor but it used to be common practice to change the motor oil of forestry machines in the forests (Westbye et al., 2013). The demand for land in remote parts of the Jämtland region is often too low for companies to be interested in investments in soil remediation without enforcement of legal imperatives (Westbye et al., 2013). The small scale of these polluted sites in proportion to the costs of traditional remediation practices contribute to the low priority (Jonsson and Haller 2014). Conventional methods for soil remediation such as soil excavation and landfill disposal are often expensive and energy consuming (Mohee and Mudhoo 2012). The high costs involved in removal of toxic substances from contaminated soils may prevent remediation from being carried out; especially in areas of little economic value (Boopathy 2000), which is often the case both in developing countries and in sparsely populated regions in industrialized countries. Previous research at Mid Sweden University (MIUN) has revealed that organic amendments such as whey and fermented whey can enhance the naturally occurring degradation of different compounds found in diesel fuel (Jonsson and Östberg, 2011, Östberg et al., 2007a, Östberg et al., 2006, Östberg et al., 2007b). *In situ* whey application is a relatively energy-efficient and mostly non-soil-disruptive method based on a readily available by-product, which makes it a candidate for appropriate *in situ* bioremediation in low prioritized regions (Tajam et al., 2010, Jonsson and Haller, 2014).

1.4 Objectives and scope

The scope of this doctoral thesis project is appropriate soil remediation methods (see section 3.3) for marginalized regions that go beyond pollution reduction targets and include strategies to support sustainable development. This means that, in addition to assuring that the pollution of a particular site is taken to harmless levels, appropriate bioremediation projects should support sustainable development and must not contribute to violations of sustainability principles (see section 3.5).

The overall goal of this project is to address problems related to sustainable development and bioremediation in marginalized regions by answering the following questions:

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1. What bioremediation methods may be considered appropriate (section 3.3) in marginalized regions?
2. What concerns need to be considered in order to approach sustainable development that goes beyond pollution reduction targets in polluted areas in marginalized regions?
3. What factors may limit the success of bioremediation projects in marginalized areas?
4. What is the efficiency of some appropriate bioremediation technologies?
5. What constraints are related to the use of such technologies and how can these be overcome?

Theoretical frameworks such as ecological engineering and the framework for strategic sustainable development were explored as tools to make bioremediation projects meet the criteria of sustainability and feasibility in terms of degradation and bioaccumulation rates. The capacity to meet these criteria were evaluated by literature studies as well as laboratory-, pilot- and field-scale experiments.

1.5 Outline of the thesis

This doctoral thesis is based on four journal manuscripts produced in a research project on bioremediation in marginalized regions. A book chapter (Jonsson and Haller 2014) and four conference papers (Pronoza et al., 2016, Haller and Jonsson, 2013, Iraguha et al., 2016, Haller et al., 2012) has also been produced as a result of this research project. The thesis is divided into seven sections. After the introductory section, potentially appropriate bioremediation technologies in marginalized regions are discussed in general terms. In section 3, theoretical concepts for the design of projects that support sustainable development are discussed (**Paper I**). In the same section biophysical/chemical topics (such as metabolic pathways, environmental fate, and degradation and bioaccumulation mechanisms) that affect the success of a bioremediation technology are discussed. Such topics have been assessed experimentally (**Paper II, III and IV**) in order to assist the identification of appropriate bioremediation methods in marginalized regions. The design of the pilot-scale experimental station, lab and field experiments are described in section 4 together with the systems theoretical tools used for sustainability assessment during the project. In section 5 the results from experiments on diesel degradation conducted in the experimental station are presented together with results from field and laboratory experiments on microbial transport of compost tea organisms and bioaccumulation (**Paper II, III and IV**). Finally, the most significant findings of the project are discussed and suggestions for future research are provided in sections 6 and 7.

2. Bioremediation in marginalized regions

2.1 Soil pollution – magnitude and environmental fate

The last 200 years of industrialization have caused widespread soil contamination on a global scale (Science Communication unit, 2013) and at least one third of the world's ecosystems are currently suffering from pollution (Nellemann and Corcoran, 2010). In addition to the detrimental effects on animal health, ecosystem functions and food security, soil contamination may pose direct hazards to human health. The most frequent contaminants of soil in Europe are heavy metals and mineral oil (Science Communication Unit, 2013). Three million sites are estimated to have been potentially polluted in Europe and approximately 250,000 of these may need urgent remediation (Jones et al., 2012). In developing countries, a lot less is known about the magnitude of the soil contamination but large-scale application of persistent pesticides is still affecting large territories (Ongley and Booty, 1999, Ortiz-Hernández et al., 2014, Wesseling et al., 2001).

Soil contaminants can enter the human body via three main routes: eating, inhalation and dermal absorption. The exposure through *eating* can either happen indirectly by eating plants grown on contaminated soil, which are subsequently consumed, either by humans or by agricultural livestock which are a food source for humans or by direct ingestion of the soil (geophagia). In particular, children under three years of age are prone to this kind of exposure which is one important pathway for human exposure to soil contamination. *Inhalation* effects mainly agriculture workers who may be exposed to particles that may lodge in the lungs and subsequent absorption into the bloodstream. *Dermal absorption* (through skin contact) is primarily a concern for volatile organic compounds and less of a problem for non-volatile soil contaminants such as heavy metals. (Science Communication Unit, 2013).

The life cycle of a pollutant after its release into the environment is referred to as environmental fate. The environmental fate and persistence of the pollutant depend on the physico-chemical properties of the compound itself but many external factors such as oxygen supply, temperature and sun exposure are equally important. Organic pollutants can be degraded completely into carbon dioxide and water or undergo a partial degradation in which toxic or nontoxic metabolites are formed. Heavy metals and some recalcitrant organic pollutants may persist unmodified in the environment indefinitely. Depending on whether the pollutant is organic or inorganic, persistent or easily degradable, complex or simple, its environmental fate varies considerably. Many environmental factors affect the degradation rates of organic pollutants. In tropical clay soils that are poor in nutrients, the context in which the experimental parts of the project have been carried out, two of the most determining factors are: availability of nutrients, and the physio-chemical properties of the contaminated soil (**Paper II,** Alexander, 1999).

2.1.1 The influence of soil properties on biodegradation

Clay soils have a greater capacity for physico-chemical attenuation of pollutants than sandy soils, but their fine texture tends to be unfavourable for most soil life (Alexander 1999). Soil texture also affects the microbial migration (Abu-Ashour et al., 1994) and in dense soils, the mass transfer may be a limiting factor for biodegradation. The limited pore space in compacted soils restricts the diffusion of gases, which may lead to poorly aerated soils, especially if the soils are saturated with water. Non-polar substances such as PAH tend to sorb to organic matter and clay particles, thereby decreasing their bioavailability (Megharaj et al., 2011). Soil organic matter is vital to microorganisms, and the vertical distribution of microorganisms is typically characterized by higher microbial activity in the upper soil horizons where most soil organic matter and oxygen is available. The presence of earthworms and macrofauna is generally favourable for degradation (Sinha et al., 2010).

2.1.2 The influence of nutrients availability on biodegradation

When physico-chemical factors such as temperature, pH and bioavailability of the pollutant are apt for bacterial activity and degradation, the limiting factor of degradation of organic pollutants in soil is habitually deficient concentrations of nitrogen and phosphorus (Welander, 2005). By adding organic by-products, different nutrients are supplied that may increase the degradation rates in different ways. The great excess of degradable organic carbon from diesel in a contaminated soil, however, creates an unfavourable C:N ratio for the degradation. In these nitrogen-limited circumstances, the supply of nitrogen and phosphorous is crucial for the biodegradation (Alexander 1999). There are different suggested application rates in the literature and the optimal application of N and P is likely to be specific for each site. Pritchard, Mueller et al. (1992) advocate applying as much fertilizer as possible without exceeding toxic concentrations, whereas Margesin and Schinner (1997) recommend C:N:P ratios of 50/5/1 for degradation of diesel oil. Urea is commonly used as a source of N, but when applied in high doses, toxic effects such as rise in pH, drop in bacterial viability and increased nitrification rates may occur. Slow-release sources of N, such as methylene urea, tend to show much less negative effects (Kauppi et al., 2011). Other macronutrient-based substances that stimulate degradation rates include organic compounds such as peptone, calcium lactate, yeast extract, nicotinamide, riboflavin, pyridoxine, thiamine, and ascorbic acid. At too high concentrations however, such substances have a retarding effect on degradation (Alexander 1999). Below, some individual characteristics and degradation mechanisms of the pollutants that are the focus of this project are discussed: diesel fuel hydrocarbons, POPs and heavy metals.

2.1.3 Diesel fuel

Petroleum hydrocarbons are complex, yet typically biodegradable, mixtures of aliphatic and aromatic hydrocarbons (Atlas, 1981, Atlas, 1995, Alexander, 1999). Diesel fuel is predominantly composed of paraffins with chain-lengths from C10 to C28 (referred to as diesel range organics, DRO), alkylbenzenes, naphtalenes and low concentrations of polycyclic aromatic hydrocarbons (PAH). The inherently high exergy stored in these molecules could potentially be utilized to fuel the microbial degradation process (Jonsson and Haller, 2014). As diesel is a complex mixture of compounds, its degradation usually involves consortiums of microorganisms, able to metabolize different molecular structures. When diesel is degraded by microorganisms, a range of intermediate metabolites are produced, most of which are nontoxic, polar molecules (Riser-Roberts, 1998). However, the water-solubility of polar compounds them subject to an increased mobility, and leads to an increased risk for ground water contamination. In some cases, microbial transformation has been reported to lead to an increased toxicity, especially under oxygen-limited conditions (Long and Aelion, 1999, Alexander, 1999).

