Phytoremediation of heavy metal polluted soils in marginalised regions: opportunities, limitations and sustainable development
ABSTRACT

Soil pollution is one of the problems that obstruct sustainable development in the affected regions, posing a threat to the local environment, ecosystems and human wellbeing. Phytoremediation is one of the techniques used to clean polluted soils. It relies on the ability of some plants to absorb or stabilize certain substances from soil, including organic and inorganic pollutants. Amaranth was chosen as a potential candidate for the proposed phytoremediation project in Chinandega region, Nicaragua, an area that was heavily used for cotton production in the last century, and is now characterised as having high levels of soil pollution, Cd being one of the most common one. The aspects such as opportunities and limitations for the designing of such a project, as well as its contribution to the sustainable development of the region were examined in this thesis. To further support the investigation, the laboratory experiment was performed to study the uptake rate of Cd by amaranth in a greenhouse conditions.

The results of the study show that the main opportunities for the design of this project would be the possibility of combining the cleaning of soil with co-benefits such as producing food and energy, as well as additional removal of organic pollutants. The main limitations would be the lack of initial data about the pollution, and possible economic losses due to inability of using amaranth for food. The implementation of this project in real life would mean a support of sustainable development of the Chinandega region on many levels, including social, environmental and economic benefits. The results of the laboratory pot experiment are yet to be included in the study, as the experiment still continues.
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Appendix 2. Height of collected plants from the second sampling, cm.
1 INTRODUCTION

1.1 Soil pollution in marginalised regions and sustainable development

Soil pollution is one of the problems that obstruct sustainable development in the affected regions, posing a threat to the local environment, ecosystems and human wellbeing. Toxic heavy metals such as Cd, Hg, Pb, As, etc. are common and dangerous pollutants (Batty, 2013). Polluted soil creates lack of arable land, depriving local communities of economic profits, as well as posing a health risk.

Marginalised regions are especially affected. These regions are characterised by many social, ecological and economic challenges, such as the lack of economic support and incentives, weak governance, lack of knowledge and skills, and the inhabitants of these regions have limited possibilities in participating or influencing the decision-making process of the society (Haller, 2017). These factors make the implementation of remediation technologies in those areas more difficult.

In Nicaragua, the soil pollution problem exists on agricultural fields, namely on former cotton fields in Chinandega region, which were heavily exploited in the 1960s - 70s (up until 1990s) with heavy application of different chemicals in forms of fertilisers and pesticides. This resulted in today’s contamination of soil with organic and inorganic pollutants. Heavy metal contamination is of a big concern, with Cd being one of the pollutants. Consequently, this makes those valuable fields unfit to safe exploitation, but still, some activities do occur on such soils, perhaps even unintentionally (Haller, 2017). In Nicaragua, the polluted cotton fields are still used by poor local communities to produce food. This puts in danger the wellbeing of people who have no other choice but to use that soil. It also further affects the environment by polluting rivers and lakes, and allowing the pollutants to move further up the food chain.

Given the overall world trend of trying to step on the sustainable development path, problems such as those described above hardly seem like a fitting phenomenon. Thus, cleaning of soil becomes obvious and necessary, as well as finding a way to make the cleaning process fit to the local specifications of the marginalised region, thus contributing to the sustainable development.

Phytoremediation is one of the techniques to clean polluted soils. It relies on the ability of some plants to absorb or stabilize certain substances from soil, including organic and inorganic pollutants. It is a cost-effective technology, which seems suitable to the circumstances of the marginalised region.
Amaranth is explored as one of the locally appropriate plants with potentially good ability to clean soils from both organic and inorganic pollutants. It is a plant commonly grown in Asia and South America, used mainly for its edible seeds, leaves and stems. Historically, several species of amaranth provided cultures such as the Aztecs with the majority of their diet and also dyes for fabrics, therefore playing a huge part in the indigenous culture (Fomsgaard et al., 2010). The costs of establishing and harvesting an Amaranth crop are relatively low when compared to conventional crops like wheat (South African Department of Agriculture and Fisheries, 2010). Taking into account its availability and simplicity in cultivating, it was chosen like a suitable study object.

1.2 Goals and scope of the thesis

The overarching goal, or the focal point of this thesis was to see how a phytoremediation project will contribute to the sustainable development of the affected region in Nicaragua. Three main objectives were chosen based on that:

1. To determine the uptake rate of Cd by amaranth;
2. to investigate the potential of using amaranth in a remediation of heavy metal polluted soils (particularly with Cd);
3. to define opportunities and limitations for the design of a phytoremediation project on the polluted agricultural fields of Chinandega, Nicaragua.

