Trabajo Fin de Master

Master Universitario en Ciencia y Tecnología del Agua y del Terreno

Universidad Politécnica de Cartagena

Identification of soil physical and chemical properties involved in aided phytostabilization efficiency of a tailing pond

Author: Aurore Neveu

Directors: Jose Alberto Acosta Avilés

Silvia Martínez Martínez

July 2016

Acknowledgements

I would like to thank my two directors, Jose Alberto Acosta Avilés and Silvia Martínez Martínez for their help, their availability and their advices during all the project.

Moreover, I would like to thank people of the Grupo de Gestión, Aprovechamiento y Recuperación de Suelos y Agua (GARSA) for their advices and their availability during my experiments in the laboratory.

Table of contents

Acknowledgements ... 3

Abstract ... 6

1. Introduction ... 7
  1.1. Previous history ... 7
    1.1.1. Mining activity in district of Cartagena-La Union ... 7
    1.1.2. Environmental impact of the mining operation ... 8
    1.1.3. Rehabilitation techniques of soils using phytoremediation ... 9
  1.2. Objectives ... 17

2. Material and method ... 18
  2.1. Study area ... 18
    2.1.1. Geographic location ... 18
    2.1.2. Geology ... 19
    2.1.3. Topography ... 19
    2.1.4. Climatology ... 20
    2.1.5. Rehabilitation of the study area ... 21
  2.2. Experimental design ... 24
  2.3. Analyses method ... 26
    2.3.1. Physical and chemical analyses of soil ... 26
    2.3.2. Heavy metal analyses ... 34
    2.3.3. Statistics analyses ... 39

3. Results and discussion ... 40
  3.1. Evaluation of the vegetation cover and biodiversity ... 40
  3.2. Physical properties of the soil ... 43
    3.2.1. Apparent density ... 43
    3.2.2. Real density ... 44
    3.2.3. Soil texture ... 44
    3.2.4. Aggregate stability in water ... 45
  3.3. Chemical properties of the soil ... 47
    3.3.1. pH ... 47

3.3.2. Electric conductivity 48
3.3.3. Inorganic carbon 49
3.3.4. Organic carbon 49
3.3.5. Sulphur 50
3.3.6. Total nitrogen 51
3.3.7. Available phosphorus 52
3.4. Total metals, soluble metals and available metals with $CaCl_2$ 54
3.4.1. Total metals 54
3.4.2. Water soluble heavy metals 57
3.4.3. Bioavailable metals 60
3.5. Sequential extraction of metals 64
3.5.1. Lead 65
3.5.2. Copper 66
3.5.3. Zinc 67
3.5.4. Cadmium 68

4. Conclusions 70

5. References 71

6. Annexes 88
Annex 1: Soil classification according to the density 88
Annex 2: Soil classification according to the percentage of CaCO3 89
Annex 3: Results of laboratory analysis. 90
Annex 4: Pictures of plants species mentioned in the report. Source: Google Images 95

Abstract

The mineral extraction in the mining mountain of Cartagena-La Unión has been developed during 2500 years by different population who have colonized the area. Now, we can find many polluted mining residues by heavy metal in this area due to mineral extraction. This mining residues have a negative impact on the environment and on the human health. To decrease these impacts, it must decontaminate this zone.

In the mining mountain of Cartagena-La Unión, there is an area restored by a technique of phytoremediation. The technique used is the aided phytostabilization. The principle of this technique is to use tolerant plants with metal to immobilize heavy metals by an accumulation in the root or a precipitation in the rhizosphere.

As the physical and chemical characteristics in the area of study aren't appropriate for the majority of plants, it must use amendments to facilitate the growth of plants. For this project, the amendments are: pig slurry, pig manure and marble mud.

Two years after the end of the rehabilitation of Santa Antonieta tailing pond, we can see that exist differences in the growth of the plants. There are plots where there aren't plants and others areas where plants are developed. The objective of this project is to study the differences of soil physical and chemical properties among the plots where there is a good vegetation cover and areas where there aren't plants. After carrying out the lab analyses and visits to the studied area we conclude that the pH influences on the vegetation development due to its acidity. Also, the electric conductivity negatively influences the development of the vegetation when the values are very high for that plants can absorb the water and the nutrients. The inorganic carbon is too low in order that the vegetation grow, due to it prevents the biological activity and the nutrients retention of the soil. The available phosphorus is also too low, it's an important component for the plants growth but when the pH is acid, the available phosphorus become unavailable. Regarding the heavy metals concentrations in the studied plots we observed that copper, zinc and cadmium affect the efficiency of the aided phytostabilization.

Keywords: Phytostabilization; Polluted mining residue; Heavy metals; Mixing of marble mud, pig slurry and pig manure.

1. Introduction

1.1. Previous history

1.1.1. Mining activity in district of Cartagena-La Union

The mining district of Cartagena-La Union is one of the most important of Spain and it is the most representative of the Murcia Region. The mining district of Cartagena-La Union is a coastal mountain about 26 km length which is one of the most important mining districts in the Iberian Peninsula [1].

In this district, there were a large deposit of iron, lead and zinc [2]. This concentration of metals is associated with volcanic activity that took place in the south-eastern of Spain during the Upper Miocene. The alteration of potassic calc-alkaline rocks (andesite, dacite and rhyodacites) resulted in the precipitation of minerals in faults and cavities of the mountain [1].

The mining activity of Cartagena-La Union has been developed for more than 2500 years. It has been one of the most important in Spain and in Europe [2].

The mining extraction has started in the 3rd century BC by the Carthaginians. Their objective was to extract precious metals in order to finance their wars campaign in the Iberian Peninsula and in the Mediterranean Sea. Then, during the Roman era, the Roman civilization has started to exploit in a way more intensive the mining resources. The Roman civilization has mainly extracted the silver to commercialize it. During the Middle Age, the mining activity has deeply decrease due to the barbaric invasion and afterwards the Muslim occupation [2].

In the 19th century, the mining activity started again and it was superior at the mining activity of Roman. This recovery is mainly due to the industrial revolution with the promulgation of the Mine Law in 1825. It’s also due to the discovery of a vein Jaroso de Almagrera in 1839 which will provoke an increase of the mining activity. What will enable to the Spain to be one of the first international exporter of lead and silver in the middle of the 19th century. During this period, the zinc and iron were exploited too. At the end of the First World War, the mining activity has deeply decrease by the fact that the commercial value of metals had decreased, the deposits were over-exploited and that there were few investments in the technology of mining activity [2; 3].

In the middle of the 20th century, the mining activity started again with a new exploitation technique: the opencast mining operation with the separation of mineral through wells by differential flotation using the cutting method in order to increase the profitability of the exploitation. In the end of the 1980s, the mining exploitation has reduced a lot due to the exhaustion of mining resources, a weak need in metals and a reduction of the metals price. In 1991, the mining activity of Cartagena-La Union definitively stopped [2; 3].

The mining exploitation with the separation of mineral through wells by differential flotation has created several million tonnes of sterile residues which contain lead, zinc, copper or iron. We can see on the Photo 1 the sterile residues after the mining exploitation [3].

Photo 1: Landscape after the mining exploitation. Source: www.panoramio.com

1.1.2. Environmental impact of the mining operation

First of all, the opencast mining operation creates a large disfigurement of the landscape like we can see on the Photo 1. It requires to excavate one or several huge hole on the shore to extract the metals contained in the mountain. This operation is done at open air because it is not possible to find metals in high concentrations in veins as before. The metals extracted are found in small concentrations and diffused in vast stretches of subsoil [4].

The main environmental impacts caused by the mining operation are widely known. The mining operation at large scale has harmful impact. We will see the different impacts which has been identified in several opencast mining operations [4]:

- Large scale pollution of surface water and groundwater with toxic substances, including: arsenic, lead, cadmium, chromium, cyanide and mercury. And some radioactive substances with catastrophic impact on the fauna, the flora and the human health

- Acid rock drainage: a process that acidifies the surface water and groundwater and favours the water contamination with heavy metals, including several centuries after the end of the mining operation

- Massive deforestation and drought, which create a desertification and a depletion of water resources

- Destruction of habitats of animal species and plants species in danger of extinction and certain entire ecosystems.
- Harmful impacts to the protected areas
- Air pollution due to the dust and the noise generated by the recurring explosions produced in the excavation. The noise of the mills and the constant vehicular traffic can create impacts on the flora and the wildlife
- Emissions of greenhouse gases
- Soil pollution

Moreover, the soils conditions of mining deposits are unfavourable for plant growth: acid pH, high salinity, high concentrations of heavy metals and metalloids, low content of organic matter and nutrients [5]. However, some species are able to colonize this type of soil for example, *Hyparrhenia hirta* or *Zygophyllum fabago* [6]. But the ingestion of these plants by herbivorous animals can make entry the heavy metals into the food chain [7].

Furthermore, in certain weather conditions, the structure of mining deposit can be vulnerable to the collapse. The stability of the tailing ponds is affected by the collapse of dams, sliding and erosion of the slope foot and gully erosion. The sliding of the slope can occur during periods of torrential rains which provoke the erosion of the slope foot and the overflow water above it. Moreover, the tailing pounds are not waterproof and hasn't interior system of drainage, so, before a periods of torrential rains, the only way out of the water is the evaporation, the infiltration or exceed the slope. Besides, the high gradient of the slopes facilitates the material loss by the erosion [8].

1.1.3. Rehabilitation techniques of soils using phytoremediation

There are different techniques of soil rehabilitation like physical and chemical treatments (for example: soil washing technique, electronic remediation or physical separation), heating treatments with the vitrification technique and biological treatments (the bioaugmentation and phytoremediation). In this part, we will study the different technique of phytoremediation [9].

The phytoremediation is a process which use plants to eliminate, transfer, stabilise and destroy the organic pollutants and inorganic pollutants; it's a long-term technique. There are different techniques of phytoremediation: phytoextraction, phytostimulation, phytodegradation, phytovolatilization and phytostabilization, like we can see on the Figure 1 [9].

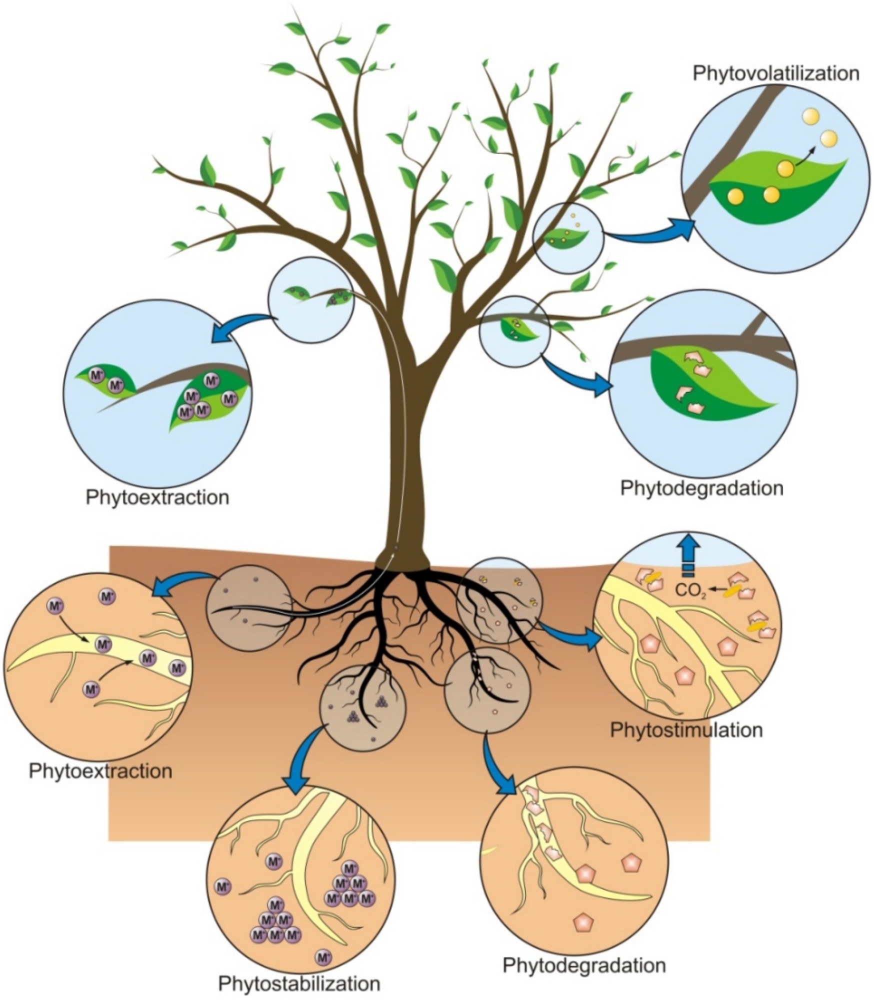

Figure 1: Phytoremediation techniques.

Phytoextraction

The phytoextraction is the uptake of contaminants by plant roots and the movement of the contaminants from the roots to aboveground parts of the plant. The contaminants are generally removed from the site by harvesting the plants. The phytoextraction accumulates the contaminants in a much smaller amount of material to be disposed of than does excavation of soil or sediment [9; 10].

The technique is mostly applied to heavy metals and radionuclides in soil, sediment, and sludges. It may use plants that naturally take up and accumulate extremely elevated levels of contaminants in their stems and leaves. It can also entail the use of plants that take up and accumulate aboveground significant amounts of contaminants only when special soil amendments are used. The plants used are highly specialized, occur naturally, and can tolerate very elevated concentrations of metals that would be toxic to other plants. Typically, these plants are small, have a small and shallow root system, and grow relatively slowly. The phytoextraction is primarily used in the treatment of soil, sediments, and sludges. The phytoextraction is generally limited to the immediate zone of influence of the roots; so, root depth determines the depth of effective phytoextraction. The root zones of most metal accumulators are limited to the top foot of the soil [9; 10].

Studies about the phytoremediation have shown that the plants the most hyperaccumulator are found in the *Brassicaceae*, *Euphorbiaceae*, *Asteraceae*, *Lamiaceae*, or *Scrophulariaceae* plant families [11]. For example, the study of Nanda Kumar et al. (1995) or the study of Raskin, I. et al. (1997) have shown that *Brassica juncea* can accumulate Pb, Cr (VI), Cd, Cu, Ni, Zn, 90Sr, B, and Se and in some case, the plants can have 10% of Pb in its roots [12; 13]. Moreover, Baker et al. (1995) have shown that it exists around 80 species of nickel-accumulating plants in the *Buxacae* family and in the *Euphoribiceae* family [11]. Some plants of the *Euphoribiceae* family can accumulate up to 5 % their dry weight in nickel [14].

The advantage of this technique is that the phytoextraction is able to trap metal and radionuclide contaminants that are in mobile chemical forms. These forms are the most threatening to human and environmental health. The disadvantages are that the most of the plants that are considered good candidates for use with this technology do not grow well under submerged conditions. So the phytoextraction has not been applied to wetlands. The accumulation of contaminants in the aboveground part of the plants may pose a risk to animals eating these plants and fences may be needed to deter grazing animals [10].

Phytostimulation

The phytostimulation is also called rhizodegradation. The phytostimulation is the breakdown of an organic contaminant in the soil through microbial activity that is enhanced by the presence of the root zone. The phytostimulation is also known as plant-assisted degradation, plant-assisted bioremediation, plant-aided *in situ* biodegradation, and enhanced rhizosphere biodegradation. The root-zone biodegradation is the mechanism for implementing rhizodegradation. The root exudates are compounds produced by plants and released from plant roots. They include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and other compounds [9; 10; 15].

The microbial populations and activity in the rhizosphere can be increased due to the presence of these exudates and can result in increased organic contaminant biodegradation in the soil. Additionally, the rhizosphere substantially increases the surface area where active microbial degradation can be stimulated. The degradation of the exudates can lead to co-metabolism of contaminants in the rhizosphere [16].

The plant roots can affect soil conditions by increasing soil aeration and moderating soil moisture content, thereby creating conditions more favourable for biodegradation by indigenous microorganisms. Therefore, they increased biodegradation could occur even in the absence of root exudates [10].

The root depth is very limited because the rhizosphere extends only about 1 mm from the root and initially the volume of soil occupied by roots is a small fraction of the total soil volume, the soil volume initially affected by the rhizosphere is limited [10]. Different studies have shown example of plants which can be used in the phytostimulation:

- Study of Federle et al. (1989) has shown that species of *Morus rubra L., Malus fusca R. Schneid* and *Maclura pomifera R. Schneid* produced exudates containing relatively high levels of phenolic compounds, at concentrations capable of stimulating growth of PCB-degrading bacteria [17].
- Study of Donnelly et al. (1994) has shown that *Mentha spicata* can extract contained a compound that induced cometabolism of a PCB [18].
- Study of Narayanan et al. (1995) has shown that the PAH degradation occurred through the use of the following mix of prairie grasses: *Andropogon gerardi, Schizachyrium scoparius, Sorghastrum nutans, Panicum virgatum, Elymus canadensis, Agropyron smithii, Bouteloua curtipendula*, and *Bouteloua gracilis* [19].

The advantages of this technique are that the contaminant destruction occurs *in situ* and a low installation and maintenance cost as compared to other remedial options. The disadvantages are the root depth can be limited due to the physical structure or moisture conditions of the soil, the plants need additional fertilization because of microbial competition for nutrients [10].

Phytodegradation

The phytodegradation is also known as phytotransformation is the breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants [9; 10].

The main mechanism is plant uptake and metabolism. Additionally, degradation may occur outside the plant, due to the release of compounds that cause transformation. Any degradation caused by microorganisms associated with or affected by the plant root is considered rhizodegradation. For phytodegradation to occur within the plant, the compounds must be taken up by the plant [10].

One study identified more than 70 organic chemicals representing many classes of compounds that were taken up and accumulated by 88 species of plants and trees [20].

Uptake is dependent on hydrophobicity, solubility, and polarity. Moderately hydrophobic organic compounds (with log kow between 0.5 and 3.0) are most readily taken up by and translocated within plants. Very soluble compounds (with low sorption) will not be sorbed onto roots or translocated within the plant. Hydrophobic compounds can be bound to root surfaces or partitioned into roots, but cannot be further translocated within the plant [21; 22].

The organic compounds are the main category of contaminants subject to phytodegradation. In general, organic compounds with a log kow between 0.5 and 3.0 can be subject to phytodegradation within the plant. Inorganic nutrients are also remediated through plant uptake and metabolism. In the organic compounds subject to phytodegradation, we can find: chlorinated solvents, herbicides, insecticides, munitions and phenols [10].

Some studies have shown that *Salix nigra, Liriodendron tulipifera* or *Taxodium distichum* are able to support some degradation of the herbicide bentazon [23].
And the degradation of the atrazine (pesticide) is possible with hybrid poplars (*Populus deltoides x nigra DN34, Imperial Carolina*) [24].

The advantage of this technique is that the contaminant degradation due to enzymes produced by a plant can occur in an environment, free of microorganisms. The plants are able to grow in sterile soil and also in soil that has concentration levels that are toxic to microorganisms. Therefore, phytodegradation potentially could occur in soils where biodegradation. The disadvantage is that toxic intermediates or degradation products may form [10].

Phytovolatilization

The phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant through contaminant uptake, plant metabolism, and plant transpiration. One of the advantages of this techniques is that the contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation [9; 10].

This technique can be used to decontaminate a soil polluted by chlorinated solvents include TCE, TCA and the carbon tetrachloride [10; 24] and some inorganic contaminants like Se or Hg [25].

Some studies have shown that the use of *Medicago sativa* participates in the phytovolatilization of the TCE [24]. *Brassica juncea* and *Brassica napus* can phytovolatilizated the selenium [26]. And *Arabidopsis thaliana* genetically modified including a gene for mercuric reductase converted mercuric salts to metallic mercury and released it to the atmosphere can be used in phytovolatilization of the mercury [27].

The advantages of this technique are that the contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas and the contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation. The disadvantage is that the contaminant or a hazardous metabolite (as vinyl chloride formed from TCE) might be released into the atmosphere [10].

Phytostabilization

The phytostabilization is defined as immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants, and the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching, and soil dispersion [9; 10].

The phytostabilization occurs through root-zone microbiology and chemistry, and/or alteration of the soil environment or contaminant chemistry. Soil pH may be changed by plant root exudates or through the production of CO_2. The phytostabilization can change metal solubility and mobility or impact the dissociation of organic compounds. The plant affected soil environment can convert metals from a soluble to an insoluble oxidation state. The phytostabilization can occur through adsorption, precipitation, complexation, or metal valence reduction [10; 28]. The plants can also be used to reduce the erosion of metal contaminated soil [10].

The most of the time, the phytostabilization is used in the decontamination of soils polluted by metals. The different metals are: arsenic, cadmium, chromium, copper, mercury, lead and zinc. For the phytostabilization of soils contaminated by heavy metals, it must use metal-tolerant plants [10].

The study of Salt et al. (1995) has shown that *Brassica juncea* can be reduce the leach of metals from soil by over 98% [29]. In climate semiarid, it must choose plants resistant to the drought and the salinity [30]. Additionally, the use of native species avoids the introduction of invasive species that can reduce regional plant biodiversity recommend find spontaneously species that have colonized mineral deposits with similar characteristics and which are entirely adapted to high levels of pollution [31; 32].

The advantages of this technique are that it recudes the mobility, and therefore the risk, of inorganic contaminants without removing them from their location, compared to other phytoremediation techniques, the using of material handling is limited and the cost are usually low. The disadvantages are that the contaminants remain in place. The vegetation and soil may require long-term maintenance to prevent rerelease of the contaminants and future leaching and the vegetation may require extensive fertilization or soil modification using amendments [10].

The use of amendments

Amendments can be used on site to increase performance phytotechnological, it is called aided or assisted phytotechnologies. There are two types [33]:

- Chemical amendments
- Organic amendments (bacteria, mycorrhizal fungi)

Their role is intended for example to increase the mobility of trace elements to facilitate their absorption by plants for the phytoextraction, decrease the mobility of trace elements to improve the immobilization by plants for the phytostabilization or to create good conditions that allow the plant growth and increase their biomass [33].

Some studies have demonstrated the beneficial use of different amendments like:

- Study of Sang-Hwan et al. (2014) which have shown that the using of red mud and *P. aquilinum* have decreased the bioavailability of Pb and reduced it to 34% of the total Pb of the soil [34]
- Study of Pérez-Sanz et al. (2004) have shown that the using of cyclonic ash increase the pH and the using of steel shots have generated a big production of biomass [35]
- Studies of Kabata-Pendias (2011) and Fernández-Caliani et al. (2010) have established that the using of marble can neutralize the acidity and decrease the mobility of heavy metals [36; 37]

- Studies of Pardo et al. (2011) and Zornoza et al. (2012) have checked that the using of pig manure can promote the plant growth because it contains a high quantify of nutrients like nitrogen or phosphorus [38; 39].

1.2. Objectives

The main objective of this work is to identify what are main the physical and chemical properties which affect the aided phytostabilization efficiency in Santa Antonieta tailing pond. This main objective can be divided in three specific objectives:

1. Estimate the vegetation cover, and biodiversity in the different study plots.

2. Determine what are the soil properties that affect the development of vegetal species which are used in the phytostabilization.

3. Know the total, bioavailable, water soluble heavy metal (Pb, Cu, Zn and Cd) concentrations and the heavy metal fractionation for determining their influence on the phytostabilization technique used in this research.