2.1.4 Persistent Organic Pollutants (POP)

POPs are hydrophobic, lipophilic, extremely stable molecules that are essentially non-reactive under normal environmental conditions (Lacayo, 2005). POPs can reach the environment in a number of ways including chemical plant discharge, losses from many consumer products such as computers, paints and household products. Pesticides used in agriculture are also important sources of POP contamination. Of the twelve POPs identified for priority action by the United Nations Environment Programme (UNEP), also known as "the dirty dozen", nine are pesticides (Lacayo, 2005). Agricultural applications of pesticides are typically unselective. In an experiment as little as 2 % of the applied pesticide was detected on the leaf surface, meaning that 98% didn't reach the target but ended up in the soil and air (Linde, 1994).

2.1.4.1 Toxaphene

Toxaphene is a pesticide that was heavily used between 1950 and the early 1990s predominantly in the USA, former Soviet Union, Central America and Brazil(Lacayo, 2005). The first signals of toxaphene as a global pollutant came from Canada and Scandinavia in the early 1980s. Although located thousands of kilometres from the main application sites, toxaphene was detected especially in lipid rich polar fish and mammals in these countries (Swackhamer et al., 1993, Muir et al., 1992). Toxaphene is a complex mixture of chlorinated terpenoids with several hundred congeners. To distinguish between these different compunds the nomenclature, developed by Harun Parlar in which toxaphene congeners is assigned a number in order of their gas chromatographic retention time, (Andrews and Vetter, 1995, Burhenne et al., 1993, Hainzl et al., 1995, Frenzen et al., 1994). The biodegradability, ecotoxicity and environmental fate vary considerably between the different congeners of toxaphene: some congeners are extremely persistent in the environment whereas others are relatively rapidly metabolized. Toxaphene is primarily degraded under anaerobic conditions but aerobic degradation is also possible although it is typically slower (Lacayo et al., 2004). Due to the chemical stability, environmental persistence, and molecular properties of many of the congeners, toxaphene compounds have been biomagnified in the food chain. The fact that POPs bioaccumulate in plant biomass suggests that plants can be used for phytoremediation purposes but very little research on the phytoremediation of POPs is available (Campos et al., 2008, Mattina et al., 2004, Mattina et al., 2003). The experiment on toxaphene uptake in amaranth (**Paper IV**) is the first study using amaranth for bioremediation of toxaphene.

In Nicaragua toxaphene was used on at least 115 000 ha of agricultural land primarily in the cotton fields of Chinandega and Leon on the Pacific coast(Carvalho et al., 2003). During the cotton boom in the 1960s and 70s Nicaragua was among the top 24 cotton producers in the world. Cotton is associated with high pesticide use and as many as 28-35 sprayings per year were common in Nicaragua. Cotton farmers have reported vicious circles in which pests becoming resistant to the pesticides were followed by higher application rates (Corriols, 2010, Lacayo, 2005). Annual application rates were as high as 31 kg ha^{-1} and the cumulative application since 1974 was 560 kg ha^{-1} on vast extensions of agricultural land. In 1984, WHO discouraged the use of toxaphene due its potential carcinogenic effects (Carvalho et al., 2003) but it wasn’t banned until 1993 (Corriols, 2010). However due its recalcitrance, high concentrations are still found in agricultural soil that consecutively leach out to rivers and estuaries. In 2002 the toxaphene (total) residues measured in soils in Nicaragua’s former cotton regions still attained 44 μg g^{-1} dw soil. Also aquatic fauna is affected and edible species such as flathead grey mullet (*Mugil cephalus)* and *Anadara Spp,* which are common components of the local diet, present toxaphene levels beyond what is safe for human consumption (Carvalho et al., 2003, Lacayo, 2005). High levels of toxaphene residues have also been found in water in wells (Appel et al., 1991) and mothers’ milk

(Calero et al., 1993). Furthermore the former cotton cultivation was located on Nicaragua's best agriculture soil in the fertile lowlands of the Pacific coast (Lacayo, 2005) whose suitability for agriculture is now significantly reduced.

2.1.5 Toxic heavy metals

Toxic heavy metals typically occur naturally in the soil environment at trace levels but due to human interference with nature's slowly occurring geo-chemical cycle of metals, many rural and urban environments contain toxic heavy metals at concentrations that may cause risks to human health, plants, animals or ecosystems (Violante et al., 2010, Wuana and Okieimen, 2011). Emissions from industrial areas, mine tailings, waste disposal, leaded gasoline and paints, land application of sewage sludge, fertilizers and pesticides cause toxic heavy metals to accumulate in the environment. Soils are the main sink for toxic heavy metals released into the environment. Since metals are elements they cannot undergo microbial or chemical degradation and they may remain in soil for a long time after introduction (Wuana and Okieimen, 2011).

The movement of heavy metals in soil environments depends on sorption/desorption reactions as well as chemical complexation with inorganic and organic ligands and biotic and abiotic redox reactions. Factors such as pH, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acid, root exudates, microbial metabolites and nutrients affect the bioavailability, leaching and toxicity of heavy metals in soil (Evangelou et al., 2007).

The metal mobility in soil depends on preferential flow and organic-facilitated transport and in favourable circumstances metals may move considerable distances through the soil (Camobreco et al., 1996, Violante et al., 2010). Heavy metals may be absorbed by and bioaccumulate in certain plants and plant-based phytoextraction is a promising low-cost technique to remediate heavy metal contaminated soil. Applications of chelating agents such as EDTA (ethylene diamine tetraacetic acid), EDDS (ethylene diamine disuccinate), NTA (nitrilotriacetic acid), natural low molecular weight organic acids and humic substances have shown enhancing effects on phytoextraction by increasing the solubility of heavy metals (Evangelou et al., 2007). The use of slowly degrading complexing agents such as EDTA may however be problematic, primarily because of their resistance to biodegradation but also due to the risk of increased leaching of toxic metals to the groundwater. A risk analysis that considers such potential hazards should be done prior to any application of chelating agents.

2.2 Bioremediation strategies supporting sustainable development in marginalized regions

The concept of marginalization is widely used in many research fields, but it is often used in a rather indistinct way to describe and deal with a wide range of processes (Breman et al., 2010). In this research project marginalized regions are defined as areas in which individuals are systematically denied full access to the opportunities, resources and rights that are available to most other individuals in the same country or region. Rural areas in developing countries and sparsely populated areas in industrialized countries are often accorded less attention by authorities and investors (Gutberlet, 1999, Macfarlane et al., 2000, Elands and Wiersum, 2001, Orcao and Cornago, 2007, Saith, 2001, Breman et al., 2010) and the inhabitants in these areas may be prevented from participating fully in the economic, social, and political life of the society. Contaminated sites in such areas need strategies that meet a different set of criteria than urban industrial sites and in many marginalized regions, soil contamination is not properly dealt with because of lack of economic incentives and lack of governance, knowledge and skills (Ongley and Booty, 1999, Westbye et al., 2013). To enable implementation of bioremediation projects in marginalized regions, the engineering solutions need to be cost-effective, compatible with the social and economic development state of the region and provide perceptible and achievable benefits.

Since marginalized regions face a number of social, ecological and economic challenges, bioremediation projects must be designed so that they do not aggravate the situation. A common assumption used to be that remediation projects are intrinsically sustainable because they reduce the risk from contamination but remediation projects (especially *ex situ*) may have a negative net effect on environmental health when factors such as CO_2 emissions, disturbance of natural habitats etc. are included in the assessment. Conventional approaches to soil remediation typically focus on the internalities of a remediation project (effectiveness of the remedy, implementability, cost considerations and time constraints etc.) (Mohee and Mudhoo, 2012). The environmental viability of specific remediation technologies has traditionally received little attention from researchers, let alone the social aspects of remediation projects. Much of the bioremediation research to date has been laboratory-based and focused on the ability of specific strains of microorganisms to degrade or mineralize a particular toxic compound (Mohee and Mudhoo, 2012, Alexander, 1999, Boopathy, 2000). Such research has increased our understanding of degradation mechanisms, kinetics and metabolic pathways, but results from the laboratory are often difficult to reproduce in field conditions due to a number of factors, including the limited survival of introduced strains (Alexander, 1999).

In this project, the aim has been to address problems related to bioremediation projects in marginalized regions that go beyond pollution reduction targets and include strategies to support sustainable development. Theoretical tools described in sections 3 and 4.1 have been used to support the design process of bioremediation strategies that aim to promote sustainable development. Three technologies were selected to be studied in more detail in this project: phytoremediation, biostimulation and bioaugmentation. They were selected because of their potential capacity to meet the criteria of cost and energy efficiency in pollutant reduction. With an appropriate implementation they may be incorporated in projects that support sustainable development. Below follows an introduction to the three types of bioremediation technologies and some characteristics that may make them appropriate candidates for marginalized regions.

2.2.1 Phytoremediation

Many plants tolerate pollutants better than humans and animals and some plants have the potential to accumulate toxic compounds in their tissue which has given rise to the practice of phytoremediation (Singh and Ward, 2004, Mohee and Mudhoo, 2012). Phytoremediation is the use of green plants to remove, contain, or render harmless environmental contaminants (Cunningham and Berti, 1993). A wealth of data from recent research has strengthened the notion that phytoremediation using suitable plants can be a cost-efficient alternative to energy intense *ex situ* project (Campos et al., 2008) and thus an interesting candidate for marginalized regions. Both inorganic and organic pollutants present in solid and liquid substrate can be remediated by phytoremediation (Salt et al., 1998). The main steps involved in phytoremediation are the following: uptake, translocation, transformation, compartmentalization, and sometimes mineralization (CarreiraL, 1995, Truu et al., 2015). Good candidates for phytoremediation are plants that accumulate high amounts of the target element and furthermore have a high growth rate, tolerate toxic effects of heavy metals, and can adapt to local soil and climate conditions, are resistant to pathogens and pests, and easy to grow. Organic soil pollutants such as chlorinated solvents and polycyclic aromatic hydrocarbons (PAHs) can be absorbed and immobilized by the plant or transpired from the shoot (Gao and Zhu, 2004, Ma and Burken, 2003, Ma et al., 2004) and some compounds may even be metabolized in the plant (phytodegradation).

