STUDY ON THE USE OF EDTA CHELATING AGENT AND SOLID AMENDMENTS TO ENHANCE NATURAL PHYTOACCUMULATION OF METALS IN A PHYTOMINING PROCESS

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Abstract

Raw materials are fundamental to Europe’s economy, growth and jobs and they are essential for maintaining and improving our quality of life. However, primary production of the 28 Member States of EU to overall materials supply can be estimated at around 9% [1]. The necessity to identify alternatives way of retrieving as firstly stated in 2008 when the European Commission launched the “Raw Materials Initiative” (RMI) whose aim was to define which were the critical raw materials for the EU’s economy considering the whole supply chain. In recent decades phytomining, a phytomanagement application focused on the recovery of metals, claimed the attention of many researchers because it appears to represent an innovative low-cost technology for the selective recovery of valuable trace elements from secondary resources. However, two decades after its inception and numerous successful experiments, commercial phytomining has not yet become a reality, that’s why a large-scale demonstration is needed to identify operational risks and provide a solid evidence of profitability. With the intention to set an important milestone in the possible implementation of this sustainable perspective and fulfilling the European initiative, in 2014 the project Bergwerk Pflanze was approved. The aim of this project is to test feasibility of a phytomining process growing metal-accumulating plants on a substrate made of waste incineration ash, harvesting the metal enriched biomass and using it as a form of bio-ore in metallurgic processes. In in this thesis are presented the results of 2 experimental researches that were set down from February 2015 to July 2015 at the Institute of Soil Research of the BOKU University of Vienna, that is a stakeholder partner of the project. The aims of the first test was to evaluate the effects of different inorganic amendments (ground bricks, zeolite, and pomice) as a substrate conditioning agents in order to improve the bioavailability of targeted elements mitigating the characteristics of high pH and electrical conductivity of the bottom ash. Second investigations were set down to identify the effects of addition of the chelating agent EDTA (Ethylenediaminetetraacetic acid) on enhancing the bioavailability of targeted metals in the substrate and consequently on enhancing the phytoaccumulation yield in the tested plants.
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1.1 The Project

Currently there are no viable technologies to recycle metals at reasonable cost from waste incineration slags, and the existing technologies require an energy input and often also various environmentally problematic chemicals. The Bergwerk Pflanze project goal is to achieve an approach different from conventional methodologies. This is a three-years lasting project born from the intentions of different stakeholders to definitely set a course of a profitable phytomining process and integrate all the step of the productive chain from the bioore production to the metallurgical work-up procedure. Around 579,000 tons of waste incineration residues were produced in 2012 in Austria, of which 455,400 tons were landfilled [2]. Thus, metals that are bound in the aggregate matrix of the residues are lost for further use, which clearly represents a loss of resource. The choice of using incineration slags as a substrate comes from the fact that annual production of waste incinerations slag in Vienna is around 150,000 tons per year and the value of critical raw materials, which are deposited annually in Vienna on Rautenweg landfill it’s over 14 million Euro. Only in Vienna landfill centre are supposed to be deposited in the form of waste incineration slag over 1100 tons of critical raw materials (Co, Cr, Ga, Mn, Mo, Nb, Ni, Sb, V, W, Zn and REE) [3]. (Table 1.1).
1 Introduction

Table 1.1 Overview of content of critical raw materials presents in two secondary streams with potential value calculation. (WSO = ashes from fluidized bed furnace, MVA = slags from waste incineration). critical raw materials are highlighted in yellow and rare earths elements (REE) in orange. [4]