2. Material and method

2.1. Study area

2.1.1. Geographic location

The study takes place in Santa Antonieta tailing pond located in Cartagena-La Unión Mining district (37º 35’ 38’’ N, 0º 53’ 11’’ W), in the southeast of Murcia Region (Spain) (Figure 2 and Photo 2). The tailing pond has a surface of 1.4 ha. The soil is a Spolic Technosol (according to Key of Soil Saxonomy, 2015) with sandy loam texture. This type of technosol has anthropogenic origin and it’s possible to recognise the presence of material extracted of the soil [7; 40].

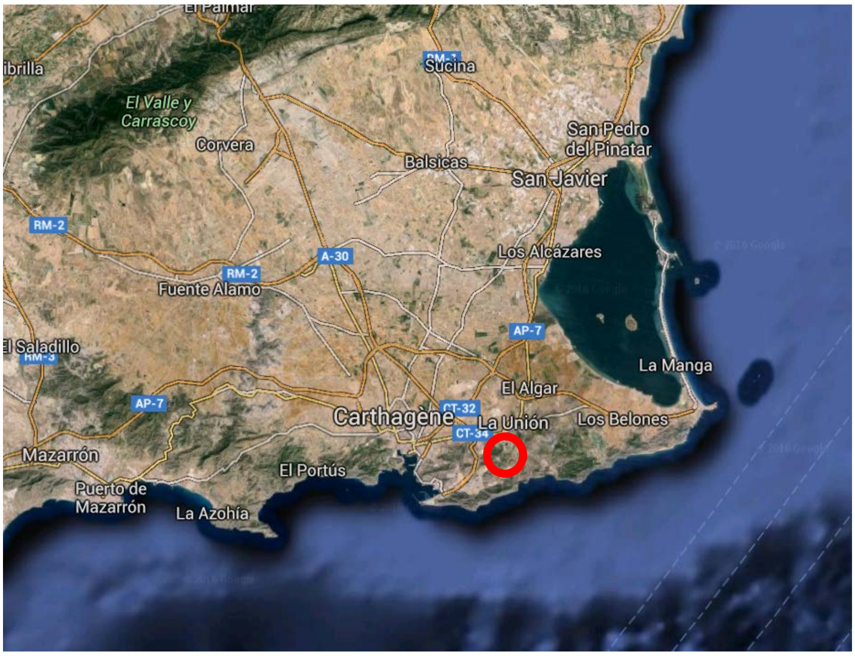

Figure 2: Mining mountain of Cartagena-La Unión. Source: Google Earth.

Photo 2: Study area: Santa Antonieta tailing pond.

2.1.2. Geology

The mining area is part of the last oriental foothills of the Betic Cordilleras emerged in the Cenozoic during the Alpine orogeny, because of the collision of the Mesomediterranean plate with the Iberian plate. Geologically, the mining mountain is composed by two tectonic superimposed components [41].

One of the tectonic components is very ancient and belongs to the nevado-filabride complex, formed during the Paleozic and composed by metamorphic materials (micaschist). It appears in form of rocks with a slaty aspect very obscure in the zone more oriental in the mountain, in the surroundings of Calblanque, Cala Reona and Cabo de Palos [42; 43].

The other component is more modern, it is formed during the Trassic and correspond to the alpujarride complex which is superimposed in form of cape. This section is composed by metamorphic rocks and sedimentary rocks. It appears in the centre of the mountain, in the Monte de las Cenizas and it stretches from the west (Portman, La Unión, the mountain of the Fausilla) to the Cartagena’s Port [41].

2.1.3. Topography

The “Campo de Cartagena” is surrounded on all its contour by mountainous elevation except for the coastal area [44]. By the north it is bounded by the mountain of Carrascoy (Figure 3), whose height gradually decreases to the east, with only some small elevation near the Mediterranean Sea. The central part of the “Campo de Cartagena” belongs to the municipalities of Torre Pacheco, Fuente Álamo and Cartagena. In this area, some hill or hillock highlight as the Cabezo Gordo (312 m), at the west of San Javier and Carmolí (117 m), further at the south and along to the Mar Menor [42].

In its southern part, it is bordered by an orographic set of east-west direction that separates the Mediterranean Sea, from Cabo de Palos to the limit of the end of Cartagena’s city. This mountainous group starts on the east by the mountain of Cabo de Palos and increase its altitude in the mining mountain, formed by the municipalities of El Llano Beal, Cartagena and La Union which occupy an area of 50 km^2 where we can find many mining operations abandoned [45].

Figure 3: Topography of the "Campo de Cartagena". Source: ArcGIS Online.

2.1.4. Climatology

Area climate is semiarid Mediterranean with a long and warm summer, a short and cold winter, moderate spring and moderate autumn. The temperatures are often softened by the proximity to the sea which enables the presence of cooler summers than the interior of the Region of Murcia [43].

The average annual temperature in the study area is of 17 °C (Figure 4). The oscillation average temperatures vary between 10.6 °C in January and 24.9 °C in August, with intermediate values in December, February, March, April and May. But is doesn't exist period of temperature equal or inferior at 5 °C so the risk of frost is low. The absolute extreme values are 40 °C because of continental tropical winds [43].

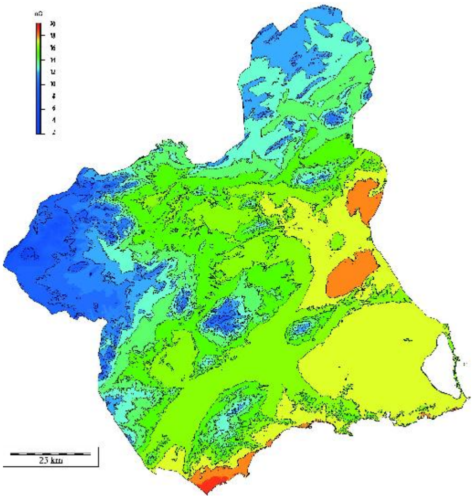

Figure 4: Temperatures in the Region of Murcia [46].

Moreover, we can observe in Figure 5 that the annual rainfall is between 300 and 350 mm. The rate of annual potential evapotranspiration exceeds 900 mm, which causes a large annual hydric deficit [7; 47].

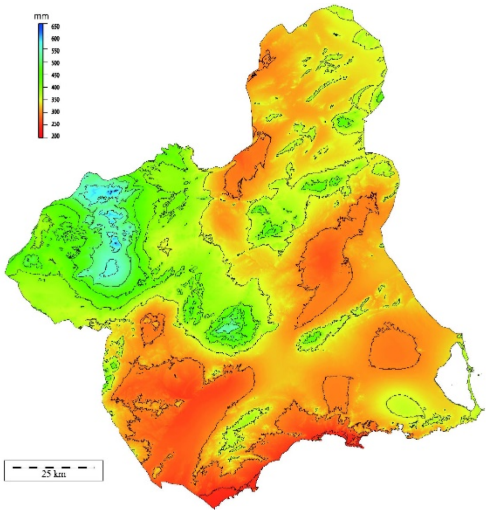

Figure 5: Precipitations in the Region of Murcia [46].

2.1.5. Rehabilitation of the study area

Firstly, the surface of the tailing pond was levelled and a drainage system was created to avoid the formation of flooded areas that can compromise the real reclamation of the tailing ponds. In addition, the amelioration of the access roads was needed to permit the passage of trucks and agricultural machinery [7].

The tailing ponds are formed by cementing agents such as oxides and hydroxides of iron, which provoke the formation of crusting, very hardened, “hardpans”, forming a coherent mass or strongly cemented, causing difficulties in water infiltration. Thus, the water infiltration and leaching of metals into groundwater is minimal. The main problem of these areas is the runoff. However, the levelling of the tailing surface, the ploughing of soil and the mixture of amendments with soil prevented that liquid flows freely into areas outside the tailings. With the prevention of sediments runoff from the ponds, it was also avoided the contamination of ground water via leaching from the streams [7].

The amelioration of the access roads and levelling of Santa Antonieta tailing pond surface were carried out in November 2010.

And the first application of pig slurry was carried out in Santa Antonieta tailing pond on 19 July 2011 (4.3 L m^{-2}) and the subsequent application on 13 September 2011 (4.3 m^{-2}) and pig manure application on 14 November 2011 (7 kg m^{-2}). The marble waste application was carried out on 18 July 2011 (8 kg m^{-2}). The application of amendment mixtures composed of several organic/inorganic wastes, instead of using a single type, can be more efficient in the rehabilitation of mining areas that contain high concentrations of several trace elements in tailings and highly contaminated leachates [48]. These amendment mixtures, which can make a Technosol in an attempt to create different soil functions, can reduce trace element availability and their concentrations in mine waste leachates [49] through the complementary effects of each amendment component.

It is of essential to know the plant species that are able to be adapted to these mining soils with high levels of metallic elements, and in recovered soils by means of valorization of marble mud and pig slurry. This technique is called phytoremediation. Thus, the presence of the vegetation serves as a barrier to the erosive effects of the wind and water impeding the loss of the soil particles, reducing the risks associated to the heavy metal spreading, leaching and run-off. The main goal of this action is the development of a vegetated landscape or ecosystem in harmony with the surrounding environment, with positive values in an aesthetic, productivity, or nature conservation context. There will be a careful selection of native plant species adapted to semiarid conditions, high potential evapotranspiration, salinity, and the presence of metals, avoiding the use of bioaccumulators, which could cause the movement of metals across the food chain. Thus, we will focus on different native plant species able to immobilize soil pollutants by absorption by roots, adsorption by roots, or precipitation in the rhizosphere. The aim is provided a primary vegetation structure capable of guaranteeing the stability of land surface. Nonetheless, the rehabilitation goal is to restore the original natural ecosystem, so that after the introduction of pioneer vegetation, a secondary succession can initialize by spontaneous colonization by the surrounded vegetation [7].

The following species were planted in Santa Antonieta tailing pond:

- *Atriplex halimus* L.
- *Cistus albidus* L.
- *Helichrysum stoechas* (L.) Moench.
- *Hyparrhenia hirta* (L.) Stapf.
- *Lavandula dentata* L.
- *Lygeum spartum* (L.) Kunth.
- *Rosmarinus officinalis* L.
- *Phagnalon saxatile* (L.) Cass.

Since the hydrological year 2011-2012, it has been extremely dry, seeding of several species was postponed to October 2012, to be successful with the plant germination and growth [7].

2.2. Experimental design

A third of the tailing pond kept unamended and unplanted, acting as a control. An area near the pond, under natural vegetation and not contaminated was used as reference of local native soil. Five plots (10 m x 10 m) were established in each zone to monitor soil evolution. In each plot, five soil samples were collected at a depth of 35 cm.

Photos (3, 4, 5, 6, 7, 8) about each plot are shown below.

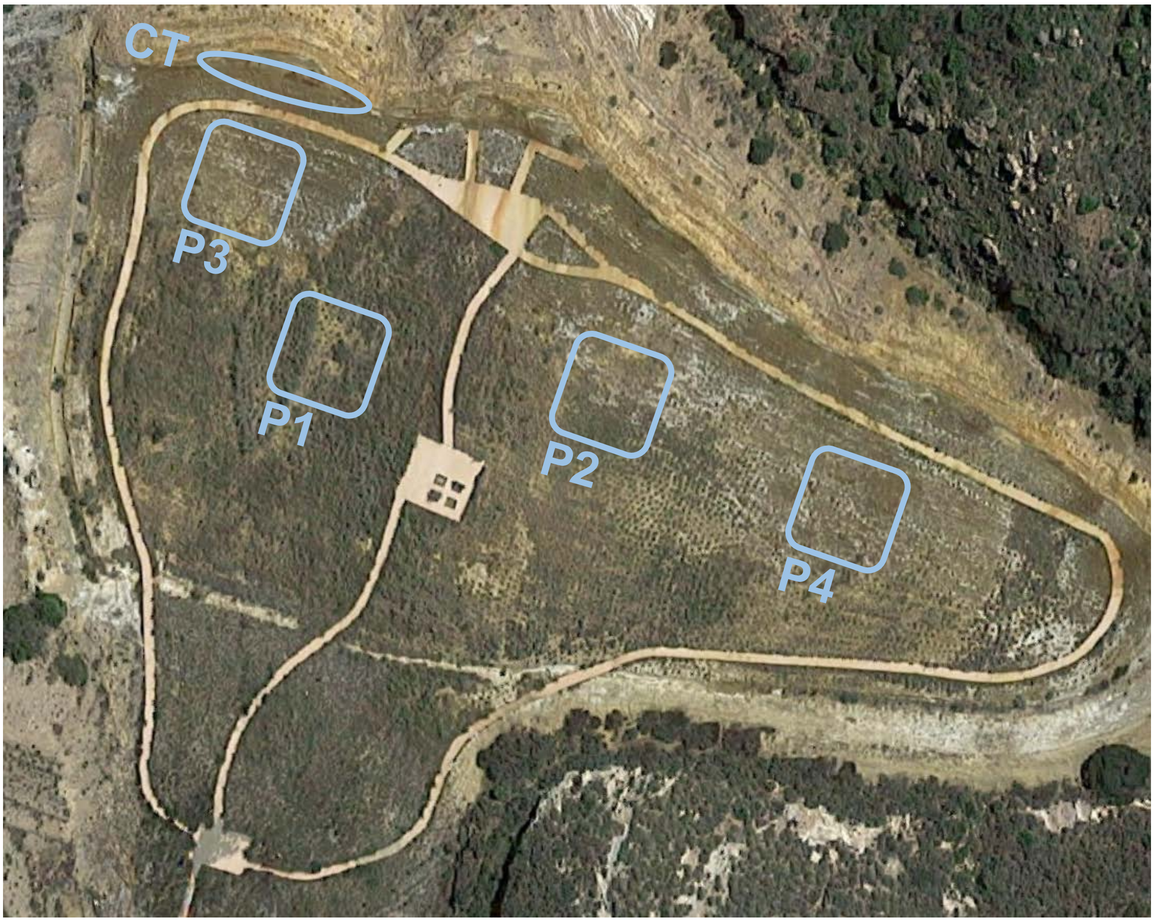

Photo 3: Location of the studied plots.

Photo 4: Plot 1.

Photo 5: Plot 2.

Photo 6: Plot 3.

Photo 7: Plot 4.

Photo 8: Plot CT.

2.3. Analyses method

2.3.1. Physical and chemical analyses of soil

Pre-treatment of samples

After removal of living material (such as mosses, roots, etc.), collected samples (preferably not less than 500 g fresh soil) were transported to the laboratory as soon as possible and were air dried or dried at a temperature of 30 °C. They were then stored until analysis.

Living macroscopic roots and all material, mineral and organic, with a diameter larger than 2 mm, should be removed from the samples by dry or wet sieving. The particles not passing the 2-mm sieve (after crushing), may be weighed separately for the determination of the coarse fragments content. The fraction smaller than 2 mm is used for the soil analysis. Only sieving above a 2 mm sieve is allowed. No further grinding was allowed except for the analysis of total carbon, total nitrogen, total sulphur and total elements.

The sample materials for storage were kept without preservative under normal room conditions with minimal temperature and humidity fluctuations, shielded from incident light.

pH

Soil pH is a measure of active soil acidity and is the most commonly used indicator of mine soil quality. The pH of a given mine soil can change rapidly as the rock fragments weather and oxidize. Pyritic minerals (FeS_2), when present, oxidized to sulphuric acid and drastically lower the pH, while carbonate ($Ca/MgCO_3$) bearing minerals and rocks tend to increase the pH as they weather and dissolve. Unweathered (or unoxidized) mine soils those contain a significant amount of pyritic-S in excess of their neutralizers (carbonates) will rapidly drop the pH to a range of 2.2 - 3.5 after exposure to water and oxygen. Vegetation achieves optimal growth in soil at a neutral pH. When the soil pH drops below to 5.5, reduced legume and forage growth occur due to metal toxicities [50].

Electric conductivity

Electrical conductivity (EC) of a soil solution or extract indicates the concentration of total soluble salts in solution, thus reflecting the degree of soil salinity. The unit of measurement is called millimhos per centimeter (mmhos/cm), or millisiemen per centimeter (mS/cm). The EC is reported to a standard temperature of 25°C by adjustment factors.

Salinity is a soil property referring to the amount of soluble salt in the soil. It is generally a problem of arid and semiarid regions. Electrical conductivity (EC) is the most common measure of soil salinity and is indicative of the ability of an aqueous solution to carry an electric current. Plants are detrimentally affected, both physically and chemically, by excess salts in some soils and by high levels of exchangeable sodium in others. Soils with an accumulation of exchangeable sodium are often characterized by low permeability making them unfavourable for plant growth.

By agricultural standards, soils with an EC greater than 4 dS/m are considered saline. Salt-sensitive plants may be affected by conductivities lower than 4 dS/m and salt tolerant species may not be impacted by concentrations of up to twice this maximum agricultural tolerance limit. Thus, the reclamation scientist must exercise care in interpretation of salinity standards. Salinity should be defined in terms of the predisturbance land use potential, the proposed postdisturbance land use, and the plant species to be seeded on the site [50].

Inorganic carbon

Inorganic carbon occurs in soils as the carbonate minerals calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), and magnesian calcites ($Ca_{1-x}Mg_x\ CO_3$). Other less common forms are aragonite ($CaCO_3$) and siderite ($FeCO_3$). Carbonate in soils can be of primary (inherit from parent material) or secondary (pedogenic) origin. Secondary carbonates are usually aggregates of silt- and clay-sized calcite crystals that are easily identified in grain mounts. Large crystals of calcite or dolomite are of primary origin [51]. The qualitative and quantitative determination, especially of Ca and Mg carbonates, is useful in studies of soil genesis and classification, and micronutrient and phosphorus sorption. Furthermore, soil carbonates affect root and water movement, soil pH [52], and the nature of the exchange complex [53].

Calcium carbonate, a major component of calcareous soils, ranges from a few percent in slightly calcareous soils to more than 80 percent in some extremely calcareous soils. The amount and form of calcium carbonate (total versus active) and its distribution down the soil profile, affect soil physical and chemical characteristics. It was found that when total $CaCO_3$ was above 20 %, active $CaCO_3$ was more than 10 %. Carter (1981) gave 11 to 30 % total carbonate and 7 to 9 % active $CaCO_3$ as the critical levels that adversely affected crop growth [54]. The authors showed that soil retention of P, Mn, Zn and Cu was directly related to carbonate content and to the distribution of total and active calcium carbonate between the clay and silt fractions. When total $CaCO_3$ was less than 20 %, the retention of those elements was affected mainly by the total amount of carbonates, but, when it was above 20 %, nature of the carbonates was more important in governing retention. The active $CaCO_3$ was more than 10 % when the total $CaCO_3$ was above 25 % in the studied soils. Therefore, one could give the limit of about 10 % active $CaCO_3$, which will contribute significantly to the retention of certain essential elements in calcareous soils.

Physical effects: The active-$CaCO_3$ portion and the distribution of $CaCO_3$ in the different particle size fraction affect the properties (soil-water relationships) of calcareous soil. Calcareous soils with 15% calcium carbonate content have higher water diffusivity and faster water movement than non-calcareous soils of similar texture. Evidently, $CaCO_3$ assists in the formation of stable soil aggregates. However, increased $CaCO_3$ content (25 % or more) tends to precipitate within the capillary tubes causing an increase in the proportion of micropores, thus reducing the percolation of water [55; 56].

Effect on plants: Continuous horizons of carbonate accumulation in soil profile may prevent root penetration and thus may retard the degree of plant development and reduce the yield [50].

Organic carbon

All organic substances, by definition, contain carbon. Soil organic matter is a complex and varied mixture of organic substances. Commonly, soil organic matter is defined as the humus in the soil. Humus is the unidentifiable residue of plants and animals that becomes fairly resistant to further decay. Reclamationists, however, include other materials that are mainly comprised of carbon as organic matter. Soil organic matter, from this point of view, can be divided into two fractions: (1) the recognizable organic material (straw, woodchips, roots, mulches, insects, etc.) with their partially decomposed remains, and (2) humus. Recognizable organic material eventually decays to the partially decomposed state and eventually to humus. The process of decomposition is carried out by heterotrophic soil organisms that obtain the carbon they need to build their cell constituents from the breakdown of organic materials. Nearly all heterotrophs also obtain their energy to live from the oxidation of the carbon in organic compounds [57; 58].

Organic matter is very important for the functioning of the soil system for various reasons. It increases soil porosity, thereby, increasing infiltration and water holding capacity of the soil, providing more water availability for plants and less runoff that may potentially become contaminated. This may be specifically helpful at mine sites where runoff may become acidic and contain high concentrations of heavy metals. The increased porosity also aids in easing tillage of the soil [57].

The establishment of cover soil on disturbed surfaces is a common reclamation strategy. Cover soils facilitate the establishment and growth of vegetation. Many times, finding enough cover soil to cover disturbed surfaces can be difficult and costly. When there is not enough soil on-site to satisfy the demand, surface soils may be hauled in from other designated sites. If surface soils are excavated to recover minerals beneath, the surface soils may be stored until reclamation of the area takes place. In these cases, surface soils may be stored for long periods, during which time, the soils may show reduced biological activity, in part due to a loss of mycorrhizae, bacteria, and invertebrates. Stored surface soils also reveal a loss of organic matter and nutrients. Therefore, organic amendments and fertilization of surface soils that have been in storage for several years are necessary to ensure rapid buildup of microbial populations and initiate nutrient cycling [57].

Determination of organic matter content is a routine procedure carried out in soil analysis because of the importance that organic matter plays in aiding plant growth and resisting the effects of erosion. However, there is no satisfactory method for determination of the organic matter content of soil. It can be determined indirectly by measuring the organic C content of the soil and multiplying the result by the ratio of organic matter to organic C normally found in soil. Different researches have suggested different ratios that range from 1.7 to 2.5, with a ratio of 2 appearing to be most universally accepted. The estimation of organic matter content from organic C concentrations is not highly accurate, because the ratio of organic matter to organic C is variable from soil to soil and with depth in the profile. Direct determination of organic matter usually involves destruction of the organic fraction by oxidation or ignition of the soil at high temperature [57].

Total nitrogen

Total soil N includes all forms of inorganic soil N. Inorganic N includes soluble forms (e.g., NO_2^- and NO_3^-), exchangeable NH_4^+, and clay-fixed nonexchangeable NH_4^+. Organic N content includes numerous identifiable and non-identifiable forms [59] and can be determined by the difference between total soil N and inorganic soil N content. Total N analyses may be divided into two main types: (i) wet digestion (e.g., Kjeldahl method) or (ii) dry combustion (e.g., Dumas method). Wet digestion techniques involve conversion of organic and inorganic N to NH_4^+ in acid and its subsequent measurement. Salts (e.g., K_2SO_4) and catalysts (e.g. Cu) are usually added to increase digestion temperatures and accelerate oxidation of organic matter [60]. The dry combustion method normally involves an initial oxidation step followed by passage of the gases through a reduction furnace to reduce NO_x to N_2. The quality of N_2 is usually determined using a thermal conductivity detector.

The total nitrogen from soil samples are analyzed using elemental analysis. Elemental analysis is a technique that provides total content of carbon, hydrogen, nitrogen, and sulphur present in a wide range of organic or inorganic samples, both solid and liquid samples. The technique is based on the full and instant oxidation of the sample through combustion with pure oxygen to an approximate temperature of 1000 °C. Different products of combustion CO_2, H_2O and N_2, are transported through the carrier gas (He) through a tube of reduction and then selectively separated into specific columns to be then thermally freed. Finally, the gases pass separately by a detector of thermal conductivity that provides a signal proportional to the concentration of each of the individual components of the mixture.