2.2.1.1 Amaranth

Amaranth (Fig. 1), having a high growth rate, high tolerance to the toxic effects of heavy metals, resistance to pathogens and pests and being easy to grow, meets many of the desirable criteria for phytoremediation candidates for marginalized regions from tropical to temperate climates (Shevyakova et al., 2011, Yuan et al., 2014, Rahman et al., 2013, Lu et al., 2014). Amaranth is also a plant that has proven to be a versatile bioaccumulator and since many agricultural soils comprise a range of different contaminants, broad-spectrum bioaccumulators are potential candidates especially if included in successional phytoremediation strategies with different plants over a number of vegetative cycles (Chunilall et al., 2005, Watanabe et al., 2009, Shevyakova et al., 2011, Rahman et al., 2013). The amaranth genus include some 60 species with different centres of domestication but the most important species of seed amaranths originated from the Americas, of which cultivated species include: A. caudatus L., (A. edulis Spegazzini syn, A. mantegacianus Passerini, hypochondriacus L., (A. leucocarpus S.Wats A. flavus L.) and A. cruentus L., (A. paniculatus L. (Stallknecht and Schulz-Schaeffer, 1993, Alemayehu et al., 2015). Amaranth was grown all over the American continent during pre-Columbian times and is presently scarcely cultivated in Nicaragua but commonly found in Bolivia, Argentina, Ecuador, Peru, Cuba, México, Guatemala, as well as in India, Pakistan, China, Malaysia, Indonesia and the USA. Being native to Central America, it is an interesting candidate for phytoremediation in Nicaragua. Apart from the results of our study (**Paper IV**), little is known about its bioaccumulation capacity and translocation pattern of toxaphene and other POPs. Ingestions of amaranth (seeds, leaves and tender stems are edible) grown on contaminated soil is a serious threat since it may accumulate many toxins (Rahman et al., 2013). However those cultivation crops that absorb toxins in their tissue without translocation to their edible parts can potentially enable farmers to obtain an income source at the same time as their soil is remediated.

***Figure 1**. Cultivated amaranth ready to harvest (“Amaranth opopopeo” by Dwight Sipler licensed under CC BY 2.0)*

2.2.2 Biostimulation

Biostimulation is a remediation technology that seeks to improve pollutant degradation by optimizing soil conditions by e.g. aeration, addition of nutrients, adjustment of pH and temperature (Adams et al., 2015, Margesin et al., 2000). Biostimulation involves a modification of the environment to be remediated in order to stimulate the autochthonous microorganisms with capacity to degrade the contaminants. The goal is to create a more favourable environment for such organisms by the addition of limiting nutrients and/or electron acceptors. Biostimulation, being simple and cost-efficient is potentially a good candidate for bioremediation in marginalized regions. Energy and labour may be saved by taking advantage of the already present native microorganisms well distributed spatially in the soil and well-suited to the local environment. The local geology of the subsurface may pose a challenge to making the nutrients available to the microorganisms since clays or other fine-grained material may limit the transport of nutrients through the soil matrix (Adams et al., 2015).

One way of providing nutrients for biostimulation in a way that may support sustainable development is by using organic by-products that would otherwise end up as waste as a source of easily available carbon, nutrients and bioactive compounds. Organic by-products are generated globally on a massive scale. Economic operations such as agriculture, livestock, fishing and forestry generate a number of residual products (Reddy and Yang, 2005, Ruane and Sonnino, 2011, Ulloa et al., 2004). A considerable number of experiments with organic by-products as amendments for bioremediation have been conducted. The most common by-products include molasses (Boopathy et al., 1994, Nikolopoulou and Kalogerakis, 2008), bagasse (Dzul-Puc et al., 2005), corn cobs (Wu et al., 2008), manure (Kästner et al., 1995), blood meal (Fischer et al., 1998, Allen et al., 2002), fish bone meal (Walworth et al., 2003) straw (Laine and Jorgensen, 1996), and rice husks (Tarley and Arruda, 2004, Forss et al., 2013). An inventory of some by-products that could potentially be used as amendments for *in situ* biostimulation in developing countries with a tropical climate was made in order to select candidates for the experimental work of this Ph. D. project (Haller et al., 2012). Two of these amendments (whey and pyroligneous acid) that were tested experimentally are described below.

2.2.2.1 Whey

Whey is produced when milk casein is removed from the milk in dairy operations to make cheese and other products. This liquid by-product constitutes between 85-95% of the milk volume, and 55% of milk nutrients remain in the whey. About half of the annual global production of 145 million metric tons is utilized for food products for animal and human use. The remaining volumes end up as potential contaminants in the environment (González Siso, 1996). The chemical content of whey is characterized by lactose, a number of essential and non-essential amino acids in different proportions, vitamin B 1,2,6,7,12, folic acid and lactic acid (Östberg et al., 2007a). Relatively few studies have been carried out on the effect of whey on biodegradation of organic pollutants (Östberg, 2006) but there is evidence that *in situ* whey application is a relatively energy-efficient and largely non-soil-disruptive method, which makes it a candidate for appropriate *in situ* bioremediation in low prioritized regions (Tajam et al., 2010, Jonsson and Haller, 2014). Research at MIUN has showed that the degradation of aliphatic and aromatic hydrocarbons can be significantly enhanced by the addition of whey and fermented whey (Östberg et al., 2007a, Östberg et al., 2006, Östberg et al., 2007b). The biodegradation-enhancing effect of whey was primarily attributed to an increased microbial biomass stimulated by the readily available carbon source (Östberg et al., 2007a). The degradation studies also showed a more complex dependence of carbon sources and growth factors, such as B-vitamins, on the degradation of an aromatic compound (phenanthrene) compared to an aliphatic compound (hexadecane) (Jonsson and Östberg, 2011). These results indicate that the presence of co-factors such as vitamins and micronutrients may be important to consider when evaluating the suitability of organic amendments for bioremediation of soil contaminated by various types of organic pollutants.

The results of the whey method have not been as promising in *in situ* experiments in Sweden as in the laboratory, presumably due to low soil temperature and low availability of the whey due to the restriction of percolation in clay soil (Vilches et al., 2010). In a tropical climate the mineralization rate is expected to be significantly higher and the method could prove more appropriate in such climate if the obstacle of low whey availability can be overcome. High concentrations of whey have been reported to inhibit biodegradation in some circumstances, and too low concentrations showed little effect (Östberg, 2006), suggesting that determination of optimum application rates is important to obtain an efficient degradation. Optimum application rates might vary however, due to factors such as soil type, precipitation, temperature etc. Repeated applications rather than a single application were reported to increase the degradation of a number of organic pollutants (Östberg, 2006).

2.2.2.2 Pyroligneous acid

Pyroligneous acid (PA) is a by-product from some charcoal production systems (Steiner et al., 2008) with a limited economic value that is also referred to as wood vinegar, pyrolysis oil, smoke extract, bio-oil etc. (Mohan et al., 2006, Zulkarami et al., 2011). Currently, the only commercially important application of PA is that of smoke flavour in food (Mohan et al., 2006). Smoke flavour is considered to be safe by the U.S. Food and Drug Administration and can be used at levels that comply with good manufacturing practice (Holley and Patel, 2005). PA is typically disposed of as waste (Zulkarami et al., 2011), but in Japan it has been used for centuries to increase crop production and to combat agricultural pests (Steiner et al., 2008, Spokas et al., 2011). At present, the majority of the charcoal consumed worldwide is produced in traditional kilns in developing countries (Ghilardi and Steierer, 2011). Such charcoal production lacks appropriate control of the gas and vapours produced in the process and causes land and air pollution (Zandersons et al., 1999). However there are production systems that recover these condensate co-products, or use them as fuel in the pyrolysis process, reducing the emission to a minimum (Mohan et al., 2006). Several low-tech kilns that recover PA from the charcoal production have been developed, including *the advanced Brazilian beehive kiln* (FAO, 1983) and the *Casamance kiln* (Nturanabo et al., 2010). In recent years the research and technology concerning PA have advanced significantly (Mohan et al., 2006), and the current trend in charcoal production is to improve the environmental performance while maintaining or improving charcoal yield and quality (Stassen, 2002).

The chemical composition of PA varies depending on several factors, such as type of feedstock, pyrolysis temperature and duration. (Jayasinghe and Hawboldt, 2012, Mohan et al., 2006). The most frequently identified compounds in PA include methanol, 2-butanonone, acetaldehyde, furan, furfural, acetic acid and other volatile organic acids (Spokas et al., 2011). PA contains phenolic compounds that are known to have antimicrobial properties (Loo et al., 2008) and PA has successfully been used to control fungal growth on wood (Mohan et al., 2008) and bacterial decay of food (Loo et al., 2008, Ma et al., 2011). At low concentrations however, PA has been shown to stimulate germination, growth and yield in a wide range of plants (Kadota and Niimi). Although the growth promoting effect of PA is not yet clearly understood, it has been attributed to nutritious components such as magnesium sulphate and nitrates of Ca, Mg, and K, but also to its stimulatory effect on symbiotic fungi and bacteria (Zulkarami et al., 2011). PA has been shown to significantly increase basal respiration and microbial biomass in highly weathered tropical soils, suggesting that microbes used PA for their metabolism (Steiner et al., 2008). Another report (Focht, 1999) reveals that all compounds typically found in smoke emitted from charcoal kilns can be metabolized by strains of phototrophic soil bacteria and that the addition of these compounds clearly intensifies respiratory metabolism in the soil. The formation of fruit bodies of edible fungi such as *Lentinus edodes, Pholiota nameko* and *Pleurotus ostreatus* was significantly increased after applications of PA to the growth medium (Yoshimura et al.). *Lentinus edodes* and *Pleurotus ostreatus* are known to be versatile degraders of many toxic compounds including PAH and organochlorine pesticides (Stamets, 2005). In a laboratory experiment, PA showed the greatest growth stimulating effect of *Pleurotus ostreatus* at concentrations of 3.0%. At higher concentration the effect declined and at 10% no fruit-bodies were produced (Yoshimura et al.). Microorganisms capable of degrading the compounds found in PA are typically not abundant in soil. However, in habitats with a permanent flow of these compounds, e.g. soil near a charcoal kiln, their presence has been reported to be increased (Focht, 1999). Continuous applications of PA could consequently be expected to increase the population of the desired microorganisms.