Knowing the uptake rate (that is, the dynamics of Cd accumulation over time during different stages of plant growth) is important to make best decisions during the project design regarding time frames and resources put into it, as well as money. The success of phytoremediation depends on many varying factors. That is why it is important to study all aspects of this process, such as physiological mechanisms of uptake and translocation patterns of pollutants in plants. A lot of research has been done in this area (Ali, 2013; Batty, 2013), but still some unexplored points remain, and the uptake rate is one of those points, therefore during this thesis work the author tried to shed some light on this matter.

The goals were chosen so as to reflect immediate and greatest needs of the ecosystem and local community, and were based on the information available about the affected region and previous research. The purpose was to look at the proposed phytoremediation method in the whole and give general and basic recommendations which would give a good start for practical real-life realisation of a project on soil remediation and sustainable development within the study region. Very detailed recommendations are not possible to give at this stage of planning, as concrete
parameters and the remediation strategy need to be chosen first. This is more to give an idea of what to think about when designing the project from the scratch.

To reach those goals, a laboratory pot experiment was performed to study the uptake rate of Cd by amaranth in a greenhouse conditions, as well as a literature review in an attempt to collect information from previous similar projects, and the assessment of the proposed project from a sustainability point of view.
2 METHODS

2.1 Review of previous work

Before starting the planning for the project, a comprehensive examination of up-to-date scientific literature was performed. Focus was made on articles which described similar research to the one we attempted to make, namely on phytoremediation of Cd polluted soils by plants such as amaranth. In this way, as much existing information and findings in this specific theme as possible were collected, and, also, “white spots” in this topic were found, where more research should be done.

The findings were summarised in a table that includes the most necessary information for easy comparison, such as species of amaranth used in studies, chemicals used to pollute soil, concentration of Cd in soil, soil type, parts of the plant analysed, harvest time, translocation and bioaccumulation of cadmium the plant. This information was used to design the pot-experiment.

2.2 Experimental setup for determination of Cd uptake rate

The laboratory experiment was setup to determine Cd uptake rates in Amaranth during the period of 4 month. The plants were grown in pots (volume 10L), with at least three amaranth plants in each pot, to get sufficient biomass amount for the subsequent analysis. Schematic representation of experimental set up is shown in table 1.

<table>
<thead>
<tr>
<th></th>
<th>90 days</th>
<th>120 days</th>
<th>150 days</th>
<th>180 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>C – control</td>
<td>X*XX</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
</tr>
<tr>
<td>Cd2 – 2 mg/kg dw soil</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
</tr>
<tr>
<td>Cd20 – 20 mg/kg dw soil</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
</tr>
</tbody>
</table>

*X – 1 pot

There were 36 pots in total, of which 12 had non-polluted soil (for characteristics see section 2.3) with 0 mg of Cd added (C - control), 12 had Cd in concentration 2
mg/kg dry weight (dw) soil (Cd2), and the remaining 12 had Cd in concentration 20 mg/kg dw soil (Cd20).

2.3 Soil characterization

The soil for the experiment was prepared by mixing sand and peat soil in ratio 6:1. The characterization of peat soil is (per m³): peat 0.95 m³, stone powder 0-2 mm 0.05m³, magnesium lime 3.5 kg, N P K 11-5-18 – 1.5 kg. The resulting substrate had 10% organic matter content and pH 5. Sand used had particle size 0.5 mm.

CdSO₄*8/3H₂O (acquired from Sigma Aldrich) was diluted in deionized water, and added to soil by spraying the needed amount of solution on soil and mixing it at the same time, to ensure even distribution of the contaminant. Each pot contained 8 kg of soil.

2.4 Growing conditions

Targeted growth conditions for the amaranth were temperature of 24-26°C and humidity of 40-50%, with 14 hours of light. A heater was placed in the experimental room to ensure the desired temperature, and some water was spread on the floor. The resulting temperature and humidity curves over time are presented in figure 1. 9 lamps were used and distributed evenly on the height of 1 m above the plants to ensure artificial lightning. Pots were watered according to the water loss, never to the point of drainage to avoid leaking of Cd. About 200 ml of water was added to each pot every 4-5 days.

![Temperature and humidity graph](image)

Figure 1. Changes of temperature and humidity in the experimental room during the duration of the plant growth.
2.5 Sample analysis

To assess the uptake rate of Cd, the plant samples were taken at different time intervals – first after 90 days from the planting day, then after 120, 150, and 180 days. These time intervals gave the opportunity to study the Cd content in plant tissue during the different plant stages – small seedling, early vegetative stage, late vegetative stage, flowering stage. Plant material was taken from the roots, stems, leaves of the plants, and flowers when they appeared. Plant material from all plants of the same pot was combined to get the sufficient amount.

Sampled plants were measured in height, extracted from the pots as intact as possible, cleaned from the soil, photographed, and divided into different parts mentioned above. The plant material was dried in an oven at 105°C during a minimum of 5 hours and stored in a desiccator to. Dried material was crushed and sieved through a 600 µm sieve to prepare it for digestion.

Then samples were digested according to EPA 200.8 for subsequent analysis by ICPMS to determine cadmium content.