<table>
<thead>
<tr>
<th>Element</th>
<th>Menge (mg/kg)</th>
<th>Menge der Metalle im Jahr (t/a)</th>
<th>Preis für Metalle od. Oxide (€/kg)</th>
<th>Wertpotential WSO</th>
<th>Wertpotential MVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>16,7</td>
<td>43</td>
<td>0,25</td>
<td>6,45</td>
<td>20,33</td>
</tr>
<tr>
<td>Cr</td>
<td>169,7</td>
<td>662</td>
<td>2,55</td>
<td>99,30</td>
<td>4,40</td>
</tr>
<tr>
<td>Ga</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>1,50</td>
<td>382,12</td>
</tr>
<tr>
<td>Mn</td>
<td>736</td>
<td>1665</td>
<td>11,04</td>
<td>249,75</td>
<td>1,94</td>
</tr>
<tr>
<td>Mo</td>
<td>24,05</td>
<td>85</td>
<td>0,36</td>
<td>1,27</td>
<td>15,20</td>
</tr>
<tr>
<td>Nd</td>
<td>140</td>
<td>220</td>
<td>2,10</td>
<td>33,00</td>
<td>13,57</td>
</tr>
<tr>
<td>Ni</td>
<td>43,8</td>
<td>72</td>
<td>0,66</td>
<td>10,80</td>
<td>7,10</td>
</tr>
<tr>
<td>V</td>
<td>7,5</td>
<td>1044</td>
<td>1,09</td>
<td>156,60</td>
<td>9,10</td>
</tr>
<tr>
<td>Zn</td>
<td>4175</td>
<td>3084</td>
<td>62,63</td>
<td>62,60</td>
<td>1,40</td>
</tr>
</tbody>
</table>

The most innovative aspects of this project is the possibility to attain an efficient use of resources and raw materials: no special energy inputs are required, plants and microorganisms work at room pressure and room temperature and at the same time CO₂ sequestration through the growth of plants is achieved. Bottom ash as can be considered a renewable substrate, thus reuse of such a material preventing sources wasting. In the end, an ecological evaluation, modelled on a life cycle assessment at the end of the project needs to be carried out. A second focal point is flexibility of production achievable trough the to growth of different plants depending on the market price of the extractable metals.

The project will be completed with a technical, economic and environmental evaluation of innovative bio-ore process.

1.1.1 Stakeholders of the project

The project requires an interdisciplinary merging of specialized knowledge, which often is new and unusual for the conventional industry. There are members of the waste management (waste disposal companies, recycling entrepreneurs, landfill operators, advisory service), expert metallurgy (non-ferrous metallurgy of the University of Leoben (MUL), Treibacher Industrie, Montanwerke Brixlegg), specialist consultations with scientific actors
1.1 The Project

(Alchemia-nova, Institute of Soil Science of the BOKU University scientific adviser: Cell Imaging and Ultrastructure Research, University of Vienna (CIUS), brought together the Institute of Microbiology of the University of Innsbruck, etc.).

1.1.2 Overview on Vienna waste management

The City of Vienna is responsible for the entire chain of waste management from collection to treatment and, finally, disposal. Approximately 200,000 tonnes of slag and ash from Vienna’s waste incineration plants are disposed of at the Rautenweg landfill. These plants transform the energy content of waste into district heating and cooling as well as electricity. Residual and bulky waste are mechanically crushed and screened. The remaining pieces, shorter than 30 centimeters, can be processed optimally in waste incineration. Simultaneously metals are sorted and sent for recycling in the metal industry.

The three waste incineration plants Flötzersteig, Spittelau and Pfaffenau as well as the fluidised bed furnace of Simmeringer dispose of an annual treatment capacity of around 780,000 tonnes together [5]. Hazardous waste are treated with two rotary kilns (Simmeringer Haide factory) and deposited outside Austria in former salt mining tunnel underground. After about an hour, non-combustible materials such as slag, ash, scrap and stones remain at the end of the combustion grate. Such residues (slags and ash) are separated by sieving in various grain sizes and freed from metals, especially ferrous and non-ferrous metal are removed separately (annually, 10,000 to 15,000 tonnes of iron and non-ferrous metals are recycled in the metal industry) [6]. The slag released from metals and the ash are processed to concrete at the city’s own waste treatment plant and deposited at the landfill in Rautenweg.

The site of the Rautenweg landfill covers an area of about 60 hectares and exists since the 1960s. With 23 million cubic meters of approved bulk volume, it is Austria’s largest landfill. In the last 55 years more than 10 million cubic meters of waste have been deposited [6]. Today, the landfill is only used to deposit treated, odourless residues from the Vienna waste incineration plants. In the last 55 years more than 10 million cubic meters of waste have been deposited [6].