2.2.3 Bioaugmentation

Bioaugmentation is the addition of degrading microorganisms to supplement the indigenous populations (Adams et al., 2015). This approach is particularly suitable for sites where the indigenous microbial populations with capacity for degradation are absent or in a stressed state as a result of the recent exposure to the contaminant. Drawbacks of this approach are problems related to the seed microorganism's survival in the new site due to competition with indigenous microorganisms, limited movement through the soil pores and maintenance of genetic stability (Atlas, 1981, Atlas, 1995). The risk of introducing potentially invasive species must also be thoroughly assessed before considering a bioaugmentation strategy. The application of compost tea is a technology that has been evaluated in this project that can be considered as both a bioaugmentation and a biostimulation strategy because it adds microorganism as well as nutrients to the soil (**Paper II and II**).

2.2.3.1 Compost Tea

Compost teas (CT) are liquid soil amendments obtained when compost is soaked and extracted in water. It is not a by-product itself, but the most commonly used ingredients are residues from different food processing activities. The ingredients can be adapted according to what is available locally. Additives (derived from by-products) such as molasses, fish hydrolysate, rock dust, yeast extract, humic acids etc. are often used to add nutrients and stimulate microbial activity. The adaptability, cost-efficiency, relative simplicity and ability to be applied over large areas makes it a potentially appropriate technology for marginalized regions. Historical evidence suggests that CT were used in agriculture by Romans and ancient Egyptians (Carballo et al., 2008), but the practice declined as synthetic pesticides became available and at present it is predominantly used in organic agriculture (Scheuerell and Mahaffee, 2006). Anecdotal evidence of disease-suppressive and soil-amending properties abounds but these testimonies typically lack supporting data. Scheuerell and Mahaffee (2002) indicate that understanding of CT is in its infancy but an increasing number of scientific reports throughout the last decades have confirmed its ability to suppress a wide range of both air- and soil-borne plant pathogens.

A number of production parameters (e.g. aeration, compost source, nutrient additives etc.) have been manipulated in order to optimize plant disease suppression (Scheuerell and Mahaffee, 2006). To date, consensus on optimum production parameters for disease suppression has not been reached, and some studies report inconsistency in the performance (Scheuerell, 2004, Ghorbani et al., 2008). However a number of reports have shown that aeration and additions of nutrients lead to significant increases of the cell mass of active bacteria (Scheuerell and Mahaffee, 2006, Pant et al., 2011, Ghorbani et al., 2008). The addition of humic acids and yeast extracts has also proved to significantly increase the fungal populations (Naidu et al., 2010).

Most research so far has focused on non-aerated compost teas (NCT), but the practice of aerating the CT in order to maintain aerobic conditions during the extraction process is gaining popularity. In recent decades, a number of companies have emerged that manufacture machines for making CT under highly aerated conditions (Pant et al., 2011, St. Martin and Brathwaite, 2012, Scheuerell and Mahaffee, 2002). The oxygen concentration in the tea has obvious consequences for the microbial communities and a distinction between aerated compost teas (ACT) and non-aerated teas (NCT) is necessary.

To date, virtually all research on CT has focused on their potential to control plant diseases in agriculture. However, the fact that CT have proved to significantly increase soil microbial respiration and dehydrogenase activity (Pant et al., 2011) makes them an interesting candidate as bioremediation amendments. Plate counts and microscopic examination studies have revealed that ACT have a bacterial population dominated by *Bacillus* sp., *Lactobacillus* sp., *Micrococcus luteus*, *Staphylococcus sciuri*, *Pseudomonas putida* , *Burkholderia glumae* and *Clavibacter agropyri,* while species of *Aspergillus*, *Penicillium* and *Trichoderma* dominated the fungal communities (Naidu et al., 2010). Many of these organisms have the capacity to degrade a number of toxic substances including polycyclic aromatic hydrocarbons (PAH) and organochlorine compounds (Fingerman and Nagabhushanam, 2005). Aeration and addition of nutrients was shown to significantly increase the population of the mentioned organisms (Naidu et al., 2010). To date, most published studies have relied on traditional culturing methods or microscopic examination to assess the microbial composition of ACT. These methods provide limited knowledge about the microbial diversity (Scheuerell, 2004), suggesting that molecular methods are needed to determine the microbial diversity of ACTs.

3. Theoretical frameworks used in this thesis project

In order to design appropriate soil remediation methods for marginalized regions that go beyond pollution reduction targets and include strategies to support sustainable development, innovative systems thinking is needed. Five theoretical frameworks/concepts were used as the main support for the innovative thinking in this project: ecotechnology integrated environmental assessment, appropriate technology, ecological engineering and framework for strategic sustainable development. The concepts are described below.

3.1 Ecotechnology

Ecotechnology is a concept that fuses technology with ecology. Technology has been defined by Encyclopaedia Britannica as:

> *"the application of scientific knowledge to the practical aims of human life or, as it is sometimes phrased, to the change and manipulation of the human environment"(2015).*

The prefix "eco" compels such manipulations of natural resources to be ecologic i.e. compatible with the long-term survival of natural ecosystems. There is no unanimity on how to define ecotechnology and many definitions and descriptions circulate in the literature (Aida, 1995, Benndorf, 2008, Jørgensen and Mitsch, 1989, Moser, 1996, Straškraba, 1993). At the department of ecotechnology and sustainable building engineering at MIUN, ecotechnology is interpreted as a scientific discipline that *deals with methods to shape future societies within ecological boundaries.* This definition of the ecotechnology concept has been central to this project since a predominant goal has been to contribute to the development of bioremediation projects in marginalized regions that go beyond the pollution reduction targets and support sustainable development. In order to create sustainable societies out of the partially degraded and polluted planet we currently inhabit, technology needs to be reshaped to fit within the ecological boundaries. In a sustainable society the ultimate goal is to limit the rate of pollution to what ecosystems can assimilate and break down without long-term negative effects on ecosystem functions or human health. Applications of ecotechnology can play an important role in the shaping of sustainable societies out of our currently degraded and polluted environment. On the path towards sustainability the disturbed ecosystems will need human intervention to boost their intrinsic capacities to recover by development of bioremediation methods and other technologies.

3.2 Integrated environmental assessment

A number of definitions of integrated environmental assessment (IEA) are found in the literature (Carr et al., 2007, Kristensen, 2004, Hisschemöller et al., 2001, Ravetz, 2000, Tol and Vellinga, 1998). IEA is typically applied to complex issues and in most of these definitions, "integrated" conveys multi- or inter-disciplinarity, and "assessment" refers to some sort of policy relevance (Tol and Vellinga, 1998). Characteristic for IEA is that it seeks to inform policy and decision making by structuring and analysing environmental problems in order to be able to communicate the findings and insights.

The Journal of Integrated Environmental Assessment and Management (IEAM), define in its aims and scope thus:

> *"devoted to bridging the gap between scientific research and the application of science in decision making, policy and regulation, and environmental management".*

Central to the IEA is the *Driver–Pressure–State–Impact–Response* (DPSIR) (Fig. 2) framework for integrated environmental reporting and assessment. The framework has been adopted as a strategy for IEA by the European Environmental Agency (EEA) and United Nations Environment Programme (UNEP) among others (Carr et al., 2007, Kristensen, 2004, Hisschemöller et al., 2001, Ravetz, 2000, Tol and Vellinga, 1998). DPSIR is designed to be used as a means to structure particular environmental problems and identify appropriate responses. It departs from the idea that there is a chain of causal links going from *driving forces* (fundamental social processes such as economic sectors, human activities etc.) through *pressures* (human activities with impact on the environment) to *states* (physical, chemical and biological) and *impacts* on ecosystems, human health and functions to *responses* by policy-makers (Kristensen, 2004, Carr et al., 2007). The DPSIR approach was used implicitly when categorizing factors related to bioremediation projects while working with the integrated planning guide (**Paper I**).