The data acquired to this day (height of the plants) were analysed using one-way analysis of variance (ANOVA).

2.6 Assessing opportunities and limitations of a phytoremediation project in Nicaragua and evaluating its contribution to the sustainable development of the region

To assess the potential of using Amaranth for phytoremediation in real-life field conditions in marginalised region, and also to find out possible opportunities and limitations of the project, an attempt was made to look at the project from different points of view (e.g. biological, agricultural, financial). Scientific literature (mostly peer-reviewed scientific articles, as well as book chapters) was used to perform this analysis.

To show the contribution that the implementation of the phytoremediation method might have to the sustainable development of the affected region, both during and after the project, the United Nation’s 17 Sustainable Development Goals (SDGs), adopted in 2015, were used to identify specific ways in which it will benefit the region.
3 RESULTS

3.1 Phytoremediation of cadmium – a literature review

3.1.1 Land contamination.

Anthropogenic activities such as mining, extraction of metals from ores and their subsequent use in different applications across industries led to the mobilization of many elements, among which heavy metals are also present. When these elements are released in the environment, they accumulate there because they are not biodegradable, and can be transferred along the food chains. Many of them pose a threat to environmental functions, living things and the human health (Ali et al., 2013).

Heavy metals are classified as essential (which are needed in very small quantities for normal functioning of physiological processes in living organisms) and non-essential (not needed for any physiological functions). Table 2 gives lists of essential and non-essential heavy metals.

Table 2. List of essential and non-essential heavy metals.

<table>
<thead>
<tr>
<th>Essential heavy metals</th>
<th>Non-essential heavy metals and metalloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, Mn, Cu, Zn, Ni</td>
<td>Cd, Pb, As, Hg, Cr</td>
</tr>
</tbody>
</table>

Land contamination is a global and increasing problem, given the current need of land for housing, food and energy production, leisure and other (Batty, 2013).


It has been reported that Cd has carcinogenic, mutagenic, and teratogenic effect on human health; acts as an endocrine disruptor; interferes with calcium regulation in biological systems; causes renal failure and chronic anemia (Ali et al., 2013).

3.1.2 Phytoremediation.

Conventional soil remediation technologies are generally not supporting sustainable development ideas, since they involve the use of potentially harmful chemicals, use of transportation (especially for ex-situ methods) which contributes to
green-house gas (GHG) emissions, or thermal treatment that negatively influences physical, chemical and biological characteristics of the soil. Phytoremediation emerges as a promising, environmentally friendly and cost effective technology (Batty, 2013; Witters et al., 2012).

Hyperaccumulators are the plants whose accumulation of heavy metals in their aboveground part surpasses the levels of those metals in the soil or in the other non-accumulating plants nearby. There is no standard for hyperaccumulators on which all scientists agree, but the most cited one (Baker and Brooks, 1989) specifies that for the plant to be considered a hyperaccumulator of Cd, it should accumulate 100 mg kg−1 dry weight Cd in its shoots when grown on Cd rich soils. Also, the shoot-to-root translocation factor of hyperaccumulators is usually higher than 1 (although it cannot be used as the only criteria for determining hyperaccumulation) (Ali, 2013).

It is hard to say for sure if amaranth is a hyperaccumulator of Cd or not. It is not mentioned anywhere except a few studies such as Li et al. (2012) and Fan (2009). The only more or less feasible conclusion that can be drawn is that some species of amaranth are potential Cd hyperaccumulators (Ding et al., 2013).

Some other plants which are reported to be the hyperaccumulators for Cd are *Azolla pinnata, Eleocharis acicularis, Rorippa globose, Solanum photeinocarpum, Thlaspi caerulescens* (Ali, 2013). It is reported that Cd can be uptaken together with Zn, since these metals tend to occur together at many contaminated sites and have chemical similarity, which means that hyperaccumulators of Zn can also be used for Cd uptake (Brooks, 1998; Ye-Tao et al., 2012).

Success of phytoremediation may depend on the initial concentration of metals in the soil. The example of this can be a study by Zhao et al. (2003), which showed that the bioaccumulation of Zn and Cd in plants decreased with increased concentration of metals in soil, making the phytoremediation of the heavy contaminated sites less successful.

3.1.3 Mechanisms of uptake of heavy metals

Usually, uptake of metals into plants occurs from the aqueous phase and consists of 5 main stages (figure 2).

In the beginning, some mobilisation of heavy metals in soil takes place. After uptake of metal ions by plant roots, they can either accumulate there, or move further through xylem vessels, sometimes reaching the shoots, where they are deposited in vacuoles. Heavy metal sequestration is performed as one of the ways to remove excess
metal ions from cytosol. Finally, compartmentalization of complexed metal in vacuoles is a part of the tolerance mechanism in hyperaccumulating plants. In his paper, Ali (2013) lists the next ways by which plant cells tolerate heavy metals: “cell wall binding, active transport of ions into the vacuole and chelation through the induction of metal-binding peptides and the formation of metal complexes”.