Sulphur

Sulphur is released to the atmosphere in the form of sulphur dioxide (SO_2) and hydrogen sulphide (H_2S) from the composition of coal and other fuels and by anaerobic decomposition of biological residues respectively. Most of this sulphur enter the soil dissolved in rain water, but small amounts may be absorbed directly from the atmosphere by the soil [61], and further small amounts may eventually enter the soil after direct absorption of sulphur dioxide from the atmosphere by growing plants [62]. Appreciable but highly variable amount of sulphur may also enter the soil as sulphates in irrigation waters [63].

Real density

The real density is measure with a pycnometer. This method consists in weighing the pycnometer alone, with soil, with water and soil and to finish filled water only. With some calculations, we can determine the real density [64; 65].

Apparent density

Apparent density is defined as the mass of a unit volume of dry soil. This volume includes both solids and pores. There are several methods for determining soil apparent density by obtaining a known volume of soil, drying it to remove the water, and weighing the dry mass. For surface soils, perhaps the simplest method is using a metallic cylinder tube to obtain a soil sample pushing deep in order to quantify the volume without disturbing the natural soil structure. The volume of soil is determined by weighting the dry soil and calculating the cylinder volume [66].

Increases in apparent density usually indicate a poorer environment for root growth, reduced aeration, and undesirable changes in hydrologic function, such as reduced water infiltration. Vegetation reestablishment is important to mine land reclamation. Apparent density can be lowered by tillage for the purposes of revegetation. On the other hand, increased apparent density indicates good compaction for foundation stability which can be important for topographic reconstruction of disturbed areas, building of roads, and building of foundations for structures [66].

Soil texture

The texture influences plant growth by its direct effect on soil aeration, water infiltration, cation exchange capacity (CEC), and erodibility. Infiltration and permeability are rapid in sandy soils, very slow in clay soils, and intermediate in loam soils. Soils that are granular, with a large diversity in particle size, have many large and small pores - a desirable characteristic for plant growth [67].

Soil texture is measured in the laboratory. The procedure commonly used involves determining particle sizes by the rates they drop in a suspension. The soil particles are first suspended in a solution. This is usually accomplished using chemical treatments along with a high-speed blender. While a set of sieves can be used to separate out the sand fraction, a sedimentation procedure is usually used to determine the amounts of silt and clay. Soil particles are denser than water and they tend to sink. Depending on their size, they will settle at different velocities with larger particles settling faster. By measuring the amount of soil still in suspension after various amounts of settling time (using a pipette or hydrometer), the percentages of each size fraction can be determined, ultimately identifying the soil textural class. The size of the particles can be determined because settling time can be related back to the diameter of a particle by Stoke's Law [67].

Soils can have any combination of gravel, sand, and clay and a textural chart is used to describe the different soil textures as shown below:

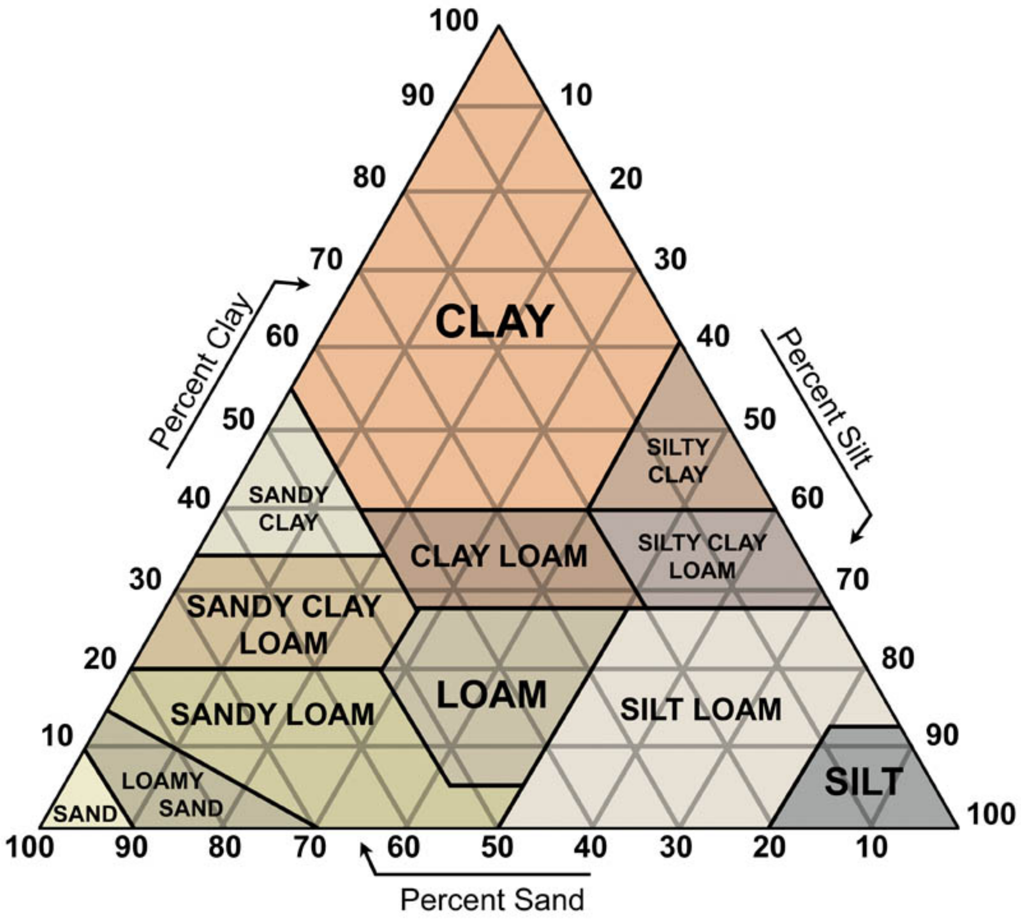

Figure 6: Soil texture triangle, showing the 12 major textural classes defined by the United States Department of Agriculture (USDA)

Soil texture is measured in the laboratory by separation of the mineral part of the soil into various size fractions and determination of the proportion of these fractions. The analysis includes all soil material, i.e. including gravel and coarser material, but the procedure below is applied to the fine earth (< 2 mm) only. Of paramount importance in this analysis is the pretreatment of the sample aimed at completing dispersion of the primary particles [67; 68].

Therefore, generally, cementing materials (usually of secondary origin) such as organic matter, salts, iron oxides and carbonates such as calcium carbonate are removed. After shaking with a dispersing agent, sand (63 µm-2 mm) is separated from clay and silt with a 63 µm sieve (wet sieving). The clay (< 2 µm) and silt (2-63 µm) fractions are determined by the pipette method (sedimentation) [67; 68].

Aggregate stability in water

An aggregate is a group of primary particles that adhere to each other more strongly than to surrounding soil particles [69]. Aggregate stability can be defined as the resistance to disruption or breakage o the bonds within the aggregates by external forces of impact, shearing, and abrasion and internal forces arising from the escape of entrapped compressed air and differential swelling [70].

Methodologies have been developed so that aggregate stability can serve as an indicator of the resistance of soils to water erosion, surface seal or crust formation, compaction leading to decreased infiltration and subsoil aeration, and as a general soil quality indicator [71 - 73]. The methodologies devised for measuring aggregate stability have been numerous and diverse (e.g. Le Bissonnais et al. (1996), Marquez et al. (2004)) [72; 74].

Soil aggregates control soil hydrology [75], affect soil oxygen diffusion and nutrient availability [76; 77], influence soil erodibility [78] and constitute a pathway of organic carbon stabilisation and long term sequestration [79]. For this analysis no crush should have be taken during the pre-treatment of the sample.

Available phosphorous

Phosphorus (P) is a naturally occurring element in the environment that can be found in all living organisms as well as in water and soils. It is an essential component for many physiological processes related to proper energy utilization in both plants and animals. Phosphorus can be added to the environment by man’s activities as point source discharges or as non-point source runoff [58; 80].

Phosphorus (P) is an essential element classified as a macronutrient because of the relatively large amounts of P required by plants. Phosphorus is one of the three nutrients generally added to soils in fertilizers. One of the main roles of P in living organisms is in the transfer of energy. Organic compounds that contain P are used to transfer energy from one reaction to drive another reaction within cells. Adequate P availability for plants stimulates early plant growth and hastens maturity. Although P is essential for plant growth, mismanagement of soil P can pose a threat to water quality [58; 80].

The concentration of P is usually sufficiently low in fresh water so that algae growth is limited. When lakes and rivers are polluted with P, excessive growth of algae often results. High levels of algae reduce water clarity and can lead to decreases in available dissolved oxygen as the algae decays, conditions that can be very detrimental to game fish populations [58; 80].

2.3.2. Heavy metal analyses

Water soluble heavy metals

Soil is a dynamic and complex system, and any change in intrinsic physico-chemical properties of heavy metal would surely change the fate of heavy metals in a soil system. Among all parameters that might affect transport of heavy metals in soil systems, redox potential is also an important parameter, although pH is usually considered to be of prime importance. Many authors have indicated and discussed its importance and ranges of possible redox conditions [81; 82].

The method proposed was designed and modified by Alloway (1990), Risser and Bailey (1992) and Heron et al. (1994). The goal of this method is to determine the amount of metal present in soluble form in the soil and, therefore the fraction that can be leached to groundwater or drag with runoff water. To achieve this goal, the metals are extracted with deionised water by shaking during a specific time [83 - 85].

Bioavailable metals

The fate of various metals, including chromium, nickel, copper, manganese, mercury, cadmium, and lead, and metalloids, including arsenic, antimony, and selenium, in the natural environment is of great concern, particularly near former mine sites, dumps, tailing piles, and impoundments, but also in urban areas and industrial centres. Soil, sediment, water, and organic materials in these areas may contain higher than average abundances of these elements, in some cases due to past mining and (or) industrial activity, which may cause the formation of the more bioavailable forms of these elements [86].

In order to estimate effects and potential risks associated with elevated elemental concentrations that result from natural weathering of mineral deposits or from mining activities, the fraction of total elemental abundances in water, sediment, and soil that are potentially bioavailable must be identified. Bioavailability is the proportion of total metals that are available for incorporation into biota (bioaccumulation). Total metal concentrations do not necessarily correspond with metal bioavailability [86].

In aquatic environments where chemically reducing conditions may prevail, metals from mining activities may be associated with sulfide minerals. These sulfide minerals are present either in the ore deposit or formed by bacterial reduction of the sulfate in oxidized tailings. Most metal sulfide minerals are quite immobile, as long as they remain in a chemically reducing environment, and they may have little impact on biota despite anomalous metal concentrations [86].

Metals of major interest in bioavailability studies, as listed by the U.S. Environmental Protection Agency (EPA), are Al, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Sb. Other metals that are presently of lesser interest to the EPA are Ag, Ba, Co, Mn, Mo, Na, Tl, V, and Zn. These metals were selected because of their potential for human exposure and increased health risk [87].

Metals can be dispersed in soil, water, and air. Geoscientists are mainly concerned with metals dispersed in soil and sediment, dissolved in ground and surface water, suspended as particles in surface water, and in pore fluid in sediment. In addition, metals can be dispersed into the atmosphere, by natural geochemical cycling and by other anthropogenic processes (such as smelting and burning leaded gasoline and coal) and by microbial activities; these metal fluxes must be considered in overall metal bioavailability studies. Bioaccumulation of metals by biota in surface water and by plants and animals in terrestrial environments can adversely affect humans. In surface and ground water, sediment and air, bioavailability is a complex function of many factors including total concentration and speciation (physicalchemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content (both particulate and dissolved fractions), and suspended particulate content, as well as volume of water, water velocity, and duration of water availability, particularly in arid and semi-arid environments. In addition, wind transport and removal from the atmosphere by rainfall (frequency is more important than amount) must be considered. Many of these factors vary seasonally and temporally, and most factors are interrelated. Consequently, changing one factor may affect several others. In addition, generally poorly understood biological factors seem to strongly influence bioaccumulation of metals and severely inhibit prediction of metal bioavailability [88].

In order to understand bioavailability, plant materials and selective chemical leaches of soil must be analyzed and the results compared. Elemental suites for which analyses are performed and the type of selective leaches utilized must be tailored to bedrock and soil types, and to suspected anthropogenic inputs. Soil pH, organic matter, and sulfur and carbonate contents should be determined to enable accurate assessment of elemental reservoirs, mobility, and bioavailability. Additional work on mineralogical residences of metals is also important because metals can be associated with several sites. The extent of bioavailability is largely controlled by elemental speciation which determines solubility. A number of soil testing methods and partial or sequential chemical extraction techniques and methods are used to determine element behaviour [89; 90]. Some of the chemical extractions are: (1) weak acid or dilute acid in buffer solution (pH 2 to 5) to release metals associated with carbonate phases, and (2) a chelating (or complexing) agent such as EDTA (ethylenediaminetetraacetic acid) [91] or DPTA (diethyenetriaminepentaacetic acid) buffered to a pH of 7 [92]. Other possible extractants include (3) hydroxylamine hydrochloride for the "reducible" fraction associated with iron and manganese oxides/hydroxides, (4) strong acid (HCl, pH 1) to identify maximum mobility of most metals [93], (5) oxidation by hydrogen peroxide to release metals associated with organic matter and (or) sulfide minerals, (6) a strong oxidizing acid (HNO3), and (7) a mixture of strong acid and HF to dissolve residual silicate minerals. The choice of extractants and the order in which they are used depends on the sediment/soil type, environmental conditions, and metals of interest.

However, these sequential/partial extractions are all "operational", since they are not completely specific to metals or chemical phases. Therefore, any determination of bioavailability should be carefully calibrated, by direct measurement, with the actual behaviour of metals in soil and plants.

The goal of this method is to determine the amount of metal present in potentially available form in the soil and, therefore could be adsorbed for vegetation in the upcoming years. It is the pool of metals which can be easily released by oxidation, reduction and hydrolysis reactions so that they could be available for biota. To achieve this goal, the metals are extracted with DTPA (diethylenetriaminepentaacetic acid), which is a chelating agent, by shaking during a specific time. After that the sample is filtered and the resulted solution measured for heavy metals by mean of atomic absorption spectrometer [94 - 97].

Sequential extraction of metals

The sequential extraction of metals is measured with the sequential extraction of Tessier et al. (1979) and modified by Li et al. (1995). The most mobile metals are removed in the first fraction and continue in order of decreasing of mobility. All the sequential extraction of metal facilitate fractionation. The different fractions are: exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound, and residual. These are also often referred to in the literature as exchangeable, weakly absorbed, hydrous-oxide bound, organic bound and lattice material components, respectively. Typically metals of anthropogenic inputs tend to reside in the first four fractions and metals found in the residual fraction are of natural occurrence in the parent rock.

The exchangeable fraction is removed by changing the ionic composition of water allowing metals sorbed to the exposed surfaces of sediment to be removed easily. A salt solution is commonly used to remove the exchangeable fraction. The carbonate-bound fraction is susceptible to changes in pH; an acid solution is used second. Metals bound to Fe and Mn oxides are particularly susceptible to anoxic (reducing) conditions so a solution capable of dissolving insoluble sulfide salts is used third. To remove metals bound in the organic phase, the organic material must be oxidized. The residual fraction consists of metals incorporated in to the crystal structures of primary and secondary minerals. This fraction is the hardest to remove and requires the use of strong acids to break down silicate structures [98; 99].

Total metals

The determination of the concentrations of heavy metals in soils is important for different reasons: i) it provides a basic understanding of the components of soil with respect to changes in its evolution, ii) reports pollution incidents that have occurred in both the present and past, and, finally, iii) is closely related to their consumption by plants and thus entering the food chain. In this sense, the method of determining these concentrations has been the focus of many discussions both in national and international forums.

The total analysis of heavy metals such as Cd, Cr, Cu, Ni, Pb, Zn and As in soils, is commonly done to evaluate the degree of contamination of aquatic and terrestrial environments. Different digestion methods are used for this purpose including various combinations of concentrated acids such as hydroflouric acid (HF), hydrochloric acid (HCl), nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4) [100; 101]. Different digestion equipment was likewise employed like open beakers heated on hot plates, digestion tubes put in the block digestors, and digestion bombs placed in conventional and microwave ovens [100; 101]. Hossner (1996) reported that the advantages of the dissolution of heavy metals in soils using concentrated inorganic acids are low cost and low salt matrix in final solution for the determination of total heavy metal content [102]. Mendoza et al. (1996) used HF-HNO_3 and *aqua regia* to digest the residual fraction following the sequential extraction of exchangeable, reducible, and oxidized fractions of Cd, Co, Cr, and Pb contained in canal and dam sediments in Mexico [103]. Chlopecka et al. (1996) used the mixture of HF and $HClO_4$ to digest the residual fraction following the sequential extraction of exchangeable, carbonate, reducible, and oxidizable fractions of Cd, Pb, and Zn in contaminated soils of Poland [104]. Martin (1996) employed the mixture of HF-$HClO_4$-HNO_3 for the total analysis of Cd, Co, Cr, Cu, Pb, and Zn in alluvial soils of Germany [105]. Charlesworth and Lees (1999) found the mixture of $HClO_4$-HNO_3-H2SO_4 suitable to digest river sediments for the determination of total contents of Cd, Cu, Ni, Pb, and Zn in England [106].

The *aqua regia* digestion method (USEPA Method 3050) was developed for the determination of heavy metals in the soils of U.S.A. [107]. In Taiwan, the *aqua regia* method is also one of the digestion methods recommended by the Environmental Protection Administration (EPA) for the total analysis of Cd, Cr, Cu, Ni, Pb, and Zn in sediments and soils

[108]. Simplicity and adaptability as a routine procedure have led to the widespread use of the *aqua regia* method for the determination of total amounts of Cd, Cr, Cu, Ni, Pb, and Zn in contaminated soils and sediments. The mixture of HF-HNO_3-$HClO_4$-$H2SO_4$ was recommended by Baker and Amacher (1982) for the total analysis of Cd, Cu, Ni, and Zn in soils [109]. A similar digestion method using the reagents of HF-HNO_3-$HClO_4$-$H2SO_4$, but modified in certain heating processes, was recommended for the total analysis of Cr in soils by Reisenauer (1982) [101]. Burau (1982) used HCl to replace H_2SO_4 in the digestion mixture of HF-HNO_3-$HClO_4$-$H2SO_4$ for the total analysis of Pb in soil samples [100]. Therefore, it is interesting and necessary to compare the different digestion methods for the total analysis of heavy metals in sediments and soils.

The procedures for dissolution and digestion using the microwave oven are replacing conventional methods because the sample is less contamination in the laboratory and can slow the process of volatilization of metals such as As, Cd, Pb, Sb, Se and Tl [110]. These procedures have been applied to the total dissolution of materials of different nature such as geological materials, coal, sewage sludge and sediment using mixtures of acids may include HF acid, so you can fully release the trace elements in the phase of aluminosilicates [111]. However, the use of HF leads to longer working tasks, tedious and dangerous, and its use is not recommended for routine analysis. Thus, environmental analytical chemistry using pseudo-total analysis has become a common procedure as an alternative to total digestion.

Finally, for a smaller number of minerals, the attack with HF alone or in combination with other acids, is ineffective to achieve total dissolution [112]. For these items and silicate materials in general, the methods of lithium metaborate fusion are useful. The most common technique is one that uses lithium metaborate and subsequent dissolution. This method is faster than the acid attack dissolves most minerals minority and provides a clear solution, ready for action. However, the disadvantage of providing solutions with a high salt content and this may influence the detection limits [113].

$HClO_4$ digestion, usually in combination with HNO_3 acid has been used in the analysis of soil [114], despite the danger of explosion that can trigger the samples with high organic matter content.

We selected the method proposed by Risser and Baker (1990) [84] that used the nitric-perchloric acid wet digestion with heating block. The goal of this method is total sample decomposition. To achieve this objective, the samples are digested with a combination of strong acids at high temperature during a specific time; after that, the sample is filtered and the resulted solution is measures for heavy metals by means of atomic absorption spectrometer. Total heavy metals concentrations are used to estimate the degree of pollution of the soil, for this the reported concentration are compared with the levels proposed by the environment laws [115 - 118].

2.3.3. Statistics analyses

The program IBM SPSS Statistics 19 enables to carry out statistics analyses of all the data of this research. The normal distribution of the data is verified by el test of Kolmogorov-Smirnov. The analysis of variance (ANOVA) with the test post-hoc of Tukey was used ($P < 0.05$), in order to determine if therewas significant differences among the results from 5 studied plots.

3. Results and discussion

3.1. Evaluation of the vegetation cover and biodiversity

The vegetation cover enables to reduce wind and water erosion due to:

- The interception of raindrops which decreases their kinetic energy and their effects on the soil.
- Plants reduce water runoff due to their roughness.
- The radicular system of plants keeps the soil structure and stimulate the infiltration.
- The reduction of wind speed on soil surface.

Moreover, it enables to increase the fertility and to decrease the imagery impact of mining industry [119 - 121].

During the sampling, the vegetation cover was evaluated. In the Table 1, we can see the percentage the vegetation cover.

Plots	Percentage of the vegetation cover (%)
P1	85
P2	45
P3	0
P4	20
CT	0

Table 1: Percentage of the vegetation cover for each plot (P1: plot 1; P2: plot 2; P3: plot 3; P4: plot 4: CT: control).

Moreover, a list of all plant species growing in each plot was done:

Plant Species	Number of plants in P1	Number of plants in P2	Number of plants in P4
Atriplex halimus L.	34	5	0
Cynodon dactylon (L.) Pers.	0	6	3
Diplotaxis erucoides (L.) DC.	0	4	0
Lobularia maritima (L.) Desv.	8	0	0
Oxalis pes-caprae L.	2	1	0

Phagnalon saxatile (L.) Cass.	6	2	0
Piptatherum miliaceum (L.) Coss.	24	10	34
Senna occidentalis (L.) Link.	1	0	0
Stipa tenacissima L.	8	4	0
Total	83	32	37

Table 2: Number of plant species in the studied plots.

With this information, we can see that new plants are colonizing these plots by comparing with the list of species planted at the beginning of the MIPOLARE project. These new plants are *Diplotaxis erucoides (L.) DC.*, *Lobularia maritima (L.) Desv.*, *Senna occidentalis (L.) Link.* or *Stipa tenacissima L*. Moreover, we can see that a lot of plants have disappeared in the 5 selected plots such as *Cistus albidus L., Helichrysum stoechas (L.) Moench, Hyparrhenia hirta (L.) Stapf, Lavandula dentata L., Limonium caesium (Girard.) Kuntze*, *Lygeum spartum (L.) Kunth or Sonchus tenerrimus L.* Likely due to the physical and chemical a conditions of the soil and plant competivity.

Some characteristics of the plants that colonize the study area are:

Atriplex halimus L. is a species usually present in Mediterranean region. This species has a good drought and salt resistance. Moreover, this species can growth in soil with weak nutrients and a poor soil structure. It produces high amounts of oxalic acid which able the species tolerate to heavy metal stress. Furthermore, this species is often used in soil remediation [122 - 124].

Cynodon dactylon (L.) Pers. is a native species which grows in tropical and subtropical regions. This species can evolve tolerance to several extreme environmental condition as salinity or high concentrations of heavy metals in soil. Moreover, it is extremely drought tolerant and well adapts to anaerobic conditions (flooding). This specieshas been used in soil remediation contaminated by Cr, Pb, Zn and Cu [125 - 127].