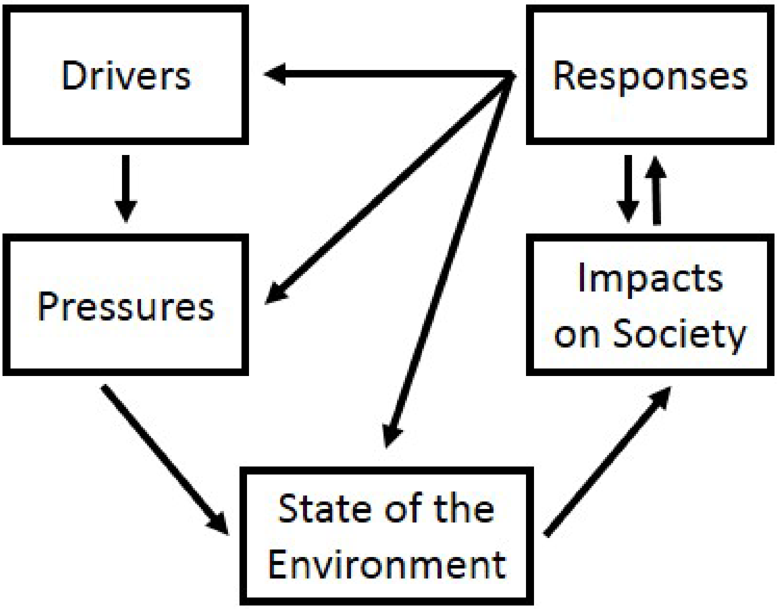

Figure 2. *A visual representation of the components and flows in the DPSIR framework.*

3.3 Appropriate technology

The focus of this project is to evaluate and assist the development of appropriate bioremediation technology for marginalized regions and the concept of appropriate technology is thus central. Appropriate technology may be defined as *(Castree et al., 2013)*:

> *"technologies that are suited to the immediate socio-economic, cultural, and environment contexts in which they are introduced".*

The appropriate technology movement emerged as a response to the perceived failings of the post-World War II technical aid efforts by the industrialized world in developing countries (Pursell, 1993). In 1973 the British economist E. F. Schumacher published *Small Is Beautiful (2011)*, subtitled - *Economics as If People Mattered* in which he calls for *intermediate technology*, i.e. technology that fits between the primitive and poverty-reinforcing tools of many of the developing countries and the large, powerful technological systems of industrialized countries (Kaplinsky, 2011, Pursell, 1993). Initially, advocates of appropriate technology prescribed that it should be small-scale, labour-intensive, low capital investment per worker, energy efficient, environmentally sound, and controlled and maintained by the local community (Evans, 1984, Hazeltine and Bull, 1998, Schumacher, 2011, Murphy et al., 2009). However many theorists like Ranis (1980) argue that appropriate technology can also be advanced, modern, capital intensive, etc. depending on the available resources, local preferences, time, and place. Ranis (*1980)*, for example rather defines appropriate technology as

> *''the joint selection of processes and products appropriate to the maximization of a society's objectives given that society's capabilities''.*

The grassroots appropriate technology movement had its all-time high during the 1970s and although the number of NGOs dedicated to the promotion of appropriate technology decreased during the following decades, the movement has not lost its momentum. The ideological intent and critique of neoclassic economics may not be as prominent as in the beginning of the movement but many technologies and ideas promoted by the movement persist today: solar energy, the generation of electricity by windmills, the utilization of abandoned dams for low-head hydroelectric generation, the development of biogenic methane gas for fuel, a reemphasis on bicycles and mass transit, recycling and the use of natural materials, composting and organic agriculture etc. (Kaplinsky, 2011, Pursell, 1993). Presumably this is because their appropriateness have made them stand the test of time.

During this project, appropriate technology has been used, essentially divorced from its ideological background, and more as an inspirational data bank for the assessment of technologies that address the issues of poverty, social equity, gender employment, and basic human needs in marginalized regions.

3.4 Ecological engineering

Ecological engineering (EE) is an approach that integrates ecology and engineering, proposed as a theoretical framework to create sustainable human-ordered ecosystems and restore natural ecosystems. The foundation of ecological engineering is often attributed to H.T. Odum (Mitsch, 2012) who defined it as "environmental manipulation by man using small amounts of supplementary energy to control systems in which the main drives are still coming from natural sources" (Odum et al., 1963.). Jørgensen and Mitch (1989) suggested an alternative definition which was subsequently refined (Mitsch and Jørgensen, 2004) to read "the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both". During recent years many others have made significant contributions to the field proposing a number of principles and concepts to guide the implementation of ecological engineering (Bergen et al., 2001, Kangas, 2003, Odum, 1996, Straškraba, 1993). Numerous disciplines (including ecological design, bioengineering, industrial ecology, agroecology etc.) share core values and goals with ecological engineering, but Mitch and Jørgensen argue that some of them lack one of the two cornerstones of ecological engineering: (i) recognition of the self-designing ability of ecosystems or (ii) a theoretical foundation that supports the approaches, not just empiricism (Mitsch and Jørgensen, 2004).

Mitsch and Jørgensen (Mitsch and Jørgensen, 2004) identify five basic concepts that have been developed to distinguish ecological engineering from other approaches such as industrial ecology, biotechnology or environmental engineering. Ecological engineering:

1. is based on the self-designing capacity of ecosystems
2. can be the acid test of ecological theories
3. relies on systems approaches
4. conserves non-renewable energy sources
5. supports ecosystem conservation

3.5 Framework for strategic sustainable development

The Framework for Strategic Sustainable Development (FSSD) is an approach that applies basic principles for sustainability and includes a planning mechanism for their application (Hallstedt et al., 2010, Broman and Robert, 2015). The framework is designed to increase the utility of individual methods, tools, and concepts by highlighting strengths and weaknesses, and enabling combinations that create more robust strategic approaches (Broman and Robert, 2015, Hallstedt et al., 2010). The FSSD consists of five interdependent but distinct levels (Missimer et al., 2016a, Missimer et al., 2016b, Broman and Robert, 2015):

1. Systems level (ecological and social principles)
2. Success level (the eight sustainability principles)
3. Strategic guidelines level
4. Action level
5. Tool level

The eight sustainability principles on the success level express boundaries within which a sustainable society can be operated: in a sustainable society, nature is not subject to systematically increasing (Broman and Robert, 2015, Missimer et al., 2016a, Missimer et al., 2016b):

1. concentrations of substances extracted from the earth's crust
2. concentrations of substances produced by society
3. degradation by physical means

And in that society, people are not subject to structural obstacles to:

4. health
5. influence
6. competence
7. impartiality
8. meaning-making

The FSSD was used in **Paper I** jointly with EE to create an integrated planning guide for design of bioremediation projects with goals for sustainable development beyond reduction targets in marginalized regions.

4. Materials and methods

As described in the section 1.4, this project set out to answer five research questions. Systems theoretical research methods were used primarily to address questions 1, 2, 3 and 5 and experimental research was conducted to provide data to answer questions 1, 3, 4 and 5.

4.1 Steering bioremediation strategies towards sustainable development

To guide the development of appropriate methods for bioremediation that has the potential to support sustainable development in marginalized regions, an "integrated planning guide" based on the application of EE within the FSSD was tested (**Paper I**). Complementary tools can advantageously be used within the FSSD (Robèrt, 2000). In **Paper I** it was demonstrated that inclusion of key concepts of ecological engineering within the framework for strategic sustainable development may give valuable input to the strategic planning process when bioremediation is used as a tool to reach sustainability goals beyond pollution reduction targets in marginalized regions. In the model for including the five concepts of ecological engineering within the FSSD (Fig. 3) the five concepts of EE are used on levels 3 and 5. On these levels they provide specific guidance and inspiration for the development of innovative energy- and resource-efficient soil bioremediation strategies (level 3) and as a qualitative monitoring tool to assess the progress towards sustainability (level 5). The integrated planning guide was applied on two cases of polluted soil in marginalized regions.

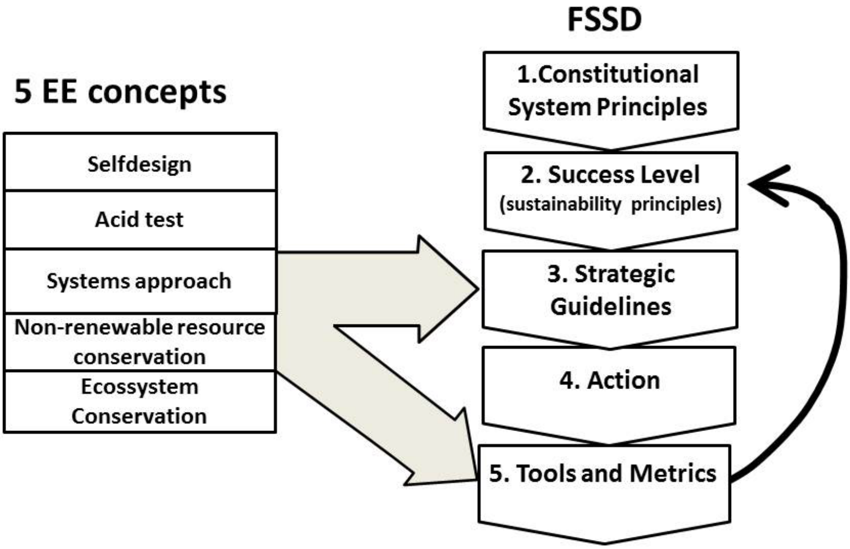

Figure 3. *A conceptual model for the inclusion of the five key concepts of ecological engineering within the framework for strategic sustainable development. The thick arrows indicate levels in FSSD where EE contributes the most. On level 3, EE provides specific guidance and inspiration for the development of innovative energy- and resource-efficient soil bioremediation strategies and on level 5 it can be used as a qualitative monitoring tool to assess the progress towards sustainability according to the vision and goals set at level 2. The thin arrow indicates feedback from the monitoring process to the strategic level (**Paper I**).*

4.2 Experimental methods

In the beginning of this project, a screening of the waste flow in tropical developing countries was produced in order to identify waste products that potentially could be used to stimulate microbial activity for bioremediation purposes (Haller et al., 2012). Emphasis was given to mapping out efficient methods that would be appropriate to economically marginalized people in such countries. Based on the screening, three amendments, whey, pyroligneous acid and compost teas, were selected to be studied in further detail. These three amendments were selected because of their liquid nature (that would facilitate transport through the soil matrix) and documented capacity to stimulate microorganisms with capacity to degrade pollutants. The three amendments were thus assessed experimentally for their potential to enhance diesel degradation (**Paper II**) at the pilot-scale experimental station described in section 4.2.3. This experiment generated further questions about the microbial transport mechanisms of the organisms present in the compost tea that were addressed in a soil column experiment (**Paper III**).

The outcomes from the integrated planning guide (**Paper I**) indicated that a strategy based on phytoextraction by plants, that could also be used for agricultural purposes by local farmers, would potentially be an appropriate technology for remediation of the contaminated soil in the Chinandega region. A field-scale experiment was thus undertaken in order to provide experimental data that would assist the feasibility evaluation of such a strategy (**Paper IV**).