![Diagram](image)

Figure 2. The schematic representation of the uptake process of metal ions in plants (reconstructed from Ali, 2013).

Information about the physiological and molecular mechanisms by which plants enhance uptake of metals is scarce in the scientific literature and is limited to only certain metals and hyperaccumulators, so there is a gap in this area. In summary, these processes include the overexpression of genes that are responsible for transportation of metals, the overproduction of amino acids such as histidine and nicotamine that act as ligands within the xylem, or enhanced activity of existing transporter mechanisms as in the case of As (Batty, 2013).

### 3.1.4 Fate of pollutants in plants and what happens to Amaranth after phytoremediation.

There are several ways that the pollutants can be treated in plants.

Phytoextraction (phytoaccumulation) means that the pollutant is taken up from soil through roots and is accumulated in the above ground parts of the plants, e.g. leaf tissues, shoots. This is one of the desirable processes, as the harvest of root biomass is generally not feasible and the removal and treatment of aboveground parts if much easier.

Phytostabilisation is the process when plants stabilise contaminants in soil, thus preventing their movement in the environment by reducing their mobility and bioavailability. It can be done through chemical reduction, oxidation or precipitation.
of the pollutant. This method provides only temporary solution, since pollutants remain in the soil.

Phytodegradation is the breakdown of pollutants in the soil with the help of symbiotic microbes in the rhizosphere. Plants excrete exudates which stimulate microbial growth. In addition, plants secrete enzymes which can degrade pollutants by themselves, not depending on rhizospheric microorganisms.

Phytovolatilization refers to the uptake of water soluble contaminants via transpiration and its subsequent conversion to a gaseous (volatile) form which is later released in the atmosphere (Ali, 2013; Batty, 2013).

If we talk about what happens specifically in amaranth, there is some information reported in scientific literature.

It was described that A. hypochondriacus leaves can alleviate Cd toxicity by bonding large amounts of accumulated Cd into pectins and proteins, which is a less harmful form compared to inorganic Cd and its water-soluble forms (Ding et al., 2013). There are also some insects which can feed on amaranth and accumulate Cd in their tissues, as well as excrete it with feces (Ding et al., 2013), and although currently it is not reported to have any beneficial effect, in theory it can be used as a food material for silkworm, for example.

The usual process of treating plants used for phytoremediation consists of following steps (according to Ali, 2013):

1. Accumulation of heavy metals in the plant biomass;
2. Harvesting of the biomass after the uptake of metals;
3. Pre-treatment and combustion of biomass to reduce volume;
4. Disposal of the resulting product in a safe way in specialized dumps.

Instead of just combusting and disposing of biomass used in phytoremediation, it can be utilized for obtaining energy, giving economic profits to the phytoremediation project and contributing to the CO₂ abatement as a result of renewable energy production (Witters et al., 2012). More and more focus is given to the use of ‘by-products’ of phytoremediation, meaning the processed plant biomass. It was calculated that using this biomass for energy production and metal recovery makes phytoremediation more financially feasible, at the same time sparing the inconvenience and risks of disposal of contaminated material (Jiang et al., 2015). In marginalised regions this way of dealing with biomass will have an especially good impact, given the scarcity and, therefore, need for energy (primarily electricity,
heating), as well as lower ability to handle the environmental risks of dealing with contaminated biomass by just disposing it.

3.1.5 Previous research

The full table with all summarised information can be found in the appendix 1. The collected information helped to design the pot experiment in the most efficient way (for example, choosing the scale of the experiment, harvest time, deciding the way and the solution to pollute soil with, choosing the concentrations of Cd, which parts of the plant to analyse, etc.) and to take into consideration some points (optimal growing conditions for the plants: temperature, light, number of plants in each pot, watering frequency, size of the pots), as well as choose the methodology for sample treatment and analysis of data.

It was noticed that most of the experiments about Cd uptake by amaranth were done with the purpose to find out the translocation (or transfer) factor (only the term ‘translocation factor’ will be used further for clarity) and bioconcentration factor, and also concentrations of Cd in different parts of the plant, such as roots, stems, leaves, shoots. Translocation factor is as the ratio of metal ion concentration in plant shoots to that in plant roots; bioconcentration factor is the ratio of metal concentration in plant roots or shoots to that in the soil (only for the added Cd treatments) (Abe, 2008; Zhang et al., 2010; Ko et al., 2014). But at the same time, only one study was found where the uptake of Cd by amaranth was studied over time to find out the rate and dynamics of this process (Li et al., 2013). So, the decision was made to focus on this aspect of the phytoremediation process in our experiment, in view of the lack of existing data.