Diplotaxis erucoides (L.) DC. is a species present in North Mediterranean region. It means that the soil condition is good for this plants if it is present. This species hasn't absorbent properties or stabilizing properties for heavy metals but it can be used to decrease the wind and water erosion of soil [128; 129].

Lobularia maritima (L.) Desv. is a species naturally present in Mediterranean region. This species growth in soil enriched in nutrients with alkaline pH and a clay or silt texture.

Moreover, this species is not tolerant to the salinity and It’s not a strong accumulator plant. However, a study has showed that this species can absorb high quantity of lead and zinc [130; 131].

Oxalis pes-caprae L. is a species naturally present in the Mediterranean region. It’s a species typical of coastal zone. It is considered an invasive plant. This species can accumulate heavy metals such as copper or nickel in high concentrations. Moreover, this plant is often used in soil remediation contaminated by heavy metals [132; 133].

Phagnalon saxatile (L.) Cass. is a native species to the Mediterranean region. It’s a species tolerant to the arid climate. Moreover, this species is very tolerant to heavy metals especially copper, lead or zinc. This species is often used in soil remediation contaminated by heavy metals [134 - 136].

Piptatherum miliaceum (L.) Coss. is native to Eurasia but it can be found in many other parts of the world. This species is a strong accumulator of lead and zinc; and therefore it is tolerant to heavy metals. For this reason, this species is often used in soil remediation contaminated by heavy metals [137; 138].

Senna occidentalis (L.) Link. is native to Egypt, North Africa and Arabia, the species has been introduced in many temperate and tropical regions. It is considered an invasive plant. This species grows in sandy claim loam soil. Some studies have showed that this species can growth in soils contaminated by copper, zinc, lead and cadmium. This plant can tolerate and absorb heavy metals [139; 140].

Stipa tenacissima L. is native to north-western Africa and the southern part of the Iberian Peninsula. It is considered an invasive plant. It is well adapted to water stress conditions. Moreover, some studies have showed that this specie can tolerate and absorb some heavy metals like copper, lead or arsenic [141 - 143].

3.2. Physical properties of the soil

3.2.1. Apparent density

The apparent density of soil is calculated as the weight of dry soil in a defined volume. The apparent density can inform about the compaction of soil, the difficulty of plant rooting, capacity of growth, and the circulation of water and air. The apparent density depends on soil texture, organic matter contents and compaction of soil [144].

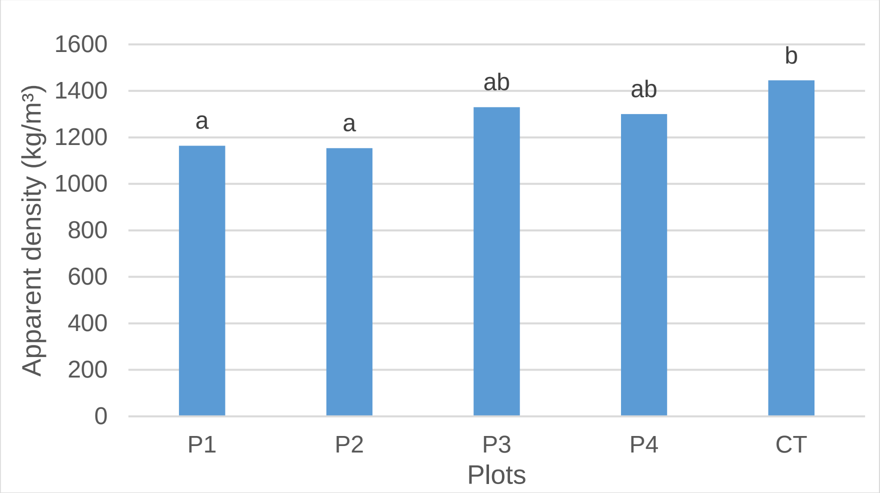

Figure 7: Apparent density in the studied plots.

The apparent density ranged between 1100 kg/m³ and 1500 kg/m³. According to the Tukey's test the apparent density is more or less identical for the plots P1, P2, P3 and P4 and similar for the plots P3, P4 and CT. This results can be explained by the fact that clay and organic matter contents in the plots P1 and P2 (with higher vegetation cover) are high to increase significantly the aggregates percentage and reduce the apparent density compared to the plot CT, so improving the physical condition for the plant growth.

3.2.2. Real density

The real density is the mean density of soil particles. It enables toestimate soil density with the pores [64].

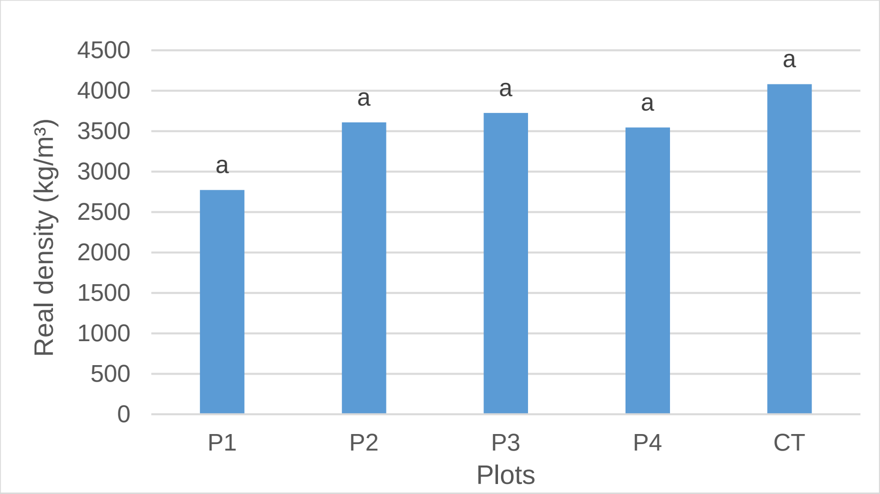

Figure 8: Real density in the studied plots;

We can see in Figure 8 according to the Tukey's test that the results of the five plots are homogeneous. The values of the real density range between 2800 and 4100 kg/m³. According to Porta et al. (1999) (annex 1), we can classify the different plots: P1 has the characteristics of a mineral horizon and P2, P3, P4 and CT have the characteristics of mineral horizons with some metal elements. These results can explain the fact that vegetation growth more in the plot P1 than in the others plots (P2, P3, P4 and CT) because metal elements can disrupt the plant development [64].

3.2.3. Soil texture

The soil texture enables to determinate the percentage of clay (< 0.002 mm), silt (0.002 - 0.05 mm) and sand (0.05 - 2 mm) in order to determinate the textural class of the soil. The soil texture enables to recognize soil properties and characteristics. For example, with the soil texture we can know the capacity of water retention available for the plants, the facility for the water circulation, the hazard of salt crust formation or the water and wind erosion [145 - 147].

Plots	% Clay	% Silt	% Sand	Textural class
P1	9	17	74	Sandy loam
P2	8	13	79	Sandy loam
P3	11	10	80	Sandy loam
P4	8	11	81	Sandy loam
CT	9	13	78	Sandy loam

Table 3: Soil texture in the studied plots.

In Table 3 we can observe that the textural class of the 5 plots is the same; it's a sandy loam texture. Particles of sandy loam are relatively spaced and enable a fast flow of the water in the soil. Substances dissolved in the water can quickly infiltrate in the depths of the soil. For example, metals dissolved in the water can infiltrate more quickly the soil. Sandy loam soils have not much stable structure what facilitate the wind erosion [148; 149].

Moreover, sandy loam soils facilitate the water erosion with water runoff which can reach a very high speed. Furthermore, the texture of studied soil has a low capacity of water and nutrient retention. So the texture of the soil can be hostile to the growth of plants [148; 149].

3.2.4. Aggregate stability in water

The aggregate stability in water is defined as the capacity of them to keep its form concerning all forces artificially induced, concretely the derivate of wetting or the impact of raindrop [47].

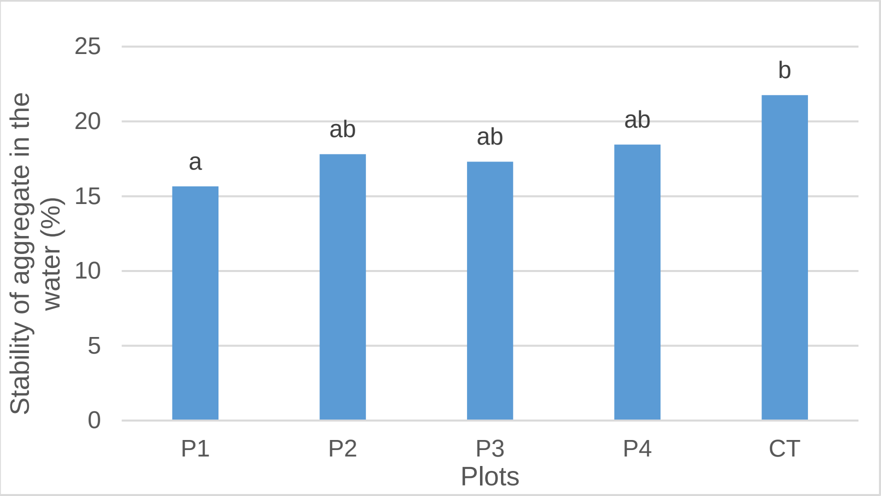

Figure 9: Aggregate stability in water in the studied plots.

The aggregate stability in water is higher in plot CT (22 %), however in plot P1 the stability of aggregate in the water is equal to 16 %. According to Tukey´s analyse two groups of aggregate stability in water are distinguished: the group with P1, P2, P3 and P4 and another group with P2, P3, P4 and CT.

These results show that the aggregates are stable in water and contribute to the improvement of the soil structure, to the increase of porosity, to the increase of the water retention capacity. Moreover, aggregates stable in the water stimulate the activity of soil fauna and decrease the erosion [145].

3.3. Chemical properties of the soil

3.3.1. pH

The values of pH enable to know the saturation of bases and the availability and the mobility of some nutrients and metals [148].

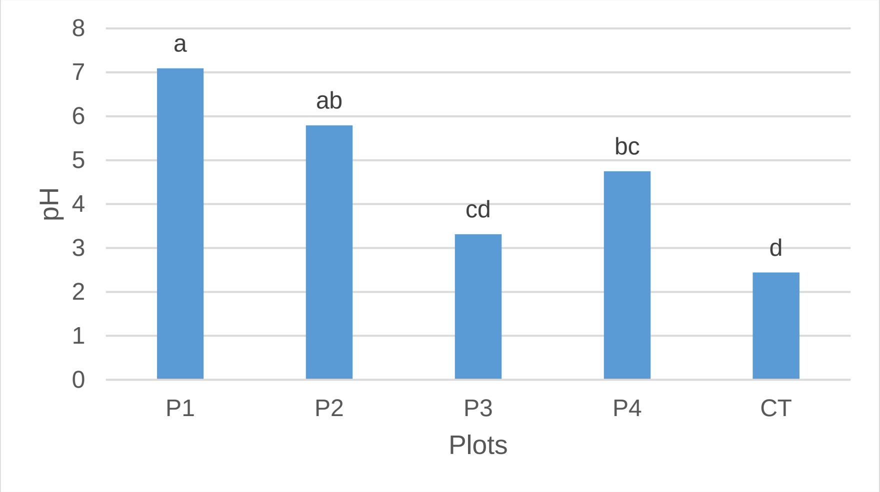

Figure 10: pH in the studied plots.

We can see in the Figure 10 that the pH value is neutral for the plot 1 (7.1); and the values are acid for P2 and P4, (5.8 and 4.8, respectively). However, for the plots P3 y CT, the pH is very acid (3.3 and 2.4, respectively). These results can explain the difference of vegetation growth in these plots because plants can’t growth at pH below 5 [149].

Furthermore, when the soil pH is very acid (pH < 3.5) a deficiency on the availability of some nutrients like calcium, magnesium, phosphorus and sulphur may be caused. The plants need these nutrients to growth. Additionally, a pH very acid increase the concentration of iron, copper and zinc for plants which is harmful for the plant development [148; 150].

Moreover, the Tukey’s test shows that different values exist among the plots. The pH value in soil from P1 is similar to P2; P2 is similar to P4; P4 is similar to P3 and P3 is similar to CT. This result can explain the difference of percentage of the vegetation cover observed in the different plots.

3.3.2. Electric conductivity

The electric conductivity determines the solution capacity to transport electric current, determining the total concentration of ionized components in the soil solution [151].

In Figure 11 we can observe that the electric conductivity is higher in the plots 3 and CT (7.4 and 7.7 mS/cm, respectively) corresponding to the plots without plants and the soluble salt contents are lower in P1, P2 and P4 (3.0; 4.0 and 4.4 mS/cm, respectively). The plots 3 and CT contain two time more ionized components than the plots P1, P2 and P4.

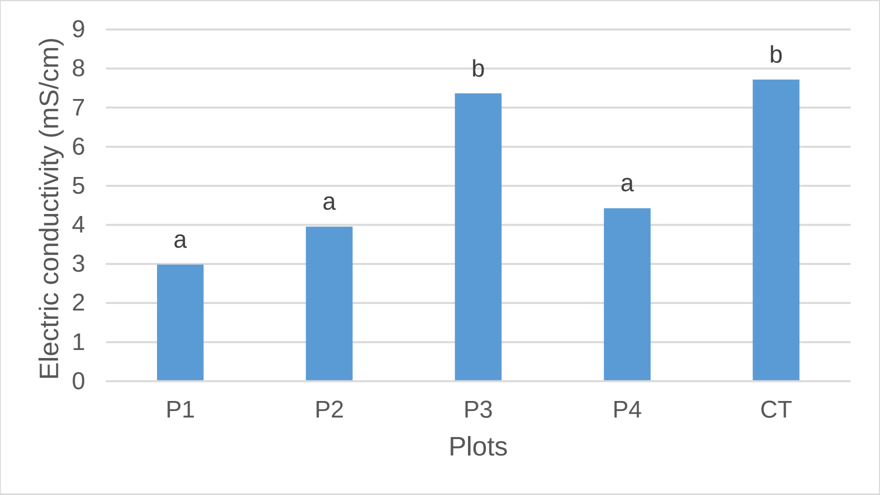

Figure 11: Electric conductivity in the studied plots.

In general, the plants can normally absorb water and nutrients until a maximum electric conductivity of 4 mS/cm. If the electric conductivity exceeds 4 mS/cm, it's more difficult for the plants to absorb all nutritional elements whose it need and it's possible that the plants die [151].

Moreover, when the electric conductivity of a soil is superior at 5 mS/cm, the electric conductivity can to cause damages at plant roots present in the soil [151].

The electric conductivity can explain the fact that we can see different vegetation in the plots. In the plots P3 and CT, it is impossible or very difficult for plants to absorb nutritional elements due to the electric conductivity. Conversely, in plots P1, P2 and P4 the vegetation can be developed without severe restrictions.

3.3.3. Inorganic carbon

The inorganic carbon corresponds to the total carbon fraction of the soil coming from mineral such as carbonates. The inorganic carbon can help to estimate different soil properties such as soil structure, biological activity, nutrient retention… [48]

We can see in Figure 12 that the inorganic carbon is higher for plots P1, P2 and P4 (2.9 %; 1.5 %; 1.3 %, respectively) than in plots 3 and CT (0.53 %; 0.47 %, respectively). From the Tukey´s test we can observe a difference in the inorganic carbon content between the plot P1 which is vegetated and the plots P3 and CT which aren't vegetated. The inorganic carbon plays a role in soil pH values because of the increase of the soil pH and therefore is a very important constituent for vegetation plant.

According to Porta et al. (1986) (Annex 2), we can classify the plots according to the percentage of inorganic carbon. For the plots P1, P2 and P4, we can say that the soil is a little limestone and for the plots 3 and CT the soil can't be consider as limestone [145].

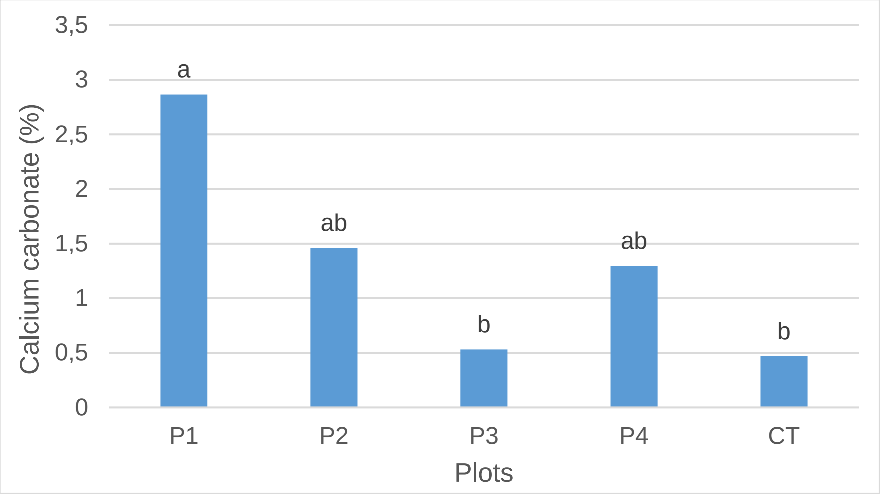

Figure 12: Inorganic carbon in the studied plots.

3.3.4. Organic carbon

The organic carbon is the principal component of the organic matter content in the soil [152]. And the organic carbon content is used like the indicator of the organic matter content in soil. The organic matter can react to heavy metals creating new complexes and increasing their availability.

In contrast, the heavy metals can be also absorbed into organic matter and therefore, they are less bioavailable for plants. So the organic matter is also an important constituent affecting solubility and availability of heavy metals in soil. In addition, organic carbon can influence on soil structure, stability of aggregate, capacity of water retention, pH… [153; 154]

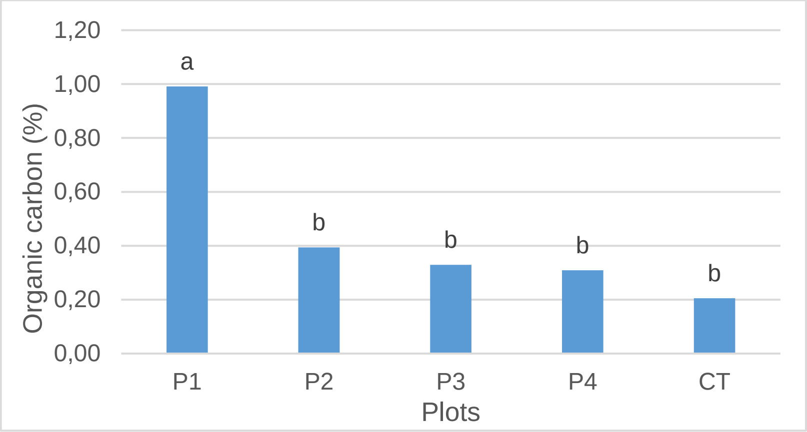

Figure 13: Organic carbon in the studied plots.

The results of the Figure 13 show that the organic carbon content is higher in the plot 1 (0.99%) than in the others plots (P2, P3, P4 and CT). According to Tukey's test, two groups can be distinguished: one for the plot 1 and one for the others plots. These differences of organic carbon content between P1 and the others plots are due to the high presence of vegetation in this plot, then when the roots, stems and leaves are decomposed in the soil the organic matter in the plot is increased. This explication is supported because the organic matter content obtained in the plot 1 is the highest registered value.

Moreover, the difference of organic carbon content between the plots can be also affected by the microorganism activity, because the organic matter from plants creates a favourable environment for microorganisms development which increase the organic carbon content of soil due to humification process [153; 154].

3.3.5. Sulphur

The majority of the sulphur in natural soil is in organic form. After its mineralization, it is transformed in sulphate. The sulphate is the assimilable form for the plants. The sulphur is an essential element for the synthesis of proteins for living organisms [155].

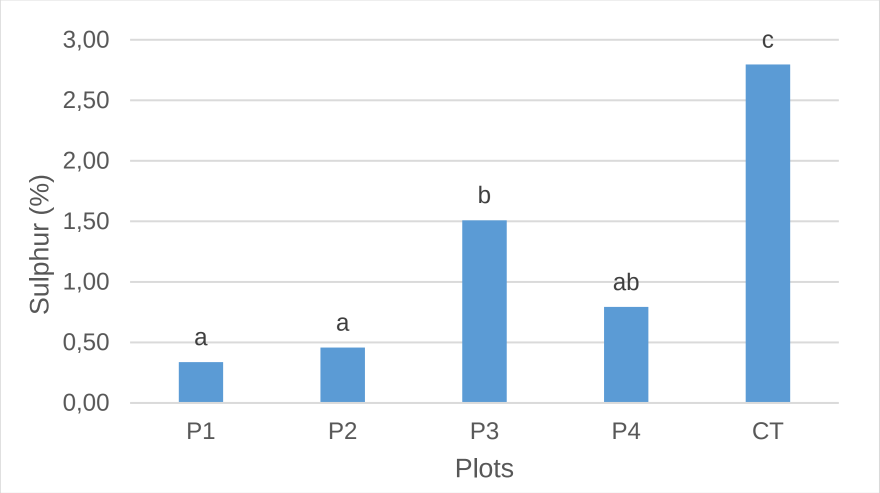

Figure 14: Sulphur in the studied plots.

With regard to the sulphur content in the studied plots, the values are lower than 0.5% in the plots P1 and P2. However, the sulphur percentages obtained in the other plots are: 0.8 in P4, 1.50 in P3 and 2.8 in CT. These results are associated to pH values, when sulphur minerals are oxidized sulphuric acid is generated and pH value decreases causing a very stress condition for plants growth (plots CT and P3). However, in plots P1, P2 and P4 the effect of sulphur is neutralized by the presence of $CaCO_3$ (supplied by marble waste) and the soil condition for plant growth are better.

3.3.6. Total nitrogen

The total nitrogen is an essential element for plants life because of it stimulates the plants growth and the microbial development. It exists a relation according to the quantity of total nitrogen and the fresh organic matter because organisms which decompose elements (plants roots; plant leaves…) in fresh organic matter need to use nitrogen [156].

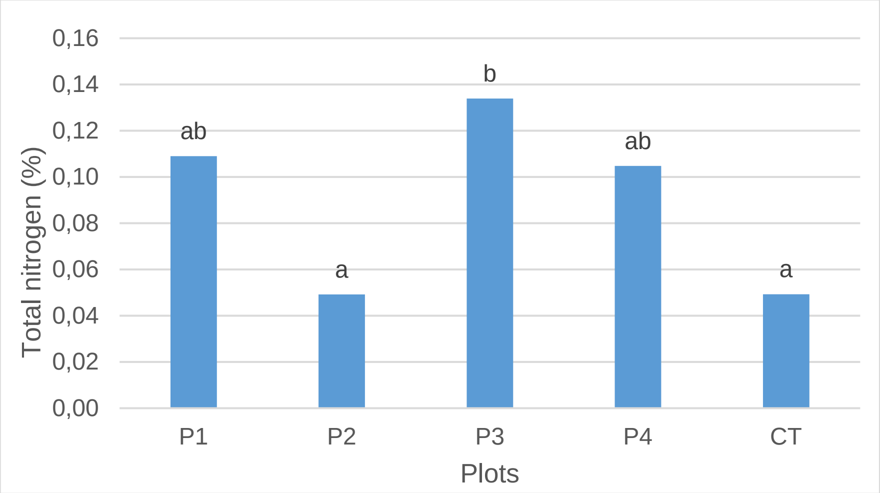

Figure 15: Total nitrogen in the studied plots;

We can see in Figure 15 that the percentage of total nitrogen varies between 0.05 % and 0.13 %. The variation is small but with the Tukey´s test is shown two different groups: one group including plots 2 and CT have the same percentages of total nitrogen around (0.5 %), and the other group including the plots P1, P3 and P4 which have very similar percentages (0.11; 0.13 and 0.10 %, respectively). Therefore, if the plots without vegetation have similar nitrogen percentages to the plots with vegetal, we can conclude that the percentage of total nitrogen in the study area doesn’t influence on the growth and the development of the plants.