4.2.1 Geographical context of the experiments

The experiments were conducted in Nicaragua (Fig. 4), a country that despite high per capita natural resources, struggle with many poverty-related problems and many areas can be described are marginalized (Kinloch Tijerino, 2008). The experiments were conducted in two administrative departments with somewhat different ecological conditions. Chontales was once part of the Americas largest continuous rainforest north of the Amazon accommodating 7 % of the world's biodiversity but today it is reduced to fragments of second-growth forests (Miller et al., 2001). Chinandega has a drier climate and encompass the best soils for agriculture in Nicaragua thanks to recent volcanic depositions of ash that has created nutrient-rich, high-quality (Joergensen and Castillo, 2001). In Chontales, on the contrary, yields are low on the highly weathered ultisols that are dominant.

Figure 4 The department of Chinandega (left) has a dry tropical climate and Chontales (right) has predominantly a humid tropical climate. "Mapa de Nicaragua mostrando al departamento de Chinandega" and "Mapa de Nicaragua mostrando al departamento de Chontales" by Vrysxyare licensed under CC BY 2.0

4.2.1.1 Las Pavas, Chontales

The scarcity of existing experimental data on sustainable remediation technology from developing countries (Ongley and Booty 1999) encouraged me to look for a pilot-scale experimental station in a tropical developing country. Las Pavas, the area where the experimental station was located belongs to the municipality of Villa Sandino in the province of Chontales, Nicaragua. Rural Chontales is a sparsely populated region in Nicaragua where the main income source is dual-purpose cattle husbandry, where whey and other organic by-products are abundant and even constitute a disposal problem. The region has a humid tropical climate with an average precipitation of 2,000 mm per year. The annual average temperature is between 25 and 28˚C (INETER, 2015). This region was included in the research project in order to explore similarities and differences of the bioremediation problems in marginalized regions from different geographic contexts.

Casa Montesano

Casa Montesano is an agroecological farm located 350 meters above sea level at which the pilot-scale experimental station was built. The farm site also functions as an educational centre where sustainable practices such as organic farming, agroforestry, green building etc. are demonstrated to the public. The farm also works as a platform for the local NGO, *Centro Integral para la Propagación de la Permacultura* (CIPP), that conducts educational projects on rural sustainable development. The farm receives volunteers and students from national and international universities who conduct research on appropriate technology.

4.2.1.2 Las Tejanas, Chinandega, Nicaragua

Las Tejanas is a rural community that belongs to the municipality of Chinandega, Nicaragua. The climate is typically dry and hot tropical with maximum temperatures as high as 42 °C. The annual precipitation is between 800 and 2000 mm. The soil type at the site is characterized as sandy loam vertisol. The land used in the experiment is currently owned by the cooperative Chinantlan who grow corn *(Zea mays),* bananas *(Musa spp.),* roselle (*Hibiscus sabdariffa),* sesame *(Sesamum indicum),* and cassava *(Manihot esculenta)* with agroecological methods. However until the early 1990s, the soil was used for cotton cultivation and was heavily sprayed with conventional fertilizers, toxaphene and other pesticides. The site was selected because it was known to have received high applications of toxaphene but no toxaphene or other POPs have been applied on the land after 1993 hence a significant field-weathering of the pesticides has taken place.

4.2.2 The pilot-scale experimental station

A pilot-scale experimental station was built in Nicaragua based on knowledge from laboratory-based research. The aim was to evaluate different amendments in circumstances that are representative of field conditions. MIUN´s previous experimental research in sparsely populated regions in the county of Jämtland was thus expanded to include another marginalized area; tropical developing countries. A site on the agroecological farm Casa Montesano, was chosen for the building of the pilot-scale experimental station with capacity for soil experiments in up to 24 compartments at a time. The experimental station for the pilot-scale bioremediation research was built between December 2012 and March 2013. I designed the experimental station

(Fig. 5) and effectuated its construction together with a fellow construction worker. The first experiment in the experimental station was started on the 4th of April 2013 (**Paper II**). The experimental station consists of 24 separate compartments in reinforced concrete and a concrete slab floor for post-treatment of soil used in the experiments at one end of the station. A roofed shed structure with a galvanized welded wire mesh was built to prevent animals and unauthorized persons from entering. The design was intended to mimic local field conditions as much as possible and facilitate the simultaneous testing of rather large quantities of contaminated soil. The soil quantity was estimated to be large enough to compensate for the inhomogeneity of the soil giving results that are representative for *in situ* remediation conditions. The soil compartments were built in two sets of 12 compartments, which was estimated as an appropriate thermal mass to buffer the soil temperature during air temperature fluctuations.

Each compartment was given a volume of 120 L: considerably larger than laboratory-scale experiments but no bigger than what one person can manage in terms of sampling and application of amendments. The depth of each compartment was 60 cm to include the first two soil horizons of common tropical soil: the organic top soil and mineral soil. The characteristics of SOM content and texture differ greatly depending on depth which affects the pollutant´s bioavailability. The inside of the compartments that comes in contact with soil was given a smooth cement plaster to prevent exchange of matter from the concrete to the soil. The compartments were provided with a lid structure of untreated wood to keep the soil from falling out. The lid was made detachable and bolted to the compartment walls to be able to remove the soil from the compartments. Further considerations regarding the design and the construction of the pilot-scale experimental station can be found in (Haller 2015).

Figure 5. *The experimental station at Casa Montesano during construction (top) and in its finished state (bottom).* *Photos by author.*

4.2.3 Soil column experiment

In response to the questions raised during the pilot-scale experiment on diesel degradation (**Paper II**), a bench-scale experiment was undertaken in order to study the vertical transport of microorganisms from ACT in soil in more detail. The failure of ACT to enhance the diesel degradation was hypothesized to be due to the difficulties for the organisms present in the ACT to migrate through the clay-rich soil and reach the contaminant **(Paper III)**. The experiment was conducted in collaboration with the *Laboratorio de Biotecnologia* (Biotechnology Laboratory) of *the National Autonomous University of Nicaragua (UNAN)* in Managua, Nicaragua[1]. Soil columns (Fig. 6) were made of galvanized steel with a volume of 1.7 l (height 32 cm and diameter (id) 8.3 cm). The column was open at the top and bottom and provided with horizontal lateral orifices with a diameter of 20 mm at 2, 10, 20 and 30 cm depth for sampling. The columns were filled by depositions of 1 cm increments of humidified soil followed by compaction with a metal pestle to reproduce field conditions of bulk density. The soil columns were sterilized in an autoclave before being placed in upright position in a UV-sterilized fume hood. Applications of 25 ml kg soil dw^{-1} of ACT were made by the use of a 10 ml pipette on the surface of the columns. Soil samples were withdrawn through the lateral orifices with a sterilized spatula, 1 and 24 h after all the compost tea had soaked into the surface of the soil columns before enumeration by culture on solid medium.

[1] This cooperation between the *Department of Ecotechnology and Sustainable Building Engineering* at MIUN and *Laboratorio de Biotecnologia* at UNAN is a result of a grant from the *Swedish Foundation for International Cooperation in Research and Higher Education,* (STINT).

Figure 6. *Application of compost teas to the soil columns (left) and sampling (right).*

Photos by author.

4.2.4 Field-scale experiment (Tejana)

The objective of the field-scale experiment (**Paper IV**) was to determine the bioaccumulation and translocation characteristics of three different cultivars of amaranth in soils contaminated with field-weathered POPs in order to identify safety issues for human consumption and/or potential for phytoremediation. Sixteen experimental plots were established with a randomized block design and four repetitions. Each plot was 6×6m with four 50 cm wide rows, 80 cm apart which were ploughed manually. Three cultivars (A. cruentus 'R127 México', A. cruentus 'Don León' and A. Caudatus 'CAC48 Perú') were selected because of their adaptation to the climatic conditions of the site. The seed were sown at 2 to 3 cm depth, 50 cm apart. After harvesting, the different parts of the plants were divided morphologically into four vegetative organs: root, stem, leaf and seed. Samples from each organ were dried at room temperature, ground and sieved through a 0.5 mm sieve. The pulverized plant samples were extracted by soxhlet extraction and analysed by GC/MS. This experiment was conducted in collaboration with the Laboratorio de Biotecnologia of the National Autonomous University of Nicaragua (UNAN) in Managua, Nicaragua[2].

[2] The study was conducted within the programme Amaranth: Future Food (project number INCO-CT-2006-032263), financed by the European Union's 6:th Framework Programme.

4.2.5. Analytical methods

Analytical methods used in the project are described below. All analysis but the GC/MS of POPs were made by myself, alone or accompanied by colleagues.

4.2.5.1 Accelerated solvent extraction

In order to deal with the large amounts of soil samples produced in the pilot-scale experiment (**Paper II**), pressurized fluid extraction was chosen as extraction method. Pressurized fluid extraction, also known as accelerated solvent extraction, permits a high extraction efficiency with a low solvent volume and a short extraction time for extracting various chemicals from a complex matrix (Luthria, Vinjamoori et al. 2004). 8 g of soil (wet weight) was mixed with 4 g diatomaceous earth and placed in 22 mL stainless steel extraction cells. Internal standards, 0.5 mL of hexamethylbenzene (HMB) solution (0.023 mol L-1 dissolved in acetone/pentane 50/50) from Sigma Aldrich® and 0.5 mL 1-chlorooctadecane solution (0.028 mol L-1 in n-hexane) from Sigma Aldrich was added to each extraction cell with a Hamilton® syringe. Pesticide quality n-hexane and acetone were used as solvents. 3 mL of the extracts were purified by column chromatography using 1 g silica gel and 2 g sodium sulphate in Pasteur pipettes stuffed with glass wool at the bottom.