There are no strong consistencies among all the papers regarding the results. While some found out that most of Cd is accumulated in leaves (Li et al., 2013; Li et al., 2012), other found that roots (Ko et al., 2014; Fan and Wei, 2014; Zhang et al., 2010; Abe et al., 2008) had the highest concentration of Cd. The same is for the transfer factor and bioaccumulation factor of amaranth. Some studies have found that the translocation factor was ≥1 (Zhang et al., 2010; Li et al., 2012), while for the rest it varied from 0.25 to 0.49. The bioaccumulation factor for all studies was approximately similar, except for one study where it was much higher (Li et al., 2012), which can be explained by the effect of applied fertilisers used in this study.
3.2 Experimental work

3.2.1 Cultivation of amaranth

The resulting set-up for the pot experiment is shown in the figure 3.

Figure 3. Finished experimental set-up (photo by the author).

The growth rate of Amaranth was slower than expected. After 45 days of cultivating, the average height of the plants was about 5-7 centimetres.

There is also a clearly visible difference in growth of plants depending on the Cd concentration in soil (figure 4). Only some of the first planted seeds in the pots with higher Cd concentration germinated after 20 days, which was not enough for the experiment. So, the second round of seed planting was done in those pots, which gave better results and most of the seeds germinated. Nevertheless, in control pots and pots with 2 mg/kg Cd plants were in a good condition, while in the pots with 20 mg/kg of Cd plants showed slower growth and smaller size, showing signs of phytotoxicity.
Figure 4. Difference in growth of Amaranth after 2 month: a – pots with 20 mg/kg Cd; b – pots with 0 mg Cd added (control) (photo by the author).

In the early stages of plant development, the problem with light distribution occurred which caused uneven vegetation, as can be seen on the figure 5.

Figure 5. Unequal vegetation (photo by the author).
The set of pots in the left lower quarter of the picture are the pots with the highest concentration of Cd so poor vegetation there is attributed to the toxicity effect and not the lack of light. On the other hand, lush vegetation in the top middle is most likely due to the higher exposure to light compared to the pots located on the edges, near the walls. Decision to move the lights higher was made, and some rearrangement of the pots was done so as to ensure equal distribution of the light, which eliminated the problem.

After 120 days since the planting date (corresponds to the second round of sampling), the height of the collected plants was measured.

These data were analysed using one-way ANOVA analysis and subsequent multiple comparison test (Tukey HSD). Results are presented in the figure 5 (raw data can be found in appendix 2).

![Graph](image)

Figure 5. Graph with the mean for each group (horizontal bold line) and a vertical “error bar” containing values within one standard deviation of the mean, cm. C – control, Cd2 – samples from pots with 2 mg of Cd per kg dw soil, Cd20 - samples from pots with 20 mg of Cd per kg dw soil.

Analysis showed significant difference between Cd20 and the other two groups (p-value <0.05), while no significant difference was observed between C and Cd2 groups. By the time of the next two sampling events, plants with Cd20 treatment did
not survive, and therefore were not included in the later analysis. The height of C and Cd2 plants was also measured and statistically compared for the last two samplings and no significant difference between them was found (therefore this data is not displayed).

In the middle point of plant cultivation some flowering occurred (figure 6). It has to be noted that flowering was registered only in the plants with Cd2 treatment.

![Flowering](image)

Figure 6. Flowering (photo by the author).

### 3.2.2 Uptake rate of Cd by amaranth

This section will be updated when the remaining result of the experiment will be available.

### 3.3 Assessing opportunities and limitations of a phytoremediation project in Nicaragua

There is no detailed information about the levels of pollution of the agricultural soils, let alone the Cd concentration in soils in Nicaragua. This is the result of a lack of governance and environmental control, typical for marginalised regions (Haller, 2017). Ideally, an investigation should be done before making a phytoremediation project, to
determine the severity of the problem and decide on the most fitting phytoremediation technique.

While designing the project, the main aim should be kept in mind (fig. 4). If the main aim is to use cultivated plant for food, then strategies to restrict metal accumulation in the plant and its further transfer along the food chain should be developed (so called phytoexclusion strategy). If the main goal is to use chosen plant for further energy production, then the preference should be given to energy crops which can tolerate metal toxicity and develop high biomass (phytostabilisation strategy). And if the main goal is to clean the soil from the toxic metal and use it in the future for cultivation of some other crops, then hyperaccumulating plants should be used. They extract metal ions from soil and accumulate them in their tissues in high concentrations, usually in the above ground parts - leaves, stems, shoots (Ye-Tao et al., 2012; see also section 3.1.2).

Figure 4. General guidelines for designing a phytoremediation project (reconstructed from Ye-Tao et al., 2012).