1.1.3 Plan of the project

In the first year laboratory trials were set down, especially substrate analysis according to standardized procedures implemented in Austria (ÖNORM) and focused on the following aspects: supporting aggregate formation, adjustment of pH, addition of nutrient, addition of organic carbon (C_{org}), decreasing salinity, leaching of easily soluble chloride and sulfate salts, possibly humus addition and inoculation with beneficial microorganisms. At the end of the first year even a first selection of plants was done according to the targeted metals identified.
The field trials start in the second year of the project and will be maintained inside the landfill Rautenweg, Vienna. Field trials will last for three years, this to guarantee the monitoring of plant performance (biomass production, metal accumulation) and optimized substrate conditions (organic matter content, nutrient supply (NPK), substrate admixtures, pest control, adjustment of water availability, etc.). The harvested biomass is used as a starting material for metal recovery tests. These last steps are performed with the help of the Chair of Nonferrous Metallurgy of the “Montan Universität Leoben”, Austria, and the “Montanwerke Brixlegg” through direct entry of the plant material in pyrometallurgical processing, further metallurgical work-ups after the concentration steps. Even various hydrometallurgical processes are being tested which ultimately lead to metal-enriched samples. Digestion tests are carried out in the laboratories of Alchemia-nova and BOKU-IBF. All data will be included in the work-up more methods with evaluation steps. Resulting streams (waste, biomass, metals, residuals) are accounted for energy and CO2 balances. Potential environmental impacts are described based on the usual criteria of an LCA based on ISO 14040. Figure 1.1 represents the steps of phytomining process as it was designed.

![Flowchart of the phytomining process as it was designed](image-url)
In the end an economic evaluation is performed on a basis of cost-benefit calculation taking in account the perspective of resource conservation and the development of strategically important secondary raw material sources.

### 1.2 Phytomanagement and phytomining

Phytomanagement describes an assemblage of related technologies that include phytomining [7][8], phytoremediation, which is divided into phytoextraction and phytostabilization as well as biofortification [8]. Phytomanagement of contaminated soils describes use of vegetation, possibly in combination with other technologies, to deliver the most cost-effective means of mitigating any environmental risks associated with the site. Phytoremediation may aim to immobilise the TEs in the soil, so that they neither leach nor enter the plant shoots (phytostabilization). Alternatively, the process may be engineered to manipulate the soil-plant system so that plants take up the contaminating TEs into the shoots, where they may be volatilised (phytovolatilization) [8], or removed from the site when the plants are harvested (phytoextraction). In soils that are deficient in essential TEs, the goal of phytomanagement is to increase the TE concentration in the edible plant organs (biofortification). Phytomining describes the phytoextraction of valuable TEs from ore bodies or metal-contaminated environments where the target metal concentration is too low for conventional mining, it can produce low volume, sulphide-free ‘bio-ore’, which can either be safely disposed or, if the target metal is of sufficient economic value, smelted, and recovered [8]. Figure 1.2 provides a schematic diagram connecting the phytomanagement technologies.

![Figure 1.2 Relationship of various “phyto” technologies to phytomanagement [8]](image-url)
Phytomining of metals can take place according to different phytoextraction approaches: continuous and induced [9] [10]. Continuous phytoextraction requires the use of plants that accumulate particularly high levels of the toxic contaminants throughout their lifetime (hyperaccumulators), while induced phytoextraction relies on the growth of high biomass crops which are induced to accumulate high concentrations of heavy metals by application of chemical amendments to the soil [9] [10]. The current criterion to define a hyperaccumulator is, a plant that can accumulate metal to a concentration of more than 1000 mg/kg (0.1%) of metal in plant tissues except for zinc (10,000 mg/kg), gold (1 mg/kg), and cadmium (100 mg/kg) [11]. Hyperaccumulating plants are taxonomically widespread throughout the plant kingdom. Approximately 400 plant species from at least 45 plant families have been reported to hyperaccumulate metals, most of which are nickel hyperaccumulators occurring in ultramafic areas all over the world, also Zn, Cd, Mn, Se, Tl and As hyperaccumulators have been clearly identified [12] [13] (Table 1.2).