3.3.7. Available phosphorus

The phosphorus is an essential element for the plants. It is important in a lot of physiological and biochemical processes. This element controls the energy exchange and favour the growth of the plants. In the most of soils, the available phosphorus represents only 0.01 % of total phosphorus. The phosphorus is usually combined with other elements, for example Al, Mn or Fe [144]. The application of organic amendments (pig slurry or pig manure) can increase the availability of the phosphorus in the soil. Moreover, the plants decomposition can increase the availability of the phosphorus in the soil [7; 157; 158].

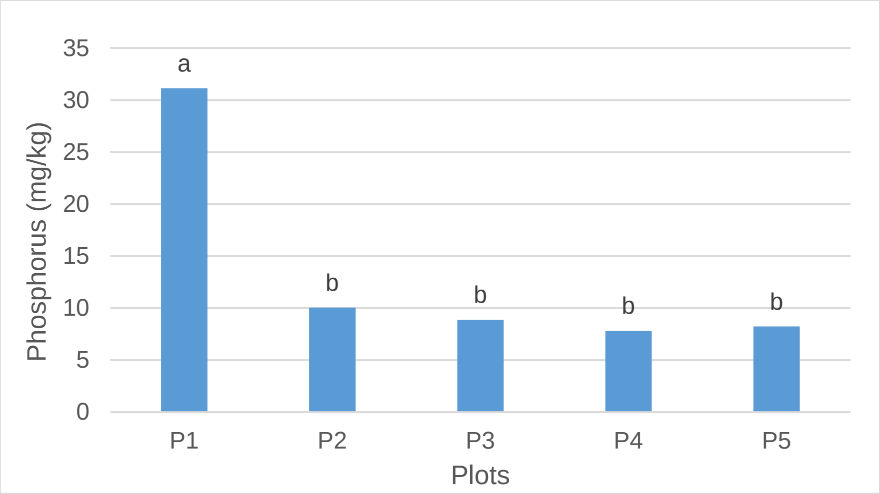

Figure 16: Available phosphorus in the studied plots.

According to Figure 16, the higher available phosphorus contents is obtained in the soil from plot 1 (31.1 mg/kg of soil). For the other plots the available phosphorus is three time less than P1 (between 7 and 10 mg/kg of soil). Moreover, with the Tukey´s test shows that there are 2 groups: the first with plot 1 and the second with plots P2, P3, P4 and CT. These results can be due to the pH of the plots P2, P3, P4 and CT is acid and when the pH is acid, the phosphorus is very little available for the plants. So, the available phosphorus can be a limiting factor for the growth and development of plants.

3.4. Total metals, soluble metals and available metals with $CaCl_2$

3.4.1. Total metals

The heavy metals are a group of chemical elements which have a high density and a certain toxicity for plants and animals when the heavy metals are in high concentration in the soil. However, in the group of heavy metals, there are two subgroups:

- The necessary heavy metals for the life, like the Zn or the Cu.
- The heavy metals which haven't any biological function known, for example Pb or Cd.

Nevertheless, if these two groups are in high concentration in the soil, they can be toxic for plants or animals. For this reason, it is necessary to determine the quantity of heavy metals in the mining waste in order to know the danger that they can create on the environment or on the human health [159].

Currently, legislation about mining soils do not exist in Spain but we have used in this study the reference levels existing in other uses of soil (Table 4) to know if the heavy metal concentrations in Santa Antonieta tailing pond are on not very high [160; 161]. In addition, we have consulted the reference levels of heavy metal registered in natural soils near to tailing pond: Zn 96 mg/kg of soil, Pb: 43 mg/kg of soil, Cu: 23 mg/kg of soil and Cd: 0,6 mg/kg of soil [162], where metal enrichment of this zone is checked and thus demonstrating the interest in the past to extract metals.

Country	**Reference level (mg/kg of soil)**	
European Union (European Communities Council Directive. 86/278/EEC)	Zn: 150 - 300 Pb: 50 - 300	Cu: 50 - 140 Cd: 1 - 3
Spain (Real Decreto 1310/1990, de 29 de octubre)	pH < 7	pH > 7
	Zn: 150 Cu: 50 Pb: 50 Cd: 1	Zn: 450 Cu: 210 Pb: 300 Cd: 3

Table 4: Reference level of polluted soil for Spain and European Union.

Total lead concentration

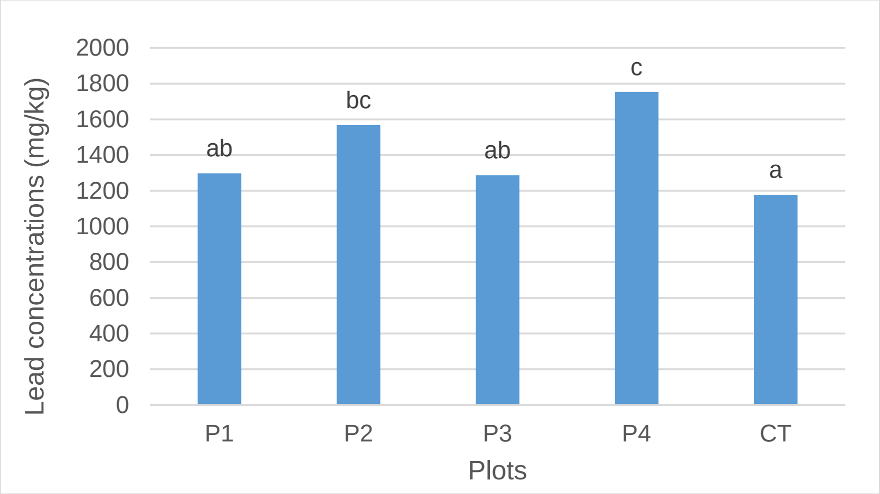

Figure 17: Pb concentrations in the studied plots.

Lead (Pb) concentrations reported in the studied soil varied between 1176 and 1753 mg/kg (Figure 17). If we compare the before values with the reference level of the Spanish legislation, we can see that the concentrations are very high. Moreover, the plot CT (without rehabilitation) contains lower lead concentration than in the others plots. The plots P2 and P4 contain the highest Pb concentration and the plots P1, P3 and CT have similar concentration according to the Tukey's test. So, the total Pb concentration doesn't affect the efficiency of aided phytostabilization due to the plot P1 with vegetation has similar Pb concentration than the plots without vegetation (P3 and CT).

Total copper concentration

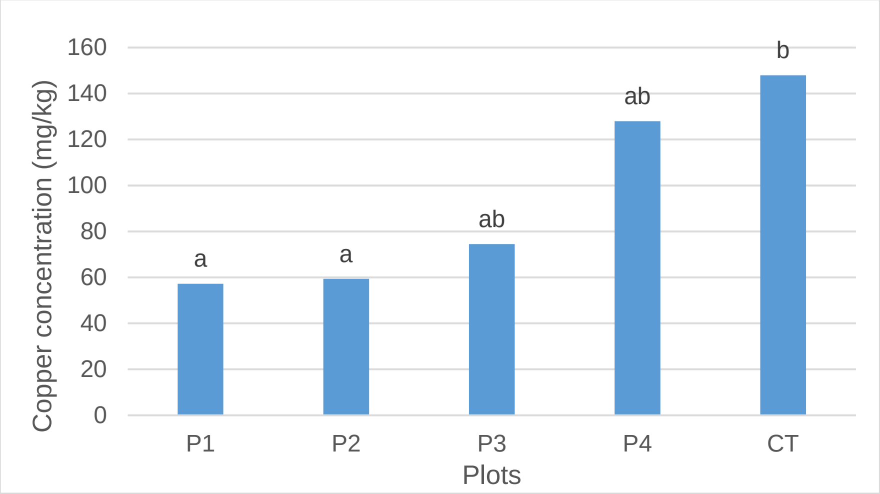

Figure 18: Cu concentrations in the studied plots.

The Cu concentrations are very high for the plots P3, P4 and CT (Figure 18). Moreover, all Cu concentrations are higher than the reference values of the Spanish legislation except for the plot P1. With the Tukey's test we can difference two groups: the Cu concentrations registered in the plots P1, P2, P3, P4 and the contents observed in the plots P3, P4 and CT. The plot CT where the rehabilitation was not carried out, has the highest Cu concentration. And the plot P3 without vegetation has similar values Cu concentrations to the plots P1 and P2 where the species plants grew. Therefore, the total Cu concentration doesn't affect efficiency of aided phytostabilization.

Total zinc concentration

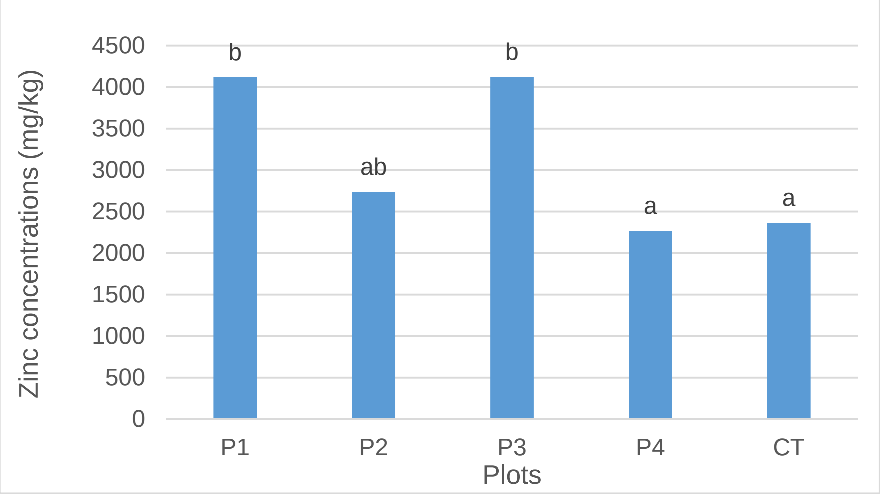

Figure 19: Zn concentrations in the studied plots.

The Zn concentrations of all the plots are very high compared to the reference levels of the Spanish legislation (Figure 19). Moreover, we can observe the Zn concentration of the plots P1 and P3 are similar according to Tukey's test, which shows that plants species can grow with a high concentration of zinc because of P1 is the most vegetated plot. The Zn concentrations in the plots P4 and CT are the lowest of all them. These results show that the total Zn concentration doesn't influence efficiency of aided phytostabilization.

Total cadmium concentration

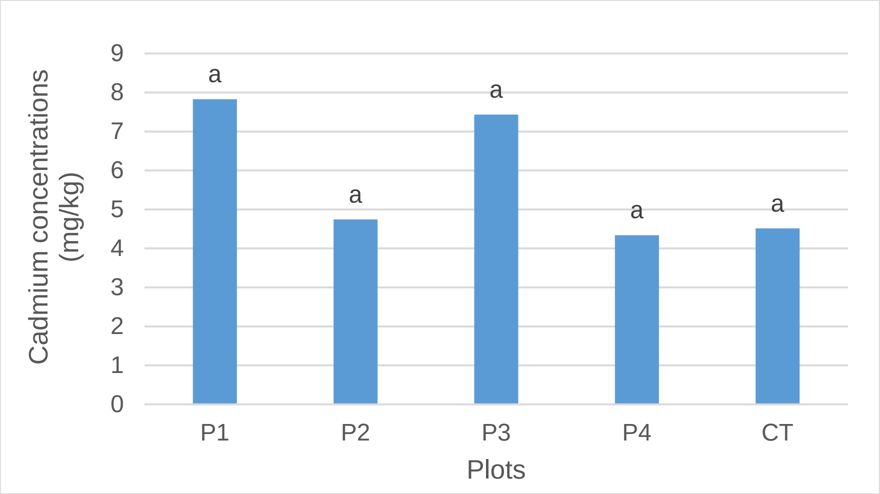

Figure 20: Cd concentrations in the studied plots.

The Cd concentrations ranged from 4 to 8 mg/kg of soil. These values are higher than the reference level of the Spanish legislation. With Tukey's test we can see that the values of all the plots are similar. That is to say that the plots with vegetation and the plots without vegetation have similar Cd concentrations. So, the total Cd concentration can't affect efficiency of aided phytostabilization.

3.4.2. Water soluble heavy metals

The use of amendments to decrease the heavy metals solubility has been demonstrated in a lot studies about rehabilitation of contaminated areas [163 - 165].

Soluble lead concentration

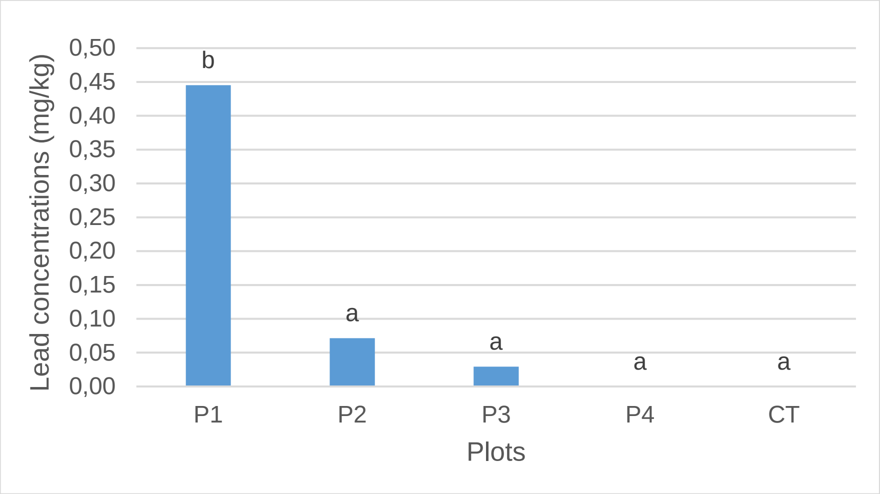

Figure 21: Pb concentrations in the studied plots.

In the above figure we check that the soils from plot P1 has the highest concentration of Pb soluble in the water (0.45 mg/kg of soil). The plots P4 and CT don't contain Pb soluble, while that in the soils from the plots P2 and P3 are observed low soluble Pb concentrations (0.07 and 0.03 mg/kg, respectively). According Tukey's test, the plots P2, P3, P4 and CT are similar, and the soil from P1 (the plot most vegetated) contains nine time more of Pb than in the others plots. Therefore, the water soluble Pb in water doesn't affect efficiency of aided phytostabilization.

Soluble copper concentration

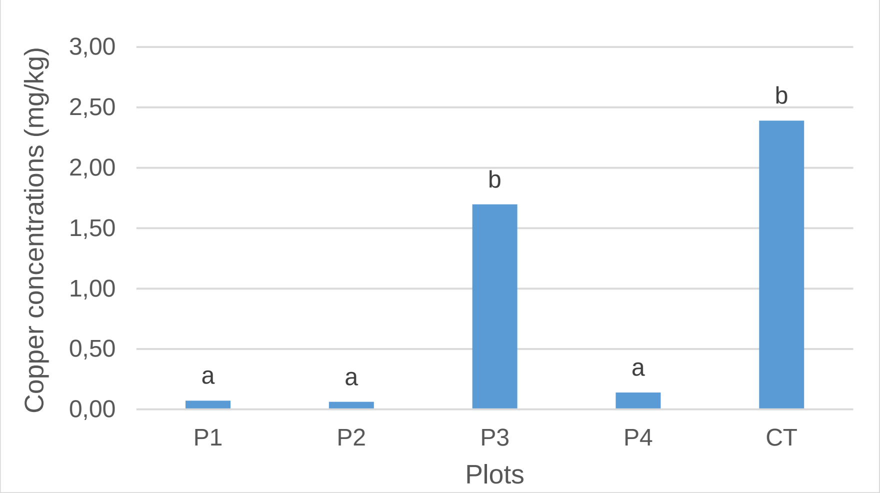

Figure 22: Cu concentrations in the studied plots.

We can see in the Figure 22 that the soils from plots P3 and CT have the highest water soluble Cu concentrations in (1.70 and 2.39 mg/kg, respectively). The Tukey's test differences two groups: on the one hand the soils from plots P1, P2 and P4 and on the other hand the soils from plots P3 and CT. In the plots P1, P2 and P4 the plants grew while in the plots P3 and CT, there weren't plant species. So, we can think that the water soluble Cu affects efficiency of aided phytostabilization and prevents the plant growth.

Soluble zinc concentration

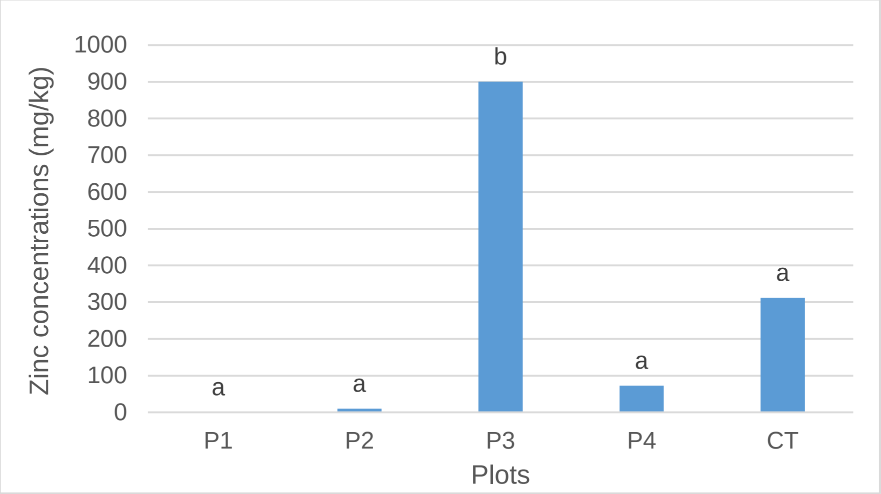

Figure 23: Zn concentrations in the studied plots.

The soil from plots P3 and CT are the most concentrated in water soluble and, particularly, the soils from the plot 3 with 900 mg/kg of soil. According to the soluble zinc concentration in the studied plots is stablished the following conclusion:

$$P1 \leq P2 \leq P4 < CT < P3$$

The plots P3 and CT have the highest water soluble Zn concentrations.

Moreover, the soluble Zn concentrations in the plot P3 is three times higher than the plot CT. This result is surprising because the plot P3 has been rehabilitated and not the plot CT, so the plot P3 must contain lower soluble Zn concentration than the plot CT. This can be because of that there was a period with intense precipitations which originated waterlogging of the plot and due to the acidic nature of these waters dried plants and plant residues enriched in heavy metals were incorporated into soil increasing the concentration of soluble metal. Other hypothesis may be that the heavy metals were transported from other contaminated adjacent areas not rehabilitated where the soluble Zn concentration was high.

Soluble cadmium concentration

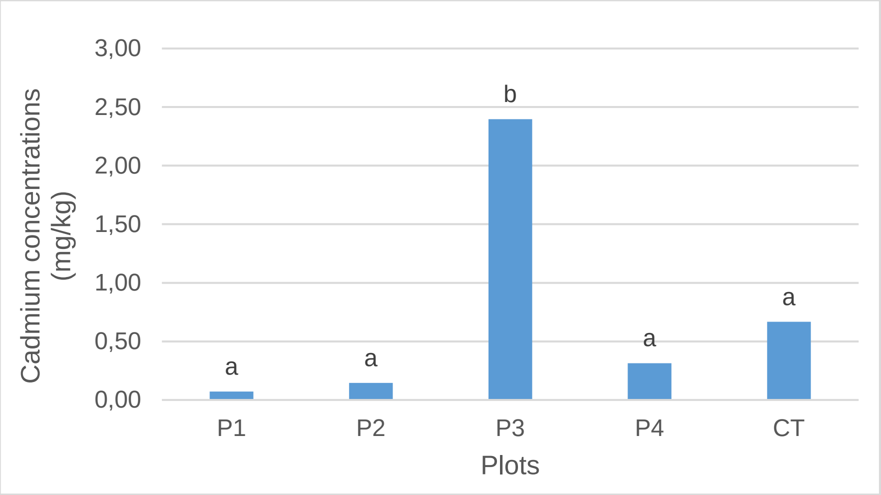

Figure 24: Cd concentrations in the studied plots.

The soil from plot P3 is the most concentrated in Cd soluble (2.40 mg/ kg). According to the soluble Cd concentration in the studied plots is stablished the following conclusion:

$$P1 \le P2 \le P4 \le CT < P3$$

The reported results are equal than the results about the soluble zinc concentration.

Moreover, we can observe a big difference of concentrations between the plots P1, P2, P4, CT and the plot P3. We can establish the same hypothesis that with the soluble zinc concentration. Therefore, we can say that the soluble cadmium in water affects efficiency of aided stabilization.

3.4.3. Bioavailable metals

In the soil, metals are associated with different fractions: (1) in solution, like free metal and soluble metallic complex, (2) absorbed in exchange places of inorganic elements of the soil, (3) bound to organic matter, (4) precipitated like oxides, hydroxides and carbonates, and (5) residual in the structure of silicate minerals. The bioavailable metals are the fraction of heavy metals which are available for the plants absorption [66].

The bioavailability depends on the solubility of metals and its adsorption capacity in the colloidal fraction of soil (only the metals associated with fractions 1 and 2 are really available for the plants uptake). The interaction among the different process like cationic exchange, adsorption/desorption, precipitation/dissolution and the formation of complex affect the distribution of metals between the soil solution and the solid phase, being responsible of their mobility and bioavailability [66].

The factors which affect the metal bioavailability are pH, electric conductivity, texture, content of clay, organic matter, oxides of Fe, Mn and Al and the presence of cations and anions in the soil solution [66].

The use of inorganic and organic amendments (pig slurry, pig manure and marble mud) decreases the bioavailable metals concentration in soil, so the toxicity for plants is decreased [7].

Bioavailable lead concentration

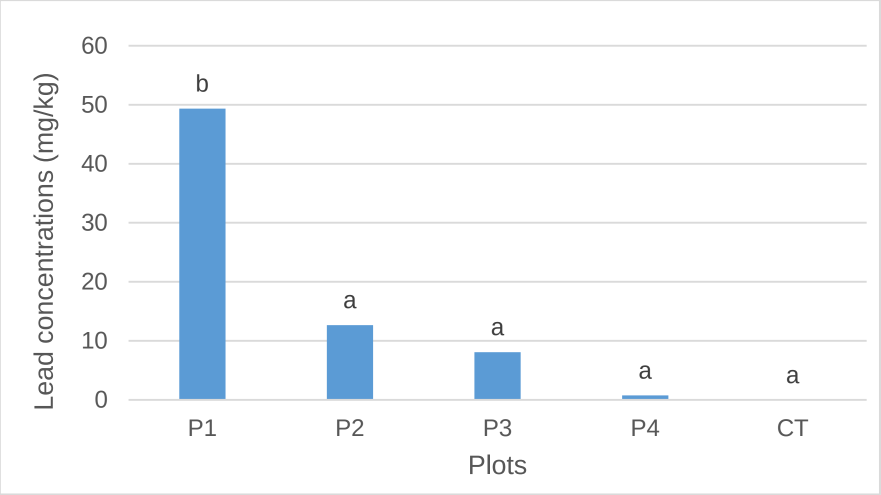

Figure 25: Pb concentrations in the studied plots.