In the case of Cd pollution and the Amaranth as the chosen plant, the main goal is soil clean-up, with the possible co-benefits of using Amaranth for animal of human food (if it will be safe) or energy production.
The application of fertilisers as well as pH amendments can be considered for the enhancement of Cd removal from the soil. It was found that lowering pH of the soil is having a facilitating effect on Cd uptake by Amaranth (Eriksson, 1989), as well as adding fertilizers (Li et al., 2012). An option for Amaranth can be an application of chicken manure (high N content) from local poultry farms. A study by Law-Jgbomo & Ajavi (2009) has shown an increase in crop yield when chicken manure is applied. Moreover, the application of manure as an organic fertiliser is contributing to lowering of soil pH. At the same time, amaranth grains can be used as a feed for the poultry.

A monoculture of amaranth may result in increasingly poor yields, so a crop rotation using nitrogen fixing plants, such as legumes, should be considered to prevent the loss of nutrients (Formsgaard et al., 2010).

Another aspect that can be considered for this project is the co-cropping system. It consists of planting a hyperaccumulator (in our case Amaranth) with a non-accumulator (typically a food crop). The end result is the increased growth of a non-accumulator plus its reduced metal uptake (allowing farmers to use it as a safe food crop), and at the same time increased metal uptake by the hyperaccumulator, which cannot be achieved in monoculture. The existing strategies for co-cropping (or intercropping) on Cd polluted soils include growing *Medicago sativa* – *Brassica juncea*, maize – *Sedum alfredii* (non-accumulator – hyperaccumulator, respectively). It should be noted that in some cases both the normal crop and the hyperaccumulator show increased uptake of Cd, which means that the normal crop cannot be used for food anymore (although the hyperaccumulator can still be used for phytoextraction), so the co-cropping plants should be chosen carefully after small-scale trials (Ye-Tao et al., 2012). Moreover, the suitable pair for co-cropping with Amaranth is yet to be found, as no research was done in this area.

Agricultural soils in the Chinandega region have been reported to be polluted with organic pollutants also, namely toxaphene. This implies the presence of co-contaminated sites, with both organic and inorganic pollutants. In this case, the interactions between substances and their combined effect should also be considered when developing the phytoremediation strategy. It has been reported that presence of organic pollutants affects metal bioavailability, and metals, in turn, can affect uptake or biodegradation of organics by microorganisms. The combination of pollutants may also affect plant growth. For example, the presence of Cd enhanced the accumulation of pyrene (an organic pollutant) in maize, and at the same time uptake of Cd in plants was reduced in the presence of pyrene (Batty, 2013).
The problem of co-contamination can be solved for example with the help of engineered bacteria or a diverse community of plants and microorganisms which complement each other. The same limitations that apply to the individual pollutants should be considered, same as the results of their combination. Although limited, there are reports of the successful remediation of both organic and inorganic pollutants (Batty, 2013).

As for the question of dealing with the amaranth biomass after the phytoremediation is over, then the combustion of plants for energy production is the most fitted option for the Nicaraguan context. Electricity is in high demand in Nicaragua and most of it is produced with the means of burning fossil fuels (namely oil) (Mayorga, 2005), so the burning of biomass will not only contribute to improvement of basic human needs but also will lower CO₂ emissions in the atmosphere (by substitution of fossil fuels) and make the project more cost effective.

The implementation of the phytoremediation on a commercial scale currently is still not satisfying (Witters et al., 2011). The decision-making process during the implementation of the phytoremediation project in real life is a crucial step. In this light, cost-benefit analysis is a useful asset in the tool box of the researchers. A two-year project is used as an example to calculate costs and benefits of phytoremediation of heavy metal polluted soil in the study of Wan et al., 2016. The costs were divided into initial capital and operational costs, and calculated in US$ per ha. Benefit included benefit during and after remediation. The end results were the following: the costs were US$75,375.2/ha, and the benefits would offset the costs in less than seven years (Wan et al., 2016). Other study has also shown that economic benefits can be drawn from the phytoremediation process when the potential income from using the cleaned lands for crop production over time is taken into account (Lewandowski et al., 2006). This could be used as a guideline for the assessment of the Amaranth project, and could persuade the municipalities in taking a closer look at this option and actually investing some money, or at least just not to obstruct its implementation.

However, it should not be forgotten that phytoremediation is just a part of the solution to manage contaminated soils, and it should be considered as an element to be included in sustainable production systems adapted to contaminated environments (Ye-Tao et al., 2012).
3.4 Phytoremediation of Cd polluted soils in Chinandega, Nicaragua, and sustainable development of the region

According to the United Nation’s 2030 Agenda for Sustainable Development, which was adopted in September 2015, there are 17 Sustainable Development Goals (SDGs) (fig. 5), which cover a broad range of issues and are supposed to ensure sustainable development of the world. The issues include poverty and hunger, climate change, protecting natural resources, social justice and peace, among others (Sustainable Development Goals…, 2015).

Thereby, making the soil clean, i.e. remediating it, would be one of many ways to contribute to the SDGs. Implementing this remediation project in real life in Nicaragua becomes an important and necessary step on the way to sustainable development, given that it would help to solve problems born as a result of Cd pollution of soil.