4.2.5.2 GC/FID and GC/MS analysis

Gas chromatography was used to analyse the samples from the pilot- and field-scale experiments (**Paper II and IV**). A flame ionization detector was used for the DRO analysis and a mass spectrometer for toxaphene and other POPs.

GC/FID

The GC/FID analysis was made following the experimental settings as stated in EPA 8015c, with minor changes. 1 μl of the sample extract injected with an MPS2 autosampler (Gerstel). A 6890 (Agilent) gas chromatograph fitted with an Agilent HP-5 column (30 m × 0.32 mm ID, 0.25 μm film thickness) was used. The following GC program was used: Inlet 200 °C, splitless, constant flow 2.2 mL min^{-1} (He), initial oven temperature 45 °C, 3 min → 275 °C (12 °C min^{-1}), hold 12 minutes.

GC/MS

The GC/MS analysis was made following the experimental settings as stated in EPA 8081B, with minor changes. 1 µl Aliquots of the samples were injected on a 30 m non-polar DB-5MS column (0.25 mm i.d., 0.25 µm film thickness) in an Agilent gas chromatograph equipped with a mass selective detector GC/MSD 6890/5973. The following temperature programme was used: 100 °C (2 min), 20 °C min-1 to 140 °C, then 5 °C min^{-1} to 300 °C. Detection was achieved by negative chemical ionization (NCI) using methane as the reagent gas at a flow of 2 ml min-1. Both full scan and selected ion monitoring (SIM) were used.

4.2.5.3. Dehydrogenase activity

Dehydrogenase activity (DHA) was used as an indirect method to detect the presence of certain microorganisms in the soil column experiment based on methods described by Margesin (2005) and Mersi & Schinner (1991). Two g of soil ww was weighed in 20 ml test tubes, in duplicates. Two g of autoclaved soil was equally prepared in duplicates. 1.5 ml of buffer (Tris HCl, pH 7.0) and substrate (2-p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride) (INT) solution was added to samples and controls which were subsequently incubated for 2 h at 40 °C. After incubation, 10ml of extraction solution (1 part N,N dimethyl formamide with 1 part ethanol 96%) was added to each test tube before shaking every 20 min for 1 h at 27 °C, in the dark. 5 standards with concentrations of 7.4, 14.8, 22.2, 29.6 and 37 ppm iodonitrotetrazolium formazan (INTF) were prepared. The contents of the tubes were then filtered with a 70 mm paper filter Ø and the INTF concentration was measured together with the calibration standards at 464 nm against the reagent blank. The concentration of INTF was calculated from the calibration curve. The DHA was expressed as "µg of released INTF per gram soil dry mass over 2 h" using the following formula (Margesin, 2005):

$$\mu g\ INTF/(g\ dry\ soil\ \times 2h) = \frac{S - C}{wm\ \times dm}$$

where S is the INTF concentration of the soil sample (μg),
C the INTF concentration of the control (μg)
wm the soil wet mass (1 g),
and dm the soil dry mass (g).

DHA was detected in all columns but the sterile control in a similar pattern to what was found by the plate count technique. However, due to a possibly contaminated soil sample, it was decided to eliminate the DHA results from the article (**Paper III**).

4.2.5.4 Enumeration and identification of microorganisms (plate count and fluorescent microscopy)

To detect and enumerate the presence of microorganisms on different depths in the soil columns, two different methods were used. Due to uncertainties with the fluorescent microscopy analysis, only the results from the plate count were included in the article (**Paper III**).

Enumeration by culture on solid medium (plate count)

The analysis was based on Foght and Aislabie (2005) with slight modifications. In essence, 1 gram of sample was diluted in sodium chloride solution 0.85% w/v. Dilutions of $10^{-1-2-3-4}$ (bacteria) and 10^{-1-2-3} (yeast and moulds) were pipetted onto plates filled with Acumedia standard methods agar (yeasts and moulds) and Saboraud 4% dextrose agar in duplicates and spread evenly using a sterile bent glass rod. After the inoculum was absorbed into agar, the plates were inverted and placed in plastic bags to incubate at 27 °C for 48 h (bacteria) 72 h (yeasts) 120 h (moulds). Colonies on the plates containing less than 300 colonies were counted to determine a mean of the replicates and expressed as colony forming units (CFU) per gram dry mass in the original sample considering the dilution factor and dry-mass correction factor.

Direct enumeration of total bacterial cells by fluorescent microscopy

The direct enumeration method was also based on Foght and Aislabie (2005). 1 gram of the initial soil sample was weighed in a sterilized 20 ml test tube to which 8 ml sodium chloride solution (0.85 %) and 1 ml formaldehyde (1%) were added as preservative. Dilutions of 10^{-1-2-3} were prepared and homogenized in a 40 gigahertz sonicator bath for 10 minutes. A 0.2 μm pore size, black polycarbonate membrane filter, moistened with filtered sterile sodium chloride solution (0.85 %), was placed in the filter unit. 2 ml of the selected homogenized dilution was filtered through the membrane. 400 μl of DAPI (4′,6-diamino-2-phenylindole) dissolved in sterilized distilled water (5 μg/ml) was added to the surface of the filter by micropipettes and left to stain in the dark for 15 min and filtered under a gentle vacuum (≤ 25 psi). The filter was then removed from the filter unit and non-fluorescent immersion oil was added to the filter before observation at 10 X and 100 X magnification with a BX43 epiflourescent microscope equipped with UV excitation filter. A drop of immersion oil was placed on the glass microscope slide with the membrane filter on top, protected with a coverslip. A minimum of 20 fields of view (FOV), each containing 20–50 cells was counted. Randomly located FOV covering a wide area of the filter were counted, avoiding its edges. Blanks consisting only of reagents were performed at the beginning and end of sample enumeration. A reference value was also obtained by a sample from compost tea on inert sand. The blank counts were subtracted from sample counts before calculation of the total numbers. Counts were calculated on the basis of wet mass of soil, corrected for background and expressed on the basis of dry mass of soil according to the following:

$$\text{Cells/g soil wet mass} = \frac{\text{total no. of cells counted}}{\text{total no. of FOV}} \times \frac{\text{total stained area}}{\text{area of FOV}} \times \frac{1}{\text{mass of soil on filter}}$$

$$\text{Cells/g soil dry mass} = (\text{cells/g soil wet mass}) \times (\text{dry-mass conversion factor})$$

5. Results

The overall research questions of this Ph. D. were answered in different degrees by one or several studies according to the following:

Research question	Addressed in
1. What bioremediation methods may be considered appropriate in marginalized regions?	**Paper I, II and IV**
2. What concerns need to be considered in order to approach sustainable development that goes beyond pollution reduction targets in polluted areas in marginalized regions?	**Paper I**
3. What factors may limit the success of bioremediation projects in marginalized areas?	**Paper I and III**
4. What is the efficiency of some appropriate bioremediation technologies?	**Paper II and IV**
5. What constraints are related to the use of such technologies and how can these be overcome?	**Paper III and IV**

The research questions (or parts of them) have been italicized in the body text below to indicate the results that contribute to answering a particular research question.

5.1 Soil bioremediation supporting sustainable development beyond pollution reduction

In order to shed light on the research question *what concerns need to be considered in order to approach sustainable development that goes beyond pollution reduction targets in polluted area in marginalized areas?* we found it useful to structure the complex sustainability issues related to bioremediation projects in an integrated planning guide (**Paper I**). The utility of the planning guide was demonstrated with two cases: one from a tropical developing country and one from a rural area in a sparsely populated subarctic region in an industrialized country. Some of the concerns that were indicated by the use of the integrated planning guide are:

- the economic and social characteristics of the region
- the social dimension and involvement of property-owners and local community
- the understanding of local ecosystems and their capacity for self-design
- the financial capacity of the region in order to avoid promotion of technologies that are incompatible with national capabilities
- the soil temperatures and availability of sunlight (in temperate climate)
- people´s capacity to influence the process e.g. obstacles to access to the social security system etc.
- any conflicts with the people´s capacity to create a meaning (obstacles to religious, cultural or political freedom).
- the demographic development and unemployment rates.

5.1.1 Application of the integrated planning guide

The application of EE within the FSSD was useful as a planning tool for design of appropriate bioremediation projects (**Paper 1**). The integrated planning guide may give valuable input to the strategic process when bioremediation is used as a tool to reach sustainability goals beyond pollution reduction targets in marginalized regions. EE provides the environmental engineer/practitioner with insights and understanding of ecosystem functions that assist the development of appropriate technologies for soil bioremediation while the five steps of the FSSD are utilized to develop a stepwise plan for sustainable development in the region. EE is most useful on two levels of the FSSD: on level 3 to provide specific guidance and inspiration for the development of innovative energy- and resource-efficient soil bioremediation strategies and on level 5, as a qualitative monitoring tool to assess the progress towards sustainability according to the vision and goals set at level 2.

The integrated planning guide provides insight on the research question *what bioremediation methods may be appropriate in marginalized regions?*, indicating that:

- solar energy and the embodied chemical energy of the organic pollutant itself should be the primary sources to power the degradation process,
- that the plants present at the polluted site are scanned for species with capacity for bioaccumulation/phytodegradation
- that locally available waste products are used to enhance microbial degradation of target soil pollutants.

5.2 Experimental research

The experimental research shed light on opportunities as well as constraints for bioremediation in marginalized regions and contributed to answering some of the research questions. The capacity of whey to enhance the degradation of diesel and amaranth to bioaccumulate POPs can be highlighted as opportunities whereas the limited vertical migration of ACT organisms is a constraint of the implementation of that technology.

5.2.1. Diesel fuel degradation

The experiment showed that treatment of diesel contaminated ultisol with 6 mL whey kg^{-1} dw significantly increased the diesel degradation rate compared to the control (**Paper 1**). The overall slow degradation rates suggest that the high clay content of the soil used in the experiment, common in many tropical regions, may be a limiting factor for diesel degradation. In marginalized regions however, where incentives are few and money is scarce, even a moderate increase in degradation rates can be significant. The experiment provided no evidence that PA or ACT would have any positive effect on diesel degradation.