We can see in Figure 25 that the bioavailable Pb concentration ranged between 0 and 49 mg/kg of soil. The plot P1 has more bioavailable Pb than the others plots (49 mg/kg of soil). According to Tukey's test, the plots P2, P3, P4 and CT are equal. The plot P1 is the most vegetated and this plot contains the highest bioavailable Pb concentrations, so we can think that the bioavailable lead doesn't affect the efficiency of aided stabilization.

Bioavailable copper concentration

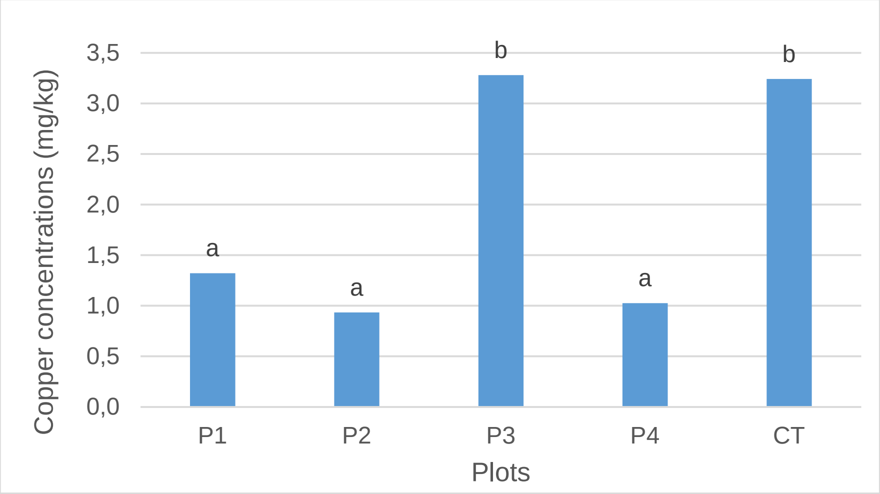

Figure 26: Cu concentrations in the studied plots.

The soil from P3 and CT have the highest bioavailable Cu concentrations (3.3 and 3.2 mg/kg of soil, respectively). Moreover, the Tukey's test distinguishes two groups: on the one hand the soils from plots P1, P2 and P4 (plots with vegetation) and on the other hand the soils from plots P3 and CT (plots without vegetation), though only a difference around 2 mg/kg exists among both groups.

Bioavailable zinc concentration

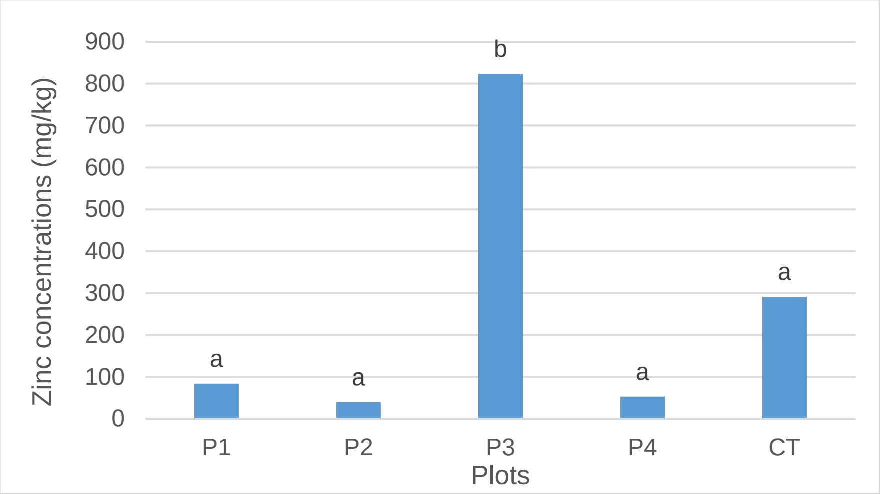

Figure 27: Zn concentrations in the studied plots.

We can see in Figure 27 that the soils from plot P3 and CT have the highest bioassimilable Zn concentrations, in particular P3 with concentrations until 824 mg/kg. According to the bioassimilable Zn concentration in the studied plots is stablished the following conclusion:

P1 ≤ P2 ≤ P4 < CT < P3

Moreover, the bioassimilable Zn concentrations in the plot P3 is three times higher than the plot CT. In the same way than water soluble Zn, likely high soluble Zn concentration come from plot CT and they are accumulated in plot P3. Therefore, the bioassimilable Zn affects efficiency of aided phytostabilization and prevent the plant growth.

Bioavailable cadmium concentration

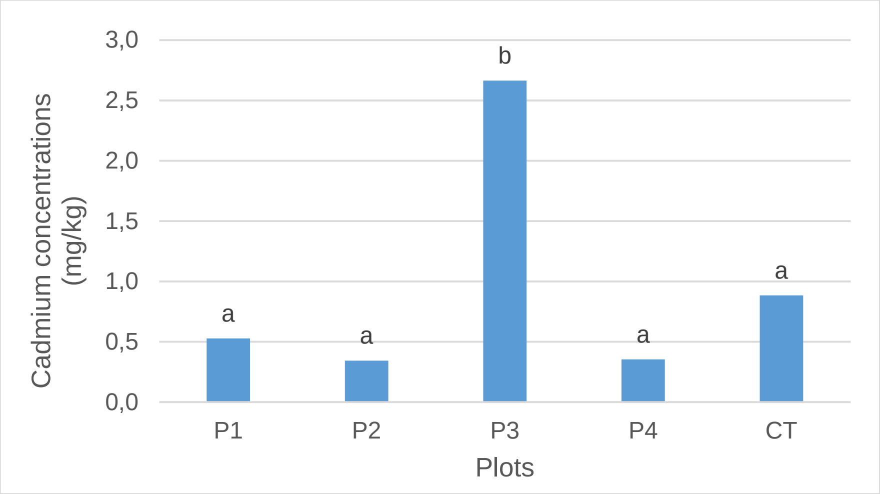

Figure 28: Cd concentrations in the studied plots.

In the Figure 28 we can see the soils from the plot P3 is the most concentrated in bioassimilable Cd with a concentration of 2.7 mg/kg of soil. According to the bioassimilable Cd concentration in the studied plots is stablished the following conclusion:

P1 ≤ P2 ≤ P4 ≤ CT < P3

The obtained results are equal than the results of bioavailable zinc concentration.

The Tukey’s test differences two groups: the plots P1, P2, P4, CT and the plots P3. We can establish the same hypothesis than with the bioassimilable zinc concentration. So, we can say that the bioassimilable cadmium affects efficiency of aided stabilization.

3.5. Sequential extraction of metals

The sequential extraction of metals enables to quantify metals concentrations present in five distinct fractions: exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound, and residual [166; 167].

The fraction of exchangeable metals represents the mobile and bioavailable part. It gathers the metals adsorbed by particles and which are easily labile by exchange of ions with the cations Ca^{2+}, Mg^{2+} or NH_4^+. This fraction is the most mobile and so the most toxic for the environment [166; 167].

The heavy metal fraction bound to carbonates is composed by component carbonated, precipitated and coprecipitated metals with natural carbonates which dissolve with a decrease of pH. So this fraction is sensible at a weak decrease of pH [166; 167].

The third fraction is the heavy metal fraction bound to Fe and Mn oxides of Fe, Al, and Mn. These oxides are in form of pellicular coating on the surface of particles. They are thermodynamically unstable in anoxic condition provoked for example while a decrease of redox potential [166; 167].

The heavy metal fraction bound to organic matter contains the metals associated with the organic matter. The connection between metals and the organic matter is the complexation. In oxidant environment, the organic matters are degraded and release heavy metals [166; 167].

The residual fraction is composed by primary minerals and secondary minerals which can contain metals in their crystalline structure [166; 167].

3.5.1. Lead

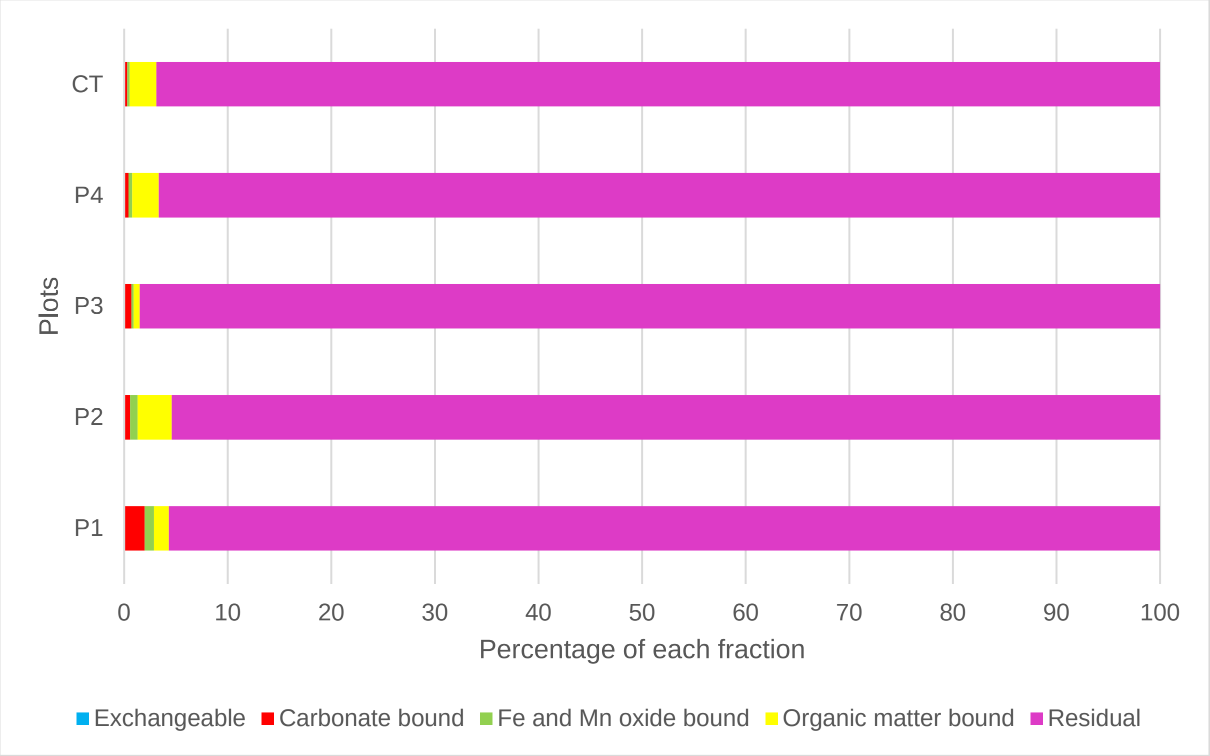

Figure 29: Pb fractionation in soils from the studied plots obtained by sequential extraction.

Lead is mostly present in the residual fraction (Figure 29), what represents 96 % in P1, 95 % in P2, 99 % in P3, 97 % in P4 and 97 % in CT. Moreover, Pb bound to organic matter in all the plants ranged between 1 % to 3 %, Pb bound to Fe and Mn oxides is around 1 % in the plots P1 and P2 and Pb bound to carbonates is higher in the plot 1 than the others plots (2 %). Furthermore, the percentage of exchangeable Pb is lower than 0.1 % for all the plots.

Lead in the residual fraction doesn't present high risks for the toxicity of plants and environment because the Pb in the residual fraction is not available and the plants can't absorb it. Therefore, probably none Pb fraction is affecting the efficiency of aided phytostabilization.

3.5.2. Copper

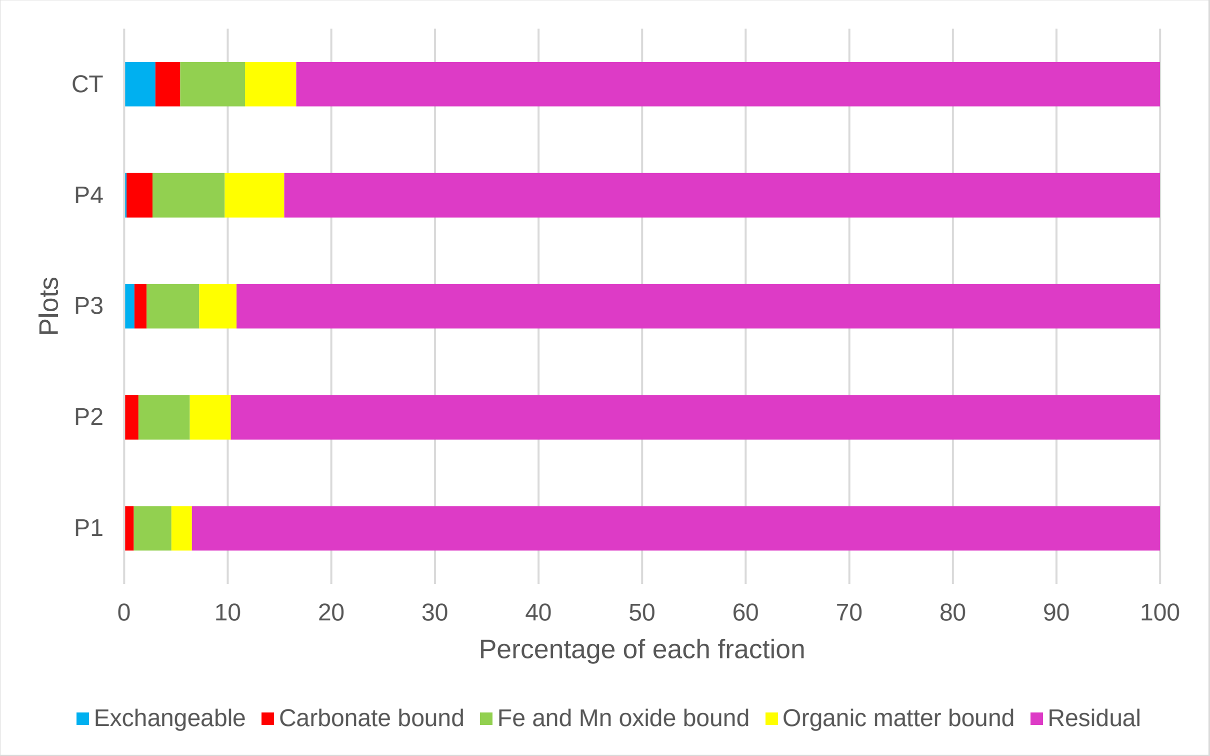

Figure 30: Cu fractionation in soils from the studied plots obtained by sequential extraction.

Cu is mostly present in the residual fraction (Figure 30), which represents 93 % in P1, 90 % P2, 89 % in P3, 85 % in P4 and 83 % in CT. Then, Cu bound to organic matter ranged between 2 % and 6 % in the studied plots and Cu bound with Fe and Mn oxide varied between 4 % and 7 %. Furthermore, Cu bound to carbonates ranged between 1 % to 2% for all the plots. And, the Cu exchangeable is present mainly in the plots P3 and CT (1 % and 3 %, respectively); in the others plots, the exchangeable Cu is lower than 0.3 %. According to the sequential extraction of Cu in the studied plots is stablished the following conclusion (except for the plot CT):

Residual > Fe and Mn oxide bound > Organic matter bound > Carbonate bound > Exchangeable

These results can confirm the hypothesis that the bioavailable Cu can affect efficiency of aided phytostabilization. We have observed one difference between plots vegetated and plots without vegetation concerning the exchangeable Cu. The exchangeable Cu fraction are the most mobile and toxic fraction for the plants, so it can influence efficiency of aided phytostabilization.

3.5.3. Zinc

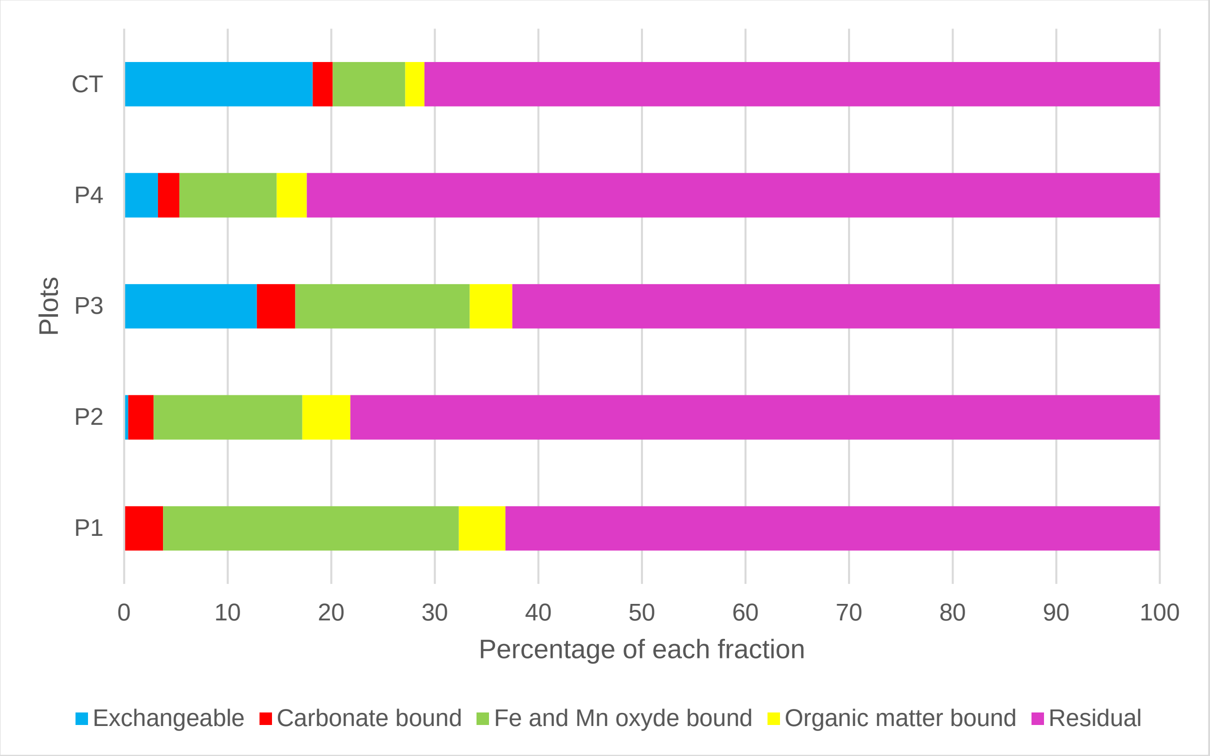

Figure 31: Zn fractionation in soils from the studied plots obtained by sequential extraction.

Zinc is mostly present in the residual fractions (Figure 31), which represents 63 % in P1, 78 % in P2, 63 % in P3, 82 % in P4 and 71 % in CT. There are some Zn percentage bound to organic matter (between 1.8 % and 4.6 %) and Cu bound to carbonates (between 1.9 % and 3.7 %) in the studied plots. Regarding to Zn bound with Fe and Mn oxides the highest concentrations are observed in the plots P1, P2 and P3 (29 %, 14 % and 17 %, respectively). And, the exchangeable zinc is higher in the plots P3 and CT (13 % and 18 %, respectively).

So, the zinc in the exchangeable fraction is mobile and present a risk for the environment and a risk for the toxicity of plants. These results are consistent with values of bioavailable Zn in the plots. Therefore, these Zn contents can affect efficiency of aided stabilization.

3.5.4. Cadmium

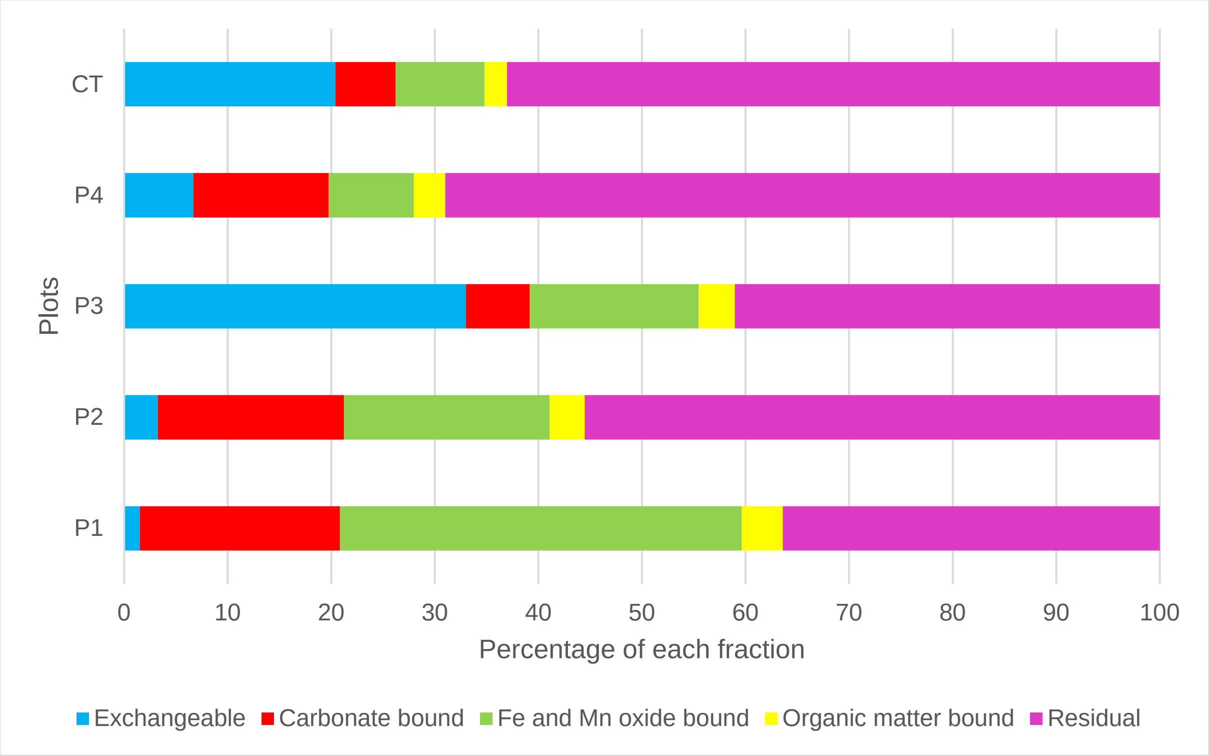

Figure 32: Cd fractionation in soils from the studied plots obtained by sequential extraction.

Cadmium residual fraction is not as predominant as with the other metals in the studied plots: 6 % in P1, 56 % in P2, 41 % in P3, 69 % in P4 and 63 % in CT (Figure 32). The percentage of Cd bound to organic matter ranged from 2 % to 4 % according to the plots. Cd bound with Fe and Mn oxides represents 39 % in P1, 20 % in P2, 16 % in P3, 8 % in P4 and 9 % in CT.

Cd bound to carbonates is higher in the plots P1, P2 and P4 (19 %, 18 % and 13 %, respectively), this fraction doesn’t present a risk for the plots P1, P2 and P4 because when the pH is higher than 5, Cd is bounded with carbonates but this metal cannot be absorbed by the plants.

Moreover, the exchangeable Cd is high for the plots P3 and CT (33 % and 20 %, respectively). For the others plots, the exchangeable Cd represents 2 % for P1, 3 % for P2 and 7 % for P4. Therefore, the exchangeable Cd is ten times more high in the plots P3 than P1 and fifteen time more high in the plots P3 than P2. In addition to, exchangeable Cd is higher in P3 than CT.

We can conclude that the cadmium in the exchangeable fraction is mobile and presents a risk for the environment and a risk for the toxicity of plants. These results are consistent with values of bioavailable Cu; and the exchangeable Cd influences efficiency of aided stabilization.