If the implementation of phytoremediation project will be successful and will reach end goals, it will contribute to:

- 1st SDG by providing local people with work and taking benefits from amaranth yields (either through burning biomass to produce energy or through using amaranth grains as food, providing it does not have a concentration of Cd that is more than allowed);
- 2nd SDG by creating possibility of food production during the remediation process through the use of either co-cropping system or the use of amaranth edible parts itself (providing it does not have a concentration of Cd that is more than allowed);
- 3rd SDG by mitigating the health risk of Cd intoxication for local community;
- 6th SDG by reducing Cd leaking to the ground waters and rivers, and subsequently drinking water;
- 7th SDG by a possibility or energy recovery from used amaranth biomass after the remediation process.
- 8th SDG by using amaranth as a relatively cheap crop; creating workplaces for local people on the amaranth plantation (providing that working conditions will be safe and fare), and giving a possibility to profit from the cleaned soils in the future by growing other crops;
- 9th SDG by developing local infrastructure needed for the project (roads, working stations), and creating opportunities for attracting innovations;
- 11th SDG by using locally available and important plant, local customs and experiences in cultivation, insuring local community participation in the decision making of the project, and making them the immediate beneficiary of the phytoremediation outcome;
- 12th SDG by using local poultry manure as a fertiliser for growing amaranth and its grain, in turn, as a feed to poultry, creating a “closed” recycling system.
- 13th SDG goal by contributing to CO₂ abatement by burning the amaranth’s biomass, instead of fossil fuels, to get energy, after the phytoremediation process; by increase of natural sinks of Cd (prevents it from spreading to adjacent ecosystems);
- 14th SDG by reducing Cd accumulation in water ecosystems;
- 15th SDG by reducing Cd accumulations in terrestrial ecosystems.
4 DISCUSSION

4.1 The phytoremediation project in Chinandega – opportunities, limitations, and contribution to the sustainable development.

From the work done so far, a couple of things become clear.

The literature review on Cd uptake by amaranth showed quite varying data. This means that even within one plant and species of plant the phytoremediation process and its success can vary, depending on the cultivar of the plant, initial condition of soil, additional amendments and pollution rates.

While investigating the design aspects of the project, some advantages were discovered. The opportunities which this project opens for everyone are quite promising. If smartly designed, the phytoremediation outcome will not be limited to just cleaning the soil from pollutants, but will also bring benefits to the society (in the form of money and work places) and environment (in the form of CO₂ abatement) by producing green energy from burning of amaranth biomass. The possibility of removing organic pollutants, namely toxaphene, together with Cd, also exists. Additional benefits from co-cropping option should also be mentioned, and they include food production and improved Cd uptake. The economic profits should also not be forgotten. It might take several years, but it is possible to make profits from the phytoremediation project as previous experience shows, and this might persuade government of the country to support development of the project, and attract other potential investors.

Limitations for the project will include lack of knowledge about the extent of the current pollution, which will make it hard to choose the best strategy. The use of monoculture of amaranth can become an issue, since soils will become depleted of nutrients and will not be able to support good yields of amaranth. This problem could be solved if a co-cropping strategy would be used. The use of edible parts of amaranth is still not clear because of the possibility of too high Cd concentration in those parts. This might mean loss of profit otherwise gained from using amaranth as animal or human food.

As it seems for now, the benefits of phytoremediation project with amaranth definitely outweigh possible drawbacks, making it a good option to solve the problem of soil pollution, and at the same time improve other aspects of human life and
environment. So, it should be given a go and tried out in real life in the Chinandega region.

Concerning the role in the sustainable development, the proposed project will contribute to most of the SDGs (12 out of 17), which shows that is has a high sustainability potential, and will greatly assists the region in different aspects of development.

It should not be forgotten that just implementing the phytoremediation project does not make it sustainable in itself. Sustainability is a complex concept, and to be so, the project should, among other things, provide safe and fair conditions to workers, strive to use energy sources other than fossil fuels, use locally available resources and experience, include education of local communities and in general be a part of a greater restoring system aimed at the sustainable development of the whole region and country.

4.2 Cultivation of amaranth

As was shown in the result section, the confirmed difference in growth rates for Cd20 plants was observed. This can be attributed to the phytotoxic effect Cd has on living organisms. Such inhibiting effect of Cd on plants was observed before (Wang et al., 2000), which suggests that it is hard for plants to tolerate high levels of Cd. What can this mean for the proposed phytoremediation project in Nicaragua? In case the contamination levels on agricultural soils in question are too high, the phytoremediation will not achieve desirable results as amaranth will have problems reaching significant biomass, which can be another limitation in the discussed project. At the same time, lower levels of Cd contamination seem to not have any significant effect on the amaranth development. Although the height of C and Cd2 plants was not significantly different, it cannot be considered as the only parameter determining the well-being of the plants. The plant can be not so high but be broader and have more biomass.