5.2.2 Microbial transport

The soil column experiment was designed as a response to the failure of ACT to enhance the degradation of diesel (**Paper II**) which was hypothesized to be due to the limited vertical transport of the introduced organisms. The experiment showed that a considerably higher fraction of the microorganisms from the CT were deposited between 2 and 10 cm in the sandy loam compared to the clay loam (**Paper III**). Bacterial deposition was concentrated to the top 2 cm in the sandy loam and then decreased abruptly after 10 cm whereas the clay loam presented an irregular pattern. Despite a favourable particle size distribution for microbial transport, the sandy soil retained a greater fraction of the microorganisms present in the CT in the top 10 cm, presumably because of the lower bulk density and higher SOM in the clay loam aided transport and growth of microorganisms. The limited migration of ACT microorganisms in sandy soil suggests that its efficiency for bioremediation or pathogen control may be limited below 10 cm depth in similar soils.

5.2.3 Bioaccumulation and translocation of POPs in amaranth

In the field experiment described in **Paper III,** concentrations of toxaphene congeners and POPs were found in all vegetative organs of the amaranth but the amounts varied considerably although a pattern of bioaccumulation in leaves and stem was evident. The average concentration of individual congeners in the four vegetative organs was strongly dominated by Parlar 39, followed by 40, 15, 31, 50, 42, 11 and 32 in descending order which approximately reflects the concentrations found in the soil. Although there was a considerable variation in bioaccumulation factor (BAF) between the different substances, many substances were accumulated to concentrations more than 10 times higher than in the soil. The concentration of toxaphene and other POPs in the edible parts of the amaranth (leaves and seeds) exceeded the maximum residue level established by the European Union for pseudo-cereals and leaf vegetables for most of the tested compounds. Of the three cultivars, *A. caudatus 'CAC48 Perú' and A. cruentus 'Don Leon'* presented the highest average BAF. None of the three cultivars can be considered a panacea hyperaccumulator for either toxaphene alone or in conjunction with other POPs but since many agricultural soils comprise a range of different contaminants, the broad-spectrum bioaccumulating capacity of amaranth can make it an interesting candidate for phytoremediation of such sites. The field-scale experiment provides valuable knowledge regarding the research questions about *the efficiency of appropriate bioremediation technologies* (phytoremediation of POPs with amaranth) suggesting that such a *method* *could be appropriate in marginalized regions* but points out a *major constraint:* the lack of knowledge on translocation patterns of various plants. This needs to be *overcome* by more experimental research.

6. Discussion

This doctoral thesis project sets out to explore the opportunities and constraints for bioremediation of contaminated soil in marginalized regions which indeed turned out to be a challenge with many unexpected constraints but also unexpected opportunities. In the introductory section of this dissertation, I begin by describing the mismanagement of human ecosystems that causes multimillion losses to society and threatens ecosystem functions, human health, and food security (Berkes et al., 2008, Rockström et al., 2009, Steffen et al., 2015, Nellemann and Corcoran, 2010). One of the cited sources, the UNEP report *Dead planet, living planet: biodiversity and ecosystem restoration for sustainable development* (Nellemann and Corcoran, 2010) essentially concludes that more than 60 % of the world's ecosystems can be considered degraded. Another, more uplifting conclusion from the same report is that it is fully possible to repair many of these degraded ecosystems. Not only is it possible, there are strong economic incentives to do so in terms of savings for the state and municipal governments, business prospects and opportunities for poverty reduction. Although there is a risk that marginalized regions will continue to receive less attention than urban industrial sites, this thesis shows that there are opportunities for appropriate bioremediation technologies to be part of the great endeavour to restore the degraded ecosystems in a way that also supports sustainable development beyond the goals of pollution reduction targets in such regions.

There are many pitfalls however that must be avoided through detailed analysis of the contaminated site using tools such as the integrated planning guide proposed in **Paper I.** Contaminated sites in marginalized regions demand a different approach than what would be suitable for a similar site in an urban industrial context. The use of the five concepts of EE within the FSSD, as an integrated planning guide, provide supportive guidelines when designing appropriate bioremediation projects for such locations by providing a stepwise guide that indicates what to do and more importantly, what not to do in order to support sustainable development. The systems theoretical research of the thesis project indicated some potential ways for making bioremediation part of a strategy that supports sustainable development such as: using solar energy and the embodied chemical energy of the pollutant as a primary energy source, screening the plants present at the polluted site for species with capacity for bioaccumulation/phytodegradation and using locally available waste products to enhance microbial degradation of target soil pollutants.

Since the sustainability principles are designed as exclusion criteria (Missimer et al., 2016a, Missimer et al., 2016b), they delineate the space within which social systems and ecosystems can function and evolve. Factors that are incompatible with the eight sustainability principles are thus *factors that may limit the success of bioremediation projects in marginalized areas.* Technologies that are not compatible with sustainable practices should be discarded and the integrated planning guide was effectively used to reject such technologies on the two cases from marginalized regions. The integrated planning guide was also tested by Iraguha, Simons et al (2016) and Pronoza, Dyer et al. (2016) who used it to assess the viability and sustainability potential of implementing amaranth in Nicaragua to clean soils polluted with toxaphene and ensure the sustainability of the bioremediation strategy based on a combination of alginates, biochar and *Jatropha curcas* L. to reduce toxaphene, heavy metals and toxic metalloids from contaminated soils in Chinandega.

Of the proposed by-products (**Paper I**, Haller, Jonsson et al. 2012; Jonsson and Haller 2014) only whey was experimentally confirmed to have a positive effect on diesel fuel degradation (**Paper II**). The effect of whey is in accordance with previously reported results from laboratory experiments (Östberg et al., 2007a, Östberg et al., 2006, Östberg et al., 2007b). Based on previous assessments of the sustainability performance of the whey method (Jonsson and Haller, 2014, Haller et al., 2012, Tajam et al., 2010), this suggests that biostimulation with whey may be an *appropriate bioremediation method in some marginalized regions.* ACT and PA showed no positive effect on diesel degradation in this type of soil but they may prove efficient for other types of contaminants or in different soil or climatic condition. The slow overall degradation rates due to the high clay-content in ultisol however, could be a considerable constraint for efficient pollutant removal in full scale applications. The soil cylinders experiment shows that the vertical migration of added ACT microorganisms is limited in soils with a high bulk density which may make bioaugmentation strategies challenging in such soils (**Paper III**). Our results indicate that in less dense soil with higher soil SOM content the efficiency may be higher. The results of the experiment point out an important *factor that may limit the success of bioremediation projects in marginalized areas:* high soil bulk densities due to the compaction of soils that limits the vertical migration of bioaugmentation organisms which limit their ability to come close to the contaminant. Contaminated sites in marginalized regions are likely to be compacted due to cattle or heavy machinery.

The three species of amaranth tested in the field experiment effectively bioaccumulated toxaphene and other POPs (**Paper IV**) which make these species potentially interesting candidates for phytoremediation in the region but the harvested crops should not be used for human or animal consumption because the toxic compounds were translocated to the edible parts.

New knowledge created during this project include (**Paper I, II, II and IV**):

- what makes remediation appropriate in marginalized regions,
- the capacity of three amendments to enhance diesel degradation,
- the vertical transport of ACT organisms in soil and
- patterns of POP bioaccumulation and translocation in three species of amaranth

There are remaining ambiguities to be unravelled however before a convincing argument can be presented to policy-makers on how to address polluted land in marginalized regions in a way that will support sustainable development. Among the constraints that remain to be overcome it is noteworthy that many are associated with lack of knowledge (about the biostimulation potential of different organic products or bioaccumulation/translocation pattern of different plants for instance) and are thus surmountable if more research is undertaken to answer the questions raised. Some of the questions or new research topics that emerged during the project that future research projects may address include:

1. the magnitude and the geographical position of polluted sites in many regions in developing countries such as Nicaragua.
2. how the biodegradation of different contaminants is affected by soil bulk density.
3. experiments on the potential of whey, ACT and PA to enhance biodegradation of contaminants other than diesel fuel.
4. the effect of the high nutrient applications (necessary to compensate for the carbon in the pollutant) on the microbial community from different supply forms and application rates of fertilizers
5. the bioaccumulation and translocation patterns in many common agriculture crops being grown and consumed in Chinandega to avoid people being exposed to toxic levels of pollutants
6. an inventory of cultivated, native and naturalized plants present on the most contaminated sites in Chinandega to identify POP-tolerant species for phytoremediation applications.

7. Conclusions

Contaminated sites in marginalized regions such as rural areas in developing countries or sparsely populated areas in industrialized countries need strategies that meet a different set of criteria than urban industrial sites in order to be appropriate. This thesis project has demonstrated that bioremediation based on readily available organic by-products as amendments or on locally present plants for phytoremediation can be appropriate choices of technology in marginalized regions. A detailed analysis of the contaminated site using tools such as the proposed integrated planning guide is necessary to design appropriate bioremediation strategies. The tested integrated planning guide was demonstrated to give valuable input to the strategic process when bioremediation is used as a tool to reach sustainability goals beyond pollution reduction targets on two cases in marginalized regions. Results from pilot-scale experiments confirm that whey can significantly increase the biodegradation rate of diesel fuel in soil, but the slow overall degradation rates due to the high clay-content in ultisol could be a considerable constraint for efficient pollutant removal in full scale applications. Results from an experiment in soil cylinders show that the vertical migration of added microorganisms was limited in dense soils. Three species of amaranth tested in the field experiment effectively bioaccumulated toxaphene and other persistent organic pollutants which make these species potentially interesting candidates for phytoremediation in the region but the harvested crops should not be used for human or animal consumption because the toxic compounds were translocated to the edible parts.

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