To summarize the above results Table 5 is shown, where we can see the physical and chemical properties which that most influence the effectiveness of aided phytostabilization.

Properties		**Influences**	**Doesn't influence**
pH		X	
Electric conductivity		X	
Apparent density			X
Real density			X
Soil texture			X
Aggregate stability in water			X
Mineralogical composition			
Inorganic carbon		X	
Organic carbon			X
Sulphur			X
Total nitrogen			X
Available phosphorus		X	
Total metals	Lead		X
	Copper		X
	Zinc		X
	Cadmium		X
Water soluble metals	Lead		X
	Copper	X	
	Zinc	X	
	Cadmium	X	
Bioavailable metals	Lead		X
	Copper	X	
	Zinc	X	
	Cadmium	X	
Sequential extraction of metals	Lead		X
	Copper	X	
	Zinc	X	
	Cadmium	X	

Table 5: Summary of physical and chemical properties that affect aided phytostabilization.

4. Conclusions

During the visits to Santa Antonieta tailing pond we observed that the vegetation growth was different in each plot. We selected 5 plots (4 plots treated by technique of aided phytostabilization and 1 plot without treatment). A list of actual vegetation growing in the plots was carried out: *Atriplex halimus L., Cynodon dactylon (L.) Pers.*, *Diplotaxis erucoides (L.) DC.*, *Lobularia maritima (L.) Desv., Oxalis pes-caprae L.*, *Phagnalon saxatile (L.) Cass.*, *Piptatherum miliaceum (L.) Coss., Senna occidentalis (L.) Link.* and *Stipa tenacissima L*; and we observed differences between the plant type and vegetation cover.

After the experiments in laboratory and the analysis of results, we can conclude that the pH affects the vegetation development due to its acidity. In addition to the electric conductivity affects the vegetation development because of that soluble salt content is very high so the plants cannot absorb the water and the nutrients. And the inorganic carbon is too low in order the vegetation grow, due to it doesn´t favour the biological activity and the nutrient retention of the soil. The available phosphorus is also too low, this nutrient is very important for the plant growth but when the pH is acid, the available phosphorus become unavailable.

With the experiments of heavy metals in laboratory (total, bioavailable, water soluble heavy metals and sequential extraction of metals), we have determined that Cu, Zn and Cd affect efficiency of the aided phytostabilization.

Finally, the quantities of organic and inorganic amendments should be increased in Santa Antonieta tailing pond in order to improve the results on aided phytostabilization, especially, available phosphorus, pH, electrical conductivity and inorganic carbon.

In addition new plants could be used to stabilize Cu, Zn and Cd such us *Diplotaxis erucoides* (L.) DC., *Senna occidentalis* (L.) Link., which have been used in similar studies and the results were very good; or *Festuca Ovina* L. and *Vetiveria zizanioides* (L.) Nash., which are species that can growth in arid and dry climate and which can to stabilize pollutants such as Cu, Zn and Cd.

5. References

[1] MANTECA, J. I. *Los yacimientos Zn, Pb, Ag-Fe del Distrito Minero de La Unión-Cartagena, Bética Oriental.* 1992. In J. M. J. García, Recursos minerales de España. Madrid: CSIC. pp 1085-1102.

[2] Region de Murcia digital. *La minería en La Unión.* URL: https://www.regmurcia.com/servlet/s.Sl?sit=c,373,m,2910&r=ReP-25317-DETALLE_REPORTAJESPADRE

[3] MARTOS MIRALLES, P. *El paisaje minero de la Sierra de Cartagena-La Unión como paisaje cultural. Del abandono y el expolio a la protección y la recupación de su patrimonio.* 2009. Fundación Sierra Minera. URL: http://www.fundacionsierraminera.org/ficheros/redes/02_Redes_04_05_2009_23_32_48.pdf

[4] ZORILLA, C., SACHER, W., ACOSTA, A. and BAEZ, M. *21 Preguntas para entender la mineria del siglo 21*. URL: http://rebelion.org/docs/138009.pdf

[5] ZORNOZA, R., FAZ, Á., CARMONA, D., ACOSTA, J., MARTINEZ MARTINEZ S. and VRENG, A. *Carbon mineralization, microbial activity and metal dynamics in tailing ponds amended with pig slurry and marble waste*. 2013. Chemosphere. Volume 90, pp 2606-2613.

[6] CONESA, H., FAZ, Á. and ARNALDOS, R. *Initial studies for the phytostabilization of a mine tailing from the Cartagena-La Unión Mining District (SE Spain).* 2007. Chemosphere. Volume 66, pp 38-44.

[7] SANCHEZ VILLALUENGA, C. Proyecto fin de Carrera. *Evolucion de la calidad del suelo, disponibilidad de metals pesados y vegetacion en un deposito minero de la sierra minera de Cartagena-La Union tras su rehabilitacion mediante fitoestabilizacion asistida.* 2015. Tesis Doctoral. Universidad Politécnica de Cartagena (Murcia, España). 133 p.

[8] RODRIGUEZ, R., GARCIA, C., ZARROCA, M., ROQUE C., LINARES, R. and OLDECOP, L. *Características geológicas y geotécnicas de los lodos de flotación en la Sierra Minera de Cartagena-La Unión (SE España).* 2001. Boletín geológico y minero. Volume 122, pp 127-144.

[9] FAVAS, P. J. C., PRATAS, J., VARUN, M., D'SOUZA, R. and PAUL, M. S. *Phytoremediation of Soils Contaminated with Metals and Metalloids at Mining Areas: Potential of Native Floral*. 2014. Environmental Risk Assessment of Soil Contamination, Dr. Maria C. Hernandez Soriano (Ed.), InTech. URL: http://www.intechopen.com/books/environmental-risk-assessment-of-soil-contamination/phytoremediation-of-soils-contaminated-with-metals-and-metalloids-at-mining-areas-potential-of-nativ

[10] SHARMA, S. and PATHAK, H. *Basic techniques of phytoremediation.* 2014. International Journal of Scientific & Engineering Research. Volume 5, pp 584-605.

[11] BAKER, A. J. M., MCGRATH, S. P., SIDOLI, C. M. D. and REEVES, R. D. *The Potential for Heavy Metal Decontamination.* 1995. Mining Environ. Manage. Volume 3, pp 12-14.

[12] NANDA KUMAR, P. B. A., DUSHENKOV, V., MOTTO, H. and RASKIN, I. *Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils.* 1995. Environmental Science & Technology. Volume 29, pp 1232-1238.

[13] RASKIN, I., SMITH, R. D. and SALT, D. E. *Phytoremediation of metals: Using plants to remove pollutants from the environment.* 1997. Current Opinion in Biotechnology. Volume 8, pp 221-226

[14] USEPA, Office of Research and Development. *Introduction to Phytoremediation*. 2000. EPA/600/R99/107.

[15] CUNNINGHAM, S. D. *The Phytoremediation of Soils Contaminated with Organic Pollutants: Problems and Promise.* 1996. International Phytoremediation Conference. May 8-10, 1996. Arlington, VA.

[16] CUNNINGHAM, S. D., BERTI, W. R. and HUANG, J. W. *Phytoremediation of Contaminated Soils*. 1995. Trends in Biotechnology. Volume 13, pp 393-397.

[17] FEDERLE, T. W. and SCHWAB, B. S. *Mineralization of Surfactants by Microbiota of Aquatic Plants.* 1989. Applied and Environmental Microbiology. Volume 55, pp 2092-2094.

[18] DONNELLY, P. K., HEGDE, R. S. and FLETCHER, J. S. *Growth of PCB-Degrading Bacteria on Compounds from Photosynthetic Plants.* 1994. Chemosphere. Volume 28, pp 981-988.

[19] NARAYANAN, M., DAVIS, L. C. and ERICKSON, L. E. *Fate of Volatile Chlorinated Organic Compounds in a Laboratory Chamber with Alfalfa Plants.* 1995. Environmental Science & Technology. Volume 29, pp 2437-2444.

[20] SCHWAB, A. P. and BANKS, M. K. *Phytoremediation of petroleum-contaminated soils*. 1999. Chapter 28. In D.C. Adriano, 1999.

[21] SHIMP, J. F., TRACYS, J. C., DAVIS, L. C., LEE, E., HUANG, W., ERICKSON, L. E. and SCHNOOR, J. L*. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials.* 1993. Critical Reviews in Environmental Science and Technology. Volume 23, pp 41-77.

[22] CUNNINGHAM, S. D., BERTI, W. R. and HUANG, J. W. *Remediation of Contaminated Soils and Sludges by Green Plants.* 1995. In HINCHEE, R. E., MEANS, J. L. and BURRIS, D. R. (eds.), Bioremediation of Inorganics. Battelle Press, Columbus, OH. pp 33-54.

[23] DEC, J. and BOLLAG, J-M. *Use of plant material for the decontamination of water polluted with phenols.* 1994. Biotechnology and Bioengineering. Volume 44, pp 1132-1139.

[24] BELLIN, C. A. and O'CONNOR, G. A. *Plant uptake of pentachlorophenol from sludge-amended soils.* 1990. Journal of Environmental Quality. Volume 19, pp 598-602.

[25] CONGER, R. M. and PORTIER, J. R*. Phytoremediation Experimentation with the Herbicide Bentazon*. 1997. Remediation Journal Spring 1997. Volume 7, pp 19-37.

[26] BANUELOS, G. S., SHANNON, M. C., AJWA, H., DRAPER, J. H., JORDAHL, J. and LICHT, L. *Phytoextraction And Accumulation Of Boron And Selenium By Poplar (Populus) Hybrid Coles*. 1999. International Journal of Phytoremediation. Volume 1, pp 81-96.

[27] BOLLAG, J-M., FRANKENBURGER JR, W. T. and SIMS, R. C. *Bioremediation of Contaminated Soils*. 1999. Agronomy Monograph series 37. American Society of Agronomy. Madison. WI. 772 pp.

[28] WANG, T. C., WEISSMAN, J. C., RAMESH, G., VARADARAJAN, R. and BENEMANN, J. R. *Parameters for removal of toxic heavy metals by water milfoil (Myriophyllum spicatum).* 1996. Bulletin of Environmental Contamination and Toxicology. Volume 57, pp 779-786.

[29] SALT, D. E., BLAYLOCK, M., KUMAR, N. P. B. A., DUSHENKOV, V., ENSLEY, D., CHET, I. and RASKIN, I. *Phytoremediation: A Novel Strategy For The Removal Of Toxic Metals.* 1995. The Environment Using Plants. Biotechnology. Volume 13, pp 468-474.

[30] CONESA, H., FAZ, Á. and ARNALDOS, R. *Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena-La Unión Mining District (SE Spain).* 2006. Science of the Total Environment. Volume 366, pp 1-11.

[31] CONESA, H., GARCIA, G., FAZ, Á. and ARNALDOS, R. *Dynamics of metal tolerant plant communities´ development in mine tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes.* 2007. Chemosphere. Volume 68, pp 1180-1185.

[32] MENDEZ, M. and MAIER, R. *Phytostabilization of mine tailings in arid and semiarid environments-an emerging remediation technology.* 2008. Environmental Health Perspectives, Volume 116, pp 278-283.

[33] SelecDEPOL. *Phytoremédiation.* 2013. URL: http://www.selecdepol.fr/fiches-techniques/phytoremediation/description

[34] SANG-HWAN, L., WONHYUN, J., WON-SEOK, L., NAMIN, K., Il HA, K., MIN-SUK, K. and JEONG-SIK, P. *Influence of amendments and aided phytostabilization on metal availability and mobility in Pb/Zn mine tailings.* 2014. Journal of Environmental Management. Volume 139, pp 15-21.

[35] PEREZ-SANZ, A., LOBO, C., PASTOR, J. and VANGRONSVELD, J. *Efficiency of Cd, Zn and Pb immobilization by soil amendments immediately after their application.* 2004. First International Meeting on Environmental Biotechnology and Engineering. México City, México; September 6-8, 2004.

[36] KABATA-PENDIAS, A. *Trace elements in soils and plants (4ª ed.).* 2011. Boca Ratón: Taylor and Francis Group.

[37] FERNANDEZ-CALIANI, J. C. and BARBA-BRIOSO, C. *Metal immobilization in hazardous contaminated minesoils after marble slurry waste application. A field assessment at the Tharsis mining district (Spain).* 2010. Journal of Hazardous Materials. Volume 181, pp 817-826.

[38] PARDO, T., CLEMENTE, R. and BERNAL, M. *Effects of compost, pig slurry and lime no trace element solubility and toxicity in two soils differently affected by mining activities.* 2011. Chemosphere. Volume 84, pp 642-650.

[39] ZORNOZA, R., FAZ, Á., CARMONA, D., KABAS, S., MARTINEZ-MARTINEZ, S. and ACOSTA, J. *Plant cover and soil biochemical properties in a mine tailing pond five years after application of marble wastes and organic amendments.* 2012. Pedosphere. Volume 22, pp 22-32.

[40] International Union of Soil Sciences (IUSS). *World reference base for soil resources 2006.* 2014. World Soil Resources Reports No. 103. FAO, Rome.

[41] ANDREO, B. and CARRASCO, F*. Excursion geologica al entorno de la Cueva de Nerja (Malaga)*. Campus de Teatinos, Malaga, España. 11 p. URL: http://www.cuevadenerja.es/images/Itinerario-geologico-1.pdf

[42] DIAZ DEL RIO, V. *Estudio Geoambiental del Mar Menor.* 1993. Monografías del Instituto Español de Oceanografía. Edita Ministerio de Agricultura, Pesca y Alimentación. Madrid. 223 p.

[43] MARTOS-MIRALLES, P., SANSANO-SANCHEZ, A., BANOS-PAEZ, P., NAVARRO-CANO, J. A. and MENDEZ-PEREZ, T. *Medio Ambiente y Empleo en la Sierra Minera de Cartagena-La Unión.* 2001. Edita Fundación Sierra Minera. La Unión (Murcia). 256 pp.

[44] CONESA-GARCIA, C. *El Campo de Cartagena. Clima e hidrología de un medio semiárido.* 1990. Editan Universidad de Murcia, Ayuntamiento de Cartagena y Comunidad de Regantes del Campo de Cartagena. Murcia. 450 pp.

[45] CONESA, H. M. *Restauración/estabilización de suelos contaminados por metales pesados como consecuencia de actividades mineras en la zona de Cartagena y La Unión.* 2005. Tesis Doctoral. Universidad Politécnica de Cartagena (Murcia, España). 398 pp.

[46] Atlas Global de la Region de Murcia. *El clima.* URL: http://www.atlasdemurcia.com/index.php/secciones/1/el-clima/4/

[47] ZORNOZA, R., FAZ, A., MARTINEZ-MARTINEZ, S., ACOSTA, J. A., GOMEZ-LOPEZ, M. D. and AVILES-MARIN, S. M. *Marble waste and pig slurry increment soil quality and reduce metal availability in a tailing pond.* 2013. Terra Latinoamericana. Volume 31, pp 105-114.

[48] KUMPIENE, J., LAGERKVIST, A. and MAURICE, C. *Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - a review.* 2008. Waste Management. Volume 28, pp 215-225.

[49] MACIAS, F. *Recuperación de suelos degradados, reutilización de resíduos y secuestro de carbono. Una alternativa integral de mejora de la calidad ambiental.* 2004. Recur. Rurais 1, pp 49–56.

[50] COBERTA, E. *Edafologia aplicada.* 1993. Ediciones Catedra SA. Madrid. 326 p.

[51] DONER, H. E. and LYNN, W. C*. Carbonate, halide, sulfate, and sulfide minerals.* 1989. *In* J.B Dixon and S.B. Weed. 1989. Minerals in the soil environment, 2nd edition. Soil Science Society of America Book Series No. 1, Soil Science Society of America, Madison, WI.

[52] NELSON, D. W. *Gaseous losses of nitrogen other than through dentriflcation.* 1982. In Stevenson, F. J. (Ed.). Nitrogen in Agricultural Soils. Agron. Monogr. 22, pp 327-363. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am., Madison, WI.

[53] ST. ARNAUD, R. J. and HERBILLON, A. J. *Occurrence and genesis of secondary magnesium-bearing calcites in soils*.1973. Geoderma. Volume 9, pp 279-298.

[54] CARTER, M. R. *Association of total CaCO3 and active CaCO3 with growth of fine tree species on chernozemic soils.* 1981. Canadian Journal of Soil Science. Volume 61, pp 173- 175.

[55] DEB, B. G. and CHADHA, S. P. *Mechanical analysis of calcareous soils and distribution of CaCO3 in various fractions.* 1970. Journal of Indian Society of Soil Science. Volume 18, pp 227-232.

[56] THABET, A. *Active and total CaCO3 in soils.* 1975 Thesis. American University of Beirut, Beirut, Lebanon.

[57] Ecosystem Restoration. *Organic Matter.* 2004. URL: http://ecorestoration.montana.edu/mineland/guide/analytical/chemical/solids/om.htm

[58] PORTA, J., LOPEZ-ACEVEDO, M. and RODRIGUEZ, R. *Técnicas y Experimentos en Edalfologia.* 1986. Collegi Oficial d'Enginyers Agronoms de Catalunya. Barcelona, 282 p.

[59] STEVENSON, F. J. *Carbon Balance of the Soil and Role of Organic Matter in Soil Fertility in Cycles of soil.* 1986. Ed Wiley and Son.

[60] BREMNER, JM. *Total nitrogen. En: Methods of soil analysis. Part 3.* 1996. Chemical methods. Sparks D. L. (Ed.), SSSA Book Series No. 5, Madison. pp 1085-1121.

[61] BERTRAMSON, B. R., FRIED, M. and TISDALE, S. L. *Sulphur studies of Indiana soils and crops.* 1950. Soil Science. Volume 70, pp 27-42.

[62] BRADY, N. C. *The nature and properties of soils (8th ed.).* 1974. Macmillan publishing Co. Inc. New York. Volume 21-25, pp 422-445.

[63] WILLIAMS, C. H., WILLIAMS, E. G. and SCOTT, N. M. *Carbon, nitrogen, sulphur and phosphorus in some Scottish soils.* 1960. Journal Soil Science. Volume 11, pp 334-346.

[64] American Society for Testing and Material. *Procedures for testing soils*. 1958. American Society for Testing and Material, Philadelphia.

[65] THOMSON L. M. and TROECH, F. R. Suelos y su Fertilidad. 2002. Ed. Reverté, Barcelona, 649 p.

[66] CAMPBELL, D. J. and HENSALL, J. K. *Bulk density*. 1991. En; Smith, K. A., Mullis, Ch. E. (Eds.), Soil analysis. Marcel Dekker, Inc. New York, pp 329-366.

[67] BRADY, N. C. and WEIL, R. R. Weil. *The Nature and Properties of Soils.* 1999. Prentice.

[68] International Soil Science Society (ISSS). *Minutes of the first Commission Meetings.* 1929. International Congress of Soil Science. Washington. Proceedings 4, pp 215-220.

Hall, Upper Saddle River, New Jersey.

[69] KEMPER, W. D. and ROSENEAU, R. C. *Aggregate stability and size distribution*. 1986. In: Methods of soil analysis. Part 1: physical and mineralogical methods. A. Klute (eds) (Monograph no.9,2nd edn). ASA, Madison, Wis, America.

[70] USDA. *Soil Quality Test Kit Guide.* 1999. United Stated Department of Agriculture (Ed.). Washington. 82 p.

[71] DORAN, J. W. and PARKIN, T. B. *Defining and assessing soil quality.* 1994. In: JW Doran; DC Coleman; DF Bezdicek & BA Stewart(eds.). Defining soil quality for a sustainable environment. SSSA Special Publication N° 35. Wisconsin, USA.

[72] LE BISSONNAIS, Y. and ARROURAYS, D. *Aggregate stability and assessment of soil crustability and erodibility: II. Application to humic loamy soils with various organic carbon contents.* 1996. Journal Soil Science. Volume 48, pp 39-48.

[73] LARNEY, F. J., JANZEN, H. H., OLSON, B. M., BULLOCK, M. S. and SELINGER, L. J. *Aggregate stability: A soil quality indicator for southern Alberta soils*. 1996. In Proc. 33rd Annual Alberta Soil Science Workshop, February 20–22, 1996, Edmonton, AB, Canada, pp 262-267.

[74] MARQUEZ, C. O., GARCIA, V. P., CAMBARDELLA, C. A., SCHULTZ, R. C. and ISENHART, T. M. *Aggregate-size stability distribution and soil stability*. 2004. Soil Science Society of America Journal. Volume 68, pp 725-735.

[75] WU, L., VOMOCIL, J. A. and CHILDS, S. W. *Pore size, particle size, aggregate size, and water retention.* 1990. Soil Science Society of America Journal. Volume 54, pp 952-956.

[76] SEXTONE, A. J., REVSBECH, N. P., PARKIN, T. B. and TIEDJE, J. M. *Direct measurements of oxygen profiles and denitrification rates in soil aggregates.* 1985. Soil Science Society of America Journal. Volume 49, pp 646-651.

[77] WANG, X., YOST, R. S. and LINGUIST, B. A. *Soil Aggregate size affects phosphorus desorption from highly weathered soils and plant growth.* 2001. Soil Science Society of America Journal. Volume 65, pp 139-146.

[78] BARTHES, B. and ROOSE, E. *Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels.* 2002. Catena. Volume 47, pp 133-149.

[79] SIX, J., BOSSUYT, H., DEGRYZE, S. and DENEF, K. *A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics.* 2004. Soil and Tillage Research. Volume 79, pp 7-31.

[80] DIEZ, J. A. *Consideraciones soble la utilizacion de la técnica extractive de Burriel-Hernando para la evaluacion de fosforo assimilable en suelos.* 1982. Anal. Edaf. Agrobiol. Volume 41, pp 1345-1353.

[81] BUURMAN, P., VAN LAGEN, B. and VELTORST E. J. *Manual for Soil and Water Analysis.* 1996. Backhuys publishers. Technical report. Leiden, Holland

[82] FRAU, F. *The formation-dissolution-precipitation cycle of melanterite at the abandoned pyrite mine of Genna Luas in Sardinia, Italy: environmental implications.* 2000. Mineralogical Magazine. Volume 64, pp 995 – 1006.

[83] ALLOWAY, B. J. *Heavy Metals in soils.* (ed). 1990 and 1995. Blackie Academic and Profesional. Londres.

[84] RISSER, J. A. and BAILEY, G. W. *Spectroscopic study of surface redox reactions with manganese oxides.* 1992. Soil Science Society of America Journal. Volume 56, pp 82-88.

[85] HERON, G., CHRISTENSEN, T. H. and TJELL, J. C. *Oxidation capacity of aquifer sediments.* 1994. Environmental Science and Technology. Volume 28, pp 153-158.

[86] ADRIANO, D.C. *Trace element in the terrestrial environment.* 1986. Springer-Verlag, New York.

[87] MCKINNEY, J. and ROGERS, R. *Metal Bio-availability*. 1992. Environmental Science and Technology. Volume 26, pp 1298-1299.

[88] LUOMA, S. N. *Can we determine the biological availability of sediment-bound trace elements?* 1989. Hydrobiologia. Volume 176-177, pp 379-396.

[89] CHAO, T. T. *Use of partial dissolution techniques in exploration geochemistry*. 1984. Journal of Geochemical Exploration. Volume 20, pp 101-135.

[90] GUNN, A. M., WINNARD, D. A. and HUNT, D. T. E. *Trace metal speciation in sediments and soils.* 1988. In Kramer, J.R., and Allen, H.E., eds., Metal Speciation: Theory, Analysis and Application, Lewis Publications, Boca Raton, Fla., pp 261-294.