Also, the fact that flowering occurred only in the Cd2 plants is saying something. Moderate levels of Cd have probably trigged stress defence mechanisms in amaranth plants and forced it to develop faster and produce seeds as soon as possible to ensure future continuation. This is a very interesting fact and can actually be a good thing as long as the concentration of Cd in not too high, shortening the time-frames for amaranth growth and accelerating phytoremediation benefits.
4.3 The uptake rate of Cd by amaranth

This section will be updated when the results of the experiment will be available.

In general, we expect the results to help us to understand at what point in time amaranth uptakes the most amount of Cd. It can be at the early stage of the plant, maybe within first month of plant development, or during the later stage when the plant reached its full biomass and is completely developed. Depending on this, it would be possible to give recommendation for the best design of the growing sessions. Does the amaranth uptake most of the Cd during the early stage? Then there is no point in wasting time for waiting for the plants to complete its growing cycle, we can just remove them after the maximum uptake and plant new ones, to make the process most efficient. Or does it reach highest uptake only at the developed stage? Then it makes sense letting the plants to grow fully, and maybe help it with the application of fertilizers.
5 CONCLUSIONS

This thesis project attempted to shed light on some of the aspects of phytoremediation in marginalised regions. To make a structured assessment, an overarching goal of seeing how this project contributes to the sustainable development of the region was made. Three objectives were defined to according to this: (1) to investigate the uptake rate of Cd by amaranth, (2) to investigate the potential of using amaranth in remediation of Cd polluted soils; and (3) to define opportunities and limitations for the designing of the project. It was found that the implementation of this project in real life would mean a support of sustainable development of the Chinandega region on many levels, including social, environmental and economic benefits.

Following conclusions regarding set goals can be made:

1) The conclusion here is yet to be done.

2) Given the existence of previous (and mostly recent) remediation cases of using amaranth with successful outcome, a number of reported researches on that topic and the local availability and suitability of amaranth, it has a promising potential for use in remediation of Cd polluted soils.

3) The main opportunities for designing of this project would be the possibility of combining the clean-up of soil with co-benefits such as producing food and energy, as well as additional cleaning of organic pollutants. The main limitations would be the lack of initial data about the pollution, difficulty of growing the monoculture of amaranth, possible economic losses due to inability of using amaranth for food, long timeframes of economic profits.
6 REFERENCES


South African Department of Agriculture and Fisheries (2010). Amaranthus Production Guideline. *Department of Agriculture, Forestry and Fisheries*.


Appendix 1. Summary of previous experimental works on phytoremediation of Cd polluted soils by amaranth.

<table>
<thead>
<tr>
<th>Article</th>
<th>Species of Amaranth</th>
<th>Chemicals used to pollute soil</th>
<th>Concentration of Cd in soil, mg/kg</th>
<th>Soil type</th>
<th>Parts of plant analysed</th>
<th>Harvest date</th>
<th>Transfer factor</th>
<th>Bioaccumulation factor</th>
<th>Concentration of Cd, mg/kg</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>root</td>
<td>shoot</td>
<td>stems</td>
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<td></td>
<td>A. caudatus</td>
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<td>2. Fan, H. L., &amp; Wei, Z. H. O. U. (2009). Screening of amaranth cultivars (Amaranthus mangostanus L.) for cadmium hyperaccumulation. Agricultural Sciences in China, 8(3), 342-351.</td>
<td>A. mangostanus</td>
<td>n.d.</td>
<td>5</td>
<td>red soil, yellow brown soil, and vegetable soil</td>
<td>roots, shoots, stems, leaves</td>
<td>45 days</td>
<td>0.20 - 0.49</td>
<td>In shoots: 6.17 - 7.82</td>
<td>In roots: 4.28 - 8.5</td>
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<td>A. spinosus</td>
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<td>A. viridis</td>
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<td>Study</td>
<td>Species</td>
<td>Treatment</td>
<td><strong>Pb</strong> (mg/kg)</td>
<td><strong>Cd</strong> (mg/kg)</td>
<td><strong>Zn</strong> (mg/kg)</td>
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<td>7. Li, N. Y., Fu, Q. L., Zhuang, P., Guo, B., Zou, B., &amp; Li, Z. A. (2012).</td>
<td><em>A. hypochondriacus</em> (3 cultivars)</td>
<td>3CdSO$_4$·8H$_2$O solution</td>
<td>5</td>
<td>paddy soil</td>
<td>roots, stems, leaves</td>
<td>60 days</td>
<td>1</td>
<td>1.4</td>
<td>17.7</td>
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*n.d.* – no data.
Appendix 2. Height of collected plants from the second sampling, cm.

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<th>Height, cm</th>
<th>C</th>
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<th>Cd20</th>
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