[91] BORGGAARD, O. K. *The use of EDTA in soil analysis*. 1976. Acta Agriculturae Scandinavica. Volume 26, pp 144-150.

[92] CROCK, J. G. and SEVERSON, R. C. *Four reference soil and rock samples for measuring element availability in the Western Energy Region: U.S.* 1980. Geological Survey Circular. Volume 841, 16 p.

[93] LEVENTHAL, J. and TAYLOR, C. *Comparison of methods to determine degree of pyritization.* 1990. Geochimica et Cosmochimica Acta. Volume 54, pp 2621-2625.

[94] BASTA, N. T. *Heavy metal and trace element chemistry in residual-treated soil: Implications on metal bioavailability and sustainable land application.* 2004. En: Susteinable land application Conference. January, 4-8, Florida. University of Florida.

[95] LASAT, M. *The uses of plants for removal of toxic metal from contaminated soil.* 2001. US-EPA.

[96] RIECHAMAN, M. S. *The responses of plants to metals toxicity: A review focusing on copper, manganese and zinc.* 2002. Australian Minerals and Energy Environment Foundation, Melbourne, Australia, 54 p.

[97] RIEUWERTS, J. S., THONTON, I., FARAGO, M. E. and ASHMORE, M. R. *Factors influencing metals bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals.* 1998. Chemical Speciation and Bioavailability. Volume 10, pp 61-75.

[98] LI, X., COLES, B. J., RAMSEY, M. H. and THORNTHON, I. *Sequential extraction of soils for multielement analysis by ICP-AES.* 1995. Chemical Geology. Volume 124, pp 109-123.

[99] TESSIER, A., CAMPBELL, P. G. C. and BISSON, M. *Sequential extraction procedure for the speciation of particulate trace metals.* 1979. Analytical Chemistry. Volume 51, pp 844-851.

[100] BURAU, R. E. Lead, in A. L. Page et al. (eds), *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed.* 1982. Agron. Monogr. Vol. 9, ASA and SSSA,Madison, WI, U.S.A., pp 347-366.

[101] REISENAUER, H. M. Chromium, in A. L. Page et al. (eds), *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed.* 1982. Agron. Monogr. Vol. 9, ASA and SSSA, Madison, WI, U.S.A., pp 337-346.

[102] HOSSNER, L. R. *Dissolution for Total Elemental Analysis*. 1996. In D. L. Sparks et al. (eds), Methods of Soil Analysis, Part 3, Chemical Methods, SSSA Book Series No. 5, ASA and SSSA,Madison, WI, U.S.A., pp 49-64.

[103] MENDOZA, C. A., CORTES, G. and MUNOZ, D. *Heavy metal pollution in soils and sediments of rural developing district 063, Mexico*. 1996. Environmental Toxicology and Water Quality. Volume 11, pp 327-333.

[104] CHLOPECKA, A., BACON, J. R., WILSON, M. J. and KAY, J. *Forms of cadmium, lead, and zinc in contaminated soils from Southwest Poland*. 1996. Journal of Environmental Quality. Volume 25, pp 69-79.

[105] MARTIN, C. W. *Heavy metal concentrations in floodplain surface soils, Lahn River, Germany.* 1996. Environmental Geology. Volume 30, pp 119-125.

[106] CHARLESWORTH, S. M. and LEES, J. A. *The distribution of heavy metals in deposited urban dusts and sediments, Coventry, England.* 1999. Environmental Geochemistry and Health. Volume 21, pp 97-115.

[107] U.S. Environmental Protection Agency. *Acid Digestion of Sediment, Sludge and Soils.* in USEPA (ed.), Test Methods for Evaluating Soil Waste SW-846. 1986. USEPA, Cincinnati, OH, U.S.A.

[108] EPA-ROC. *The Standard Methods for Determination of Heavy Metals in Soils and Plants*. 1994. National Institute of Environmental Analysis of EPA-ROC, Taipei, Taiwan, ROC (In Chinese).

[109] BAKER, D. E. and AMACHER, M. C. *Nickel, Copper, Zinc and Cadmium*. 1982. In A. L. Page et al. (eds), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, 2nd ed., Agron. Monogr. Vol. 9, ASA and SSSA, Madison, WI, U.S.A., pp 323-336.

[110] BETTINELLI, M., BARONI, U. and PASTORELLI, N. *Determinazione diretta di elementi in traccia in matrici ambientali mediante imprego dell' ETA-AAS Zeeman e midificazione di matrice.* 1989. In Minoia, C. & S. Caroli (eds), Applicazioni dell' ETA-AAS Zeeman nel laboratorio chimico e tossicologico. Eddizioni Libreria Cortina, Padova. Volume 1, pp 49-81.

[111] SANCHEZ, G., OROZCO, R. and JACINTO, M. *Estado de la Contaminación Marina en el Litoral Peruano en 1994 y 1995.* 1998. Informe Inst. Mar Perú. Volume 136, pp 7-22.

[112] INGAMELLS, C. O. *Rapid chemical analysis of silicate rocks.* 1964. Talanta. Volume 11, pp 665-666.

[113] URE, A. M. *Heavy metals in soils.* 1995. 2nd Ed. Edited by Alloway B. J. Public. Blackie Academic & Professional, London. 368 p.

[114] ARCHER, F. C. *Inorganic Pollution and Agriculture*. 1980. MAFF Reference Book 326, HMSO. London, 184 pp.

[115] CAMARA, C., FERNANDEZ, P., MARTIN-ESTEBAN, A., PEREZ-CONDE, C. and VIDAL, M. *Toma y Tratamiento de Muestras.* 2002. Ed. Sintesis S.A, Madrid. 334 p.

[116] TUZEN, M. *Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry.* 2003. Microchemical Journal. Volume 74, pp 289-297.

[117] RISSER, J. A. and BAKER, D. E. *Testing soils for toxic metals.* 1990. In: R. L. Westerman (Ed). Soil Testing and plant analysis. Soil Science Society of America Special Publication 3. 3rd Ed., Madison. pp 275-298.

[118] SCOTT, D. and THOMAS, J. H. *Comparative efficiencies of various methods for extracting trace heavy metals from soils.* 1977. Soil *science*. Volume 124, pp 319-322.

[119] ZAHER, H. *Erosion hydrique.* 2010. URL: http://www.ma.auf.org/erosion/chapitre1/III.Facteurs.html

[120] ZAHER, H. *Erosion éolienne.* 2010. URL: http://www.ma.auf.org/erosion/chapitre2/II.FacteursChap2.html

[121] ROOSE, E. *Land husbandry - Components and strategy.* 1996. 70 FAO SOILS BULLETIN. Rome

[122] MANOUSAKI, E. and KALOGERAKIS, N. *Phytoextraction of Pb and Cd by the Mediterranean saltbush (Atriplex halimus L.): metal uptake in relation to salinity.* 2009. Environmental Science and Pollution Research. Volume 16, pp 844-854.

[123] BAJJI, E., KINET, J-M. and LUTTS, S. *Salt stress effects on roots and leaves of Atriplex halimus L. and their corresponding callus cultures.* 1998. Plant Science. Volume 137, pp 131-142.

[124] AMER, N., AL CHAMI, Z., AL BITAR, L., MONDELLI, D. and DUMONTET, S. *Evaluation of Atriplex halimus, Medicago lupulina and Portulaca oleracea for phytoremediation of Ni, Pb, and Zn*. 2013. International Journal of Phytoremediation. Volume 15, pp 498-512.

[125] Istituto di Biologia Agroambientale e Forestale. *Cynodon dactylon (L.) Pers.* URL: http://www.ibaf.cnr.it/phytoremediation/cynodon-dactylon-organics-cr-pb-zn-cu.pdf

[126] SHU, W. S., YE, Z. H., LAN, C. Y., ZHANG, Z. Q. and WONG, M. H. *Lead, zinc and copper accumulation and tolerance in populations of Paspalum distichum and Cynodon dactylon.* 2002. Environmental Pollution. Volume 120, pp 445-453.

[127] SHAHANDEH, H. *Plant Screening for Chromium Phytoremediation*. 2000. International Journal of Phytoremediation. Volume 2, pp 31-51.

[128] KORBOULEWSKY, N., BONIN, G. and MASSIANI, C. *Biological and ecophysiological reactions of white wall rocket (Diplotaxis erucoides L.) grown on sewage sludge compost.* 2002. Environmental Pollution. Volume 115, pp 365-370.

[129] Observatoire Océanologique de Banyuls/Mer. *Diplotaxis erucoides.* URL: http://wwwphp.obs-banyuls.fr/UVED/module/voir_taxon/taxon_numero_781.html

[130] TESTIATI, E. *Contamination de sols par des éléments traces métalliques en zone méditerranéenne côtière : études de leur mobilité et du transferts à la phytocénose*. 2012. Université Aix-Marseille. 293 p.

[131] Group Master Management de l'Environnement, Valorisation Analyse 2006. *Report.* 2006. Université Aix-Marseille.

[132] BAYCU, G., TONULAY, D., OZDEN, H., CSATARI, I., KARADAQ, S., AGBA, T. and ROGNES, S. E. *An Abandoned Copper Mining Site in Cyprus and Assessment of Metal Concentrations in Plants and Soil.* 2014. International Journal of Phytoremediation. Volume 17, pp 622-631.

[133] DE HARO, A., PUJADAS, A., POLONIO, A. and DEL RIO CELESTINO, M. *Phytoremediation of the polluted soils after the toxic spill of the Aznalcollar mine by*

using wild species collected in situ. 2000. Fresenius Environmental Bulletin. Volume 9, pp 275-280.

[134] CONESA, H. M., GARCIA, G., FAZ, A. and ARNALDOS, R. *Dynamics of metal tolerant plant communities' development in mine tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes.* 2007. Chemosphere. Volume 68, pp 1180-1185.

[135] RUFO, L. and DE LA FUENTE, V. *Successional Dynamics of the Climatophile Vegetation of the Mining Territory of the Río Tinto Basin (Huelva, Spain): Soil Characteristics and Implications for Phytoremediation.* 2010. Arid Land Research and Management. Volume 24, pp 301-327.

[136] DE LA FUENTE, V., RUFO, L., RODRIGUEZ, N., AMILS, R. and ZULUAGA, J. *Metal Accumulation Screening of the Río Tinto Flora (Huelva, Spain).* 2010. Biological Trace Element Research. Volume 134, pp 318-341.

[137] MATHEW, A. M. *Phytoremediation of heavy metal contaminated soil.* 2001. Cochin University of Science and Technology. 90 p.

[138] GARCIA, G., FAZ, A. and CUNHA, M. *Performance of Piptatherum miliaceum (Smilo grass) in edaphic Pb and Zn phytoremediation over a short growth period.* 2004. International Bioremediation & Biodegradation. Volume 54, pp 245-250.

[139] KAMEL, M. M. and SAKR, W. R. *Response of Senna Occidentalis, Link Plants to Fertilization as Well as Citric Acid and Their Role in Remediating Soil Polluted with Cu and Pb.* 2009. World Journal of Agricultural Sciences. Volume 5, pp 784-798.

[140] RAY, J. G. and GEORGE, J. *Phytosociology of roadside communities to identify ecological potentials of tolerant species.* 2009. Journal of Ecology and the Natural Environment Volume 1, pp184-190.

[141] GARCIA, C., ROLDAN, A. and HERNANDEZ, T. *Ability of different plant species to promote microbiological processes in semiarid soil.* 2005. Geoderma. Volume 124, pp 193-202.

[142] BAGHDAD, B., NAIMI, M., BOUABLI, A., SONNET, P. and LUTTS, S. *Heavy Metals in Tailings, Soils and Vegetation of an Abandoned Lead Mine Land in Morocco.* 2006. Ecologia Mediterranea. Volume 32, pp 85-91.

[143] OYARZUN, R., CUBAS, P., HIGUERAS, P. L. and LLANOS, W. *Environmental assessment of the arsenic-rich, Rodalquilar gold–(copper–lead–zinc) mining district, SE Spain: data from soils and vegetation.* 2009. Environmental Geology. Volume 58, pp 761-777.

[144] Brady, N. *The nature and properties of soils (9nd ed.).* 1984. New York: Macmillan Publishing Company.

[145] PORTA, J., LOPEZ-ACEVEDO, M. and ROQUERO, C. *Edafologia para la agricultura y el medioambiente.* 1999. Ediciones Mundi-Prensa. 2^{a} edicion. Madrid. 849 p.

[146] Agriculture et Agroalimentaire Canada. *Texture du sol et qualité de l'eau*. URL: http://www.agr.gc.ca/fra/science-et-innovation/pratiques-agricoles/sol-et-terre/le-sol-et-l-eau/texture-du-sol-et-qualite-de-l-eau/?id=1197483793077

[147] Le site du Pôle national de compétence Éducation au développement durable. *Origines et compositions du sol.* URL: https://crdp.ac-amiens.fr/enviro/sols/sol_maj_detailp2.htm

[148] MACIEJEWSKI, J. and OSSON, B. *Productions fourragères.* Lavoisier Tec & Doc. URL: http://www.lavoisier.fr/pdf/fichiers/Productions_fourrageres_Extrait_chapitre_1.pdf

[149] RAVER, P. H., FAY, F. E. and EICHORN, S. E. *Biology of Plants*. 1999. New York: W.H. Freeman and Company.

[150] Land and Water Division. *Evaluation des Terres pour l'Agriculture Irriguée: Evaluation des Terres pour l'Agriculture Irriguée: Directives*. 1989. FAO Soils Bulletin.

[151] CARRIER, A. *Que se passe t'il dans le sol ?* 2003. URL: https://www.agrireseau.net/agriculturebiologique/documents/QUE%20SE%20PASSE%20T%20IL%20DANS%20LE%20SOL%2003%2003%2031.pdf

[152] MARTINEZ, E., FUENTES, J. P. and ACEVEDO, E. *Carbono orgánico y propiedades del suelo.* 2008. Revista de la ciencia del suelo y nutrición vegetal. Volume 8, pp 68-96.

[153] PARDO, T., CLEMENTE, R. and BERNAL, M. P. *Effects of compost, pig slurry and lime no trace element solubility and toxicity in two soils differently affected by mining activities.* 2011. Chemosphere. Volume 84, pp 642-650.

[154] KABAS, S., AROCENA, J. M., ACOSTA, J. A., MARTINEZ-MARTINEZ, S., ZORNOZA, R. and CARMONA, D. M. *Syrian Bean-Caper (Zygophyllum Fabago L.) improves organic matter and other properties of mine wastes deposits.* 2014. International Journal of Phytorremediation. Volume 16, pp 366-378.

[155] BOT, A. and BENITE, J. *The importance of soil organic matter.* 2005. 80 FAO SOILS BULLETIN. Rome. 95 p.

[156] Montana State University Bozeman. *Macronutrients.* 2004. URL: http://ecorestoration.montana.edu/mineland/guide/analytical/chemical/solids/macronutrients.htm

[157] YANG, S., LIAO, B., LI, J., GUO, T., and SHU, W. *Acidification, heavy metal mobility and nutrient accumltion in the soil-plant system of a revegetated acid mine wasteland.* 2010. Chemosphere. Volume 80, pp 852-859.

[158] VALE, M. *Perspectives d'évolution pour le raisonnement de la fertilisation phosphate.* 2010. SAS Laboratoire – AGRO-Systèmes.

[159] Region de Murcia. *Análisis de residuos. Metales pesados totales*. URL:

http://ftp.murciaeduca.es/programas_educativos/Nuevo1/LIBROETSIA/31_anlisis_de_residuos_metales_pesados_totales.html

[160] European Communities Council Directive. *(86/278/EEC) Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is used in Agriculture.* 1986. Off. J. Eur. Commun. L181, 04/07/86, 6-12.

[161] Real Decreto 1310/1990, de 29 de octubre, por el que se regula la utilización de los lodos de depuración en el sector agrario. 1990.

[162] MARTINEZ SANCHER, M. J., PEREZ SIRVENT, C., TUDELA SERRANO, M. L., LINARES MORENO, P., GARCIA LORENZO, M. L., HERNANDEZ CORDOBA, M., LOPEZ GARCIA, I. F., MOLINA RUIZ, J. NAVARRO HERVAS, C., VIDAL OTON, J., BARBERAN MURCIA, R., MANTILLA, W., TOVAR FRUTOS, P. J., SOLANO MARIN, A. M., MARIMON SANTOS, J., AGUDO JUAN, I., HERNANDEZ PEREZ, C. *Niveles de fondo y niveles genéricos de referencia de metales pesados en suelos de la Región de Murcia*. 2007.

[163] MC GOWEN, S. L., BASTA, N. T. and BROWN, G.O. *Use of Diammonium Phosphate to Reduce Heavy Metal Solubility and Transport in Smelter-Contaminated Soil.* 2000. Journal of Environmental Quality. Volume 30, pp 493-500.

[164] KUMPIENE, J., LAGERKVIST, A. and MAURICE, C. *Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. 2008. Waste management.* Volume 28, pp 215-225.

[165] SANG-HWAN, L., JIN-SOO, L., YOUN JEONG, C. and JEONG-GYU, K. *In situ stabilization of cadmium-, lead-, and zinc-contaminated soil using various amendments.* 2009. Chemosphere. Volume 77, pp 1069-1075.

[166] HLAVACKOVA, P. *Evaluation du comportement du cuivre et du zinc dans une matrice de type sol à l'aide de différentes methodologies.* 2005. L'Institut National des Sciences Appliquées de Lyon. 202 p.

[167] DURAND, C. *Caractérisation physico-chimique des produits de l'assainissement* pluvial. Origine et devenir des métaux traces et des polluants organiques. 2002. Université de Poitiers. 268 p.

[168] CHIU, K. K., YE, Z. H. and WONG, M. H. *Growth of Vetiveria zizanioides and Phragmities australis on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge: A greenhouse study.* 2006. Bioresource Technology. Volume 97, pp 158-170.

[169] SAIDI, N. *Essais de phytoremediation par Vetiver Grass (Vetiveria zizanioides).* Laboratoire des Géosciences Appliquée. Equipe: Pollution & Phytoremédiation. Faculté des Sciences. Kenitra, Maroc. 23 p.

[170] SIMON, E. and LEFEBVRE, C. *Aspects de la tolérance aux metaux lourds chez Agrostis tenuis Sibth., Festuca ovina L. et Armeria maritima. Moulin. Willd.* 1977. Oecologia Plantarum. Volume 12, pp 95-110.

6. Annexes

Annex 1: Soil classification according to the density

Clay minerals	2000-2650 kg.m^{-3}
Quartz, Feldspar	2500-2600 kg.m^{-3}
Minerals with metallic components	4900-5300 kg.m^{-3}
Mineral horizon	2600-2750 kg.m^{-3}
Organic horizon	1100-1400 kg.m^{-3}
Horizon with heavy minerals	2750 kg.m^{-3}
Mean value for minerals soils	2650 kg.m^{-3}

Annex 2: Soil classification according to the percentage of CaCO3

% of $CaCO_3$	**Classification**
< 1 %	No lime-filled
2-5 %	Little lime-filled
5-80 %	Lime-filled
> 80 %	Excessive lime-filled

Annex 3: Results of laboratory analysis.

Apparent density

Plots	Apparent density (kg/m3)
P1	1164
P2	1153
P3	1330
P4	1301
CT	1445

Real density

Plots	Real density (kg/m3)
P1	2773
P2	3608
P3	3725
P4	3544
CT	4081

Aggregate stability in water

Plots	Aggregate stability in water (%)
P1	16
P2	18
P3	17
P4	18
CT	22

pH

Plots	pH
P1	7.1
P2	5.8
P3	3.3
P4	4.7
CT	2.4

Electric conductivity

Plots	Electric conductivity (mS/cm)
P1	2.99
P2	3.95
P3	7.37
P4	4.43
CT	7.72

Inorganic carbon

Plots	Inorganic carbon (%)
P1	2.87
P2	1.46
P3	0.53
P4	1.30
CT	0.47

Organic carbon

Plots	Organic carbon (%)
P1	0.99
P2	0.39
P3	0.33
P4	0.31
CT	0.21

Sulphur

Plots	Sulphur (%)
P1	0.34
P2	0.46
P3	1.51
P4	0.79
CT	2.80

Total nitrogen

Plots	Total nitrogen (%)
P1	0.11
P2	0.05
P3	0.13
P4	0.10
CT	0.05

Available phosphorus

Plots	Available phosphorus (mg/kg of soil)
P1	31.1
P2	10.0
P3	8.9
P4	7.8
P5	8.2

Total metals

	Total metals (mg/kg of soil)			
Plots	Pb	Cu	Zn	Cd
P1	1298	57	4120	8
P2	1567	59	2738	5
P3	1286	74	4124	7
P4	1753	128	2268	4
CT	1176	148	2364	5

Water soluble heavy metals

	Water soluble heavy metals (mg/kg of soil)			
Plots	Pb	Cu	Zn	Cd
P1	0.45	0.07	0.9	0.07
P2	0.07	0.06	10	0.15
P3	0.03	1.70	900	2.40
P4	0	0.14	73	0.31
CT	0	2.39	312	0.67

Bioavailable metals

	Bioavailable metals (mg/kg of soil)			
Plots	Pb	Cu	Zn	Cd
P1	49	1.32	84	0.53
P2	13	0.93	40	0.34
P3	8	3.28	824	2.66
P4	1	1.03	53	0.35
CT	0	3.24	291	0.88

Sequential extraction of metals

	Lead (mg/kg of soil)				
Plots	1st extraction	2nd extraction	3rd extraction	4th extraction	5th extraction
P1	0.17	32.6	15.1	24	1590
P2	0.00	10.4	13.0	59	1719
P3	0.00	14.1	4.5	12	1993
P4	1.24	7.7	7.7	55	2085
CT	0.51	5.7	4.7	54	2018

	Copper (mg/kg of soil)				
Plots	1st extraction	2nd extraction	3rd extraction	4th extraction	5th extraction
P1	0.11	1.77	7.32	3.99	188
P2	0.07	1.29	4.88	3.90	88
P3	1.48	1.77	7.67	5.44	134
P4	0.17	1.79	5.01	4.14	61
CT	2.09	1.66	4.34	3.43	58

	Zinc (mg/kg of soil)				
Plots	1st extraction	2nd extraction	3rd extraction	4th extraction	5th extraction
P1	0.23	131	1001	157	2215
P2	8.4	54	317	103	1727
P3	399	116	524	128	1947
P4	60	38	172	53	1507
CT	332	35	128	34	1294

	Cadmium (mg/kg of soil)				
Plots	1st extraction	2nd extraction	3rd extraction	4th extraction	5th extraction
P1	0.13	1.61	3.24	0.33	3.04
P2	0.17	0.92	1.01	0.17	2.83
P3	2.61	0.49	1.29	0.28	3.24
P4	0.28	0.54	0.34	0.13	2.85
CT	0.84	0.24	0.35	0.09	2.59

Annex 4: Pictures of plants species mentioned in the report. Source: Google Images.

Atriplex halimus L.

Cynodon dactylon (L.) Pers.

Diplotaxis erucoides (L.) DC.

Lobularia maritima (L.) Desv.

Oxalis pes-caprae L.

Phagnalon saxatile (L.) Cass.

Piptatherum miliaceum (L.) Coss.

Senna occidentalis (L.) Link.

Stipa tenacissima L.

Festuca Ovina L.

Vetiveria zizanioides (L.) Nash.