Table 1.2 List of Hyperaccumulator plants in relation to element affinity [14]

<table>
<thead>
<tr>
<th>Element</th>
<th>Lower limit for hyperaccumulation (mg/kg)</th>
<th>No. of hyperaccumulators</th>
<th>Families of hyperaccumulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>1000</td>
<td>5</td>
<td>Pteridaceae</td>
</tr>
<tr>
<td>Cadmium</td>
<td>100</td>
<td>2</td>
<td>Brassicaceae, Asteraceae, Chenopodiaceae</td>
</tr>
<tr>
<td>Cobalt</td>
<td>1000</td>
<td>30</td>
<td>Lamiaeae, Scrophulariaceae</td>
</tr>
<tr>
<td>Copper</td>
<td>1000</td>
<td>34</td>
<td>Cyperaceae, Lamiaeae, Brassicaceae, Poaceae, Scrophulariaceae</td>
</tr>
<tr>
<td>Gold⁴</td>
<td>1</td>
<td></td>
<td>Brassicaceae</td>
</tr>
<tr>
<td>Lead⁴</td>
<td>1000</td>
<td>14</td>
<td>Compositae, Brassicaceae</td>
</tr>
<tr>
<td>Manganese</td>
<td>10,000</td>
<td>11</td>
<td>Apocynaceae, Campanulaceae, Proteaceae</td>
</tr>
<tr>
<td>Nickel</td>
<td>1000</td>
<td>320</td>
<td>Brassicaceae, Convolvulaceae, Flacourtiaceae, Violaceae, Euphorbiaceae</td>
</tr>
<tr>
<td>Selenium</td>
<td>100</td>
<td>20</td>
<td>Fabaceae, Brassicaceae</td>
</tr>
<tr>
<td>Silver⁵</td>
<td>1</td>
<td></td>
<td>Brassicaceae</td>
</tr>
<tr>
<td>Thallium</td>
<td>100</td>
<td>1</td>
<td>Brassicaceae</td>
</tr>
<tr>
<td>Uranium⁴</td>
<td>1000</td>
<td>16</td>
<td>Brassicaceae, Cramineae, Leguminosae</td>
</tr>
</tbody>
</table>

⁴ For induced hyperaccumulation.

Phytoextraction can be induce in order to cultivate high biomass plants such as Z. mays, *Brassica juncea*, *Crysopogon zizanoides*, and *Helianthus annus* with improved uptake speed of metal extraction capacities. Thus addition of chelates has been proposed. Chelates are high molecular weight compounds (aminopolyacrylic acid) and can be used to extract a wide variety of metals such as Cu, Cd, Zn, Pb, Ni, Au, Ag, etc. Chelating agents have a capacity to form water soluble metal organic complexes that bring metals into solution through desorption of sorbed species and allowing further dissolution of Fe and Mn oxides. The dissolution of precipitated compounds continues until equilibrium is reached between the complexed metal, free metal, and insoluble metal fraction. In the case of heavy metals, chelators like EDTA assist in mobilization and subsequent accumulation of soil contaminants.
such as lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn). in *Brassica juncea* (Indian mustard) and *Helianthus anuus* (sunflower) [10]. Such plants; and more in general all non-hyperaccumulator plants, are compatible with routine agricultural practices and allow repeated planting and harvesting of the metal-rich tissues. While intriguing, the plants that have these capabilities produce relatively low biomass and are not compatible with routine agricultural practices [15]. Metals that are mobilized by chelators can migrate offsite of the rhizosphere zone affecting plant metal uptake, may also affect soil ecosystem stability, its function and may also increase extraction cost [16].

The researches published up to now regarding phytomining process describe a series of negative aspects: firstly hyperaccumulator plants have a relative low biomass yield (around 10000kg per ha) [17] in order to obtain a result capable of cope with the costs at least, an intensive monoculture with high rates of fertiliser, nutrients and other chemicals inputs, should be applied, even because of low water and nutrient holding capacity of ultramafic soils. This situation may contaminate receiving waters, especially in relation to chelant agents that can make heavy metals soluble in water. For these reason Bergwerk Pflanze is focused on the use of non hyperaccumulator species that develop high biomass in relative quick period of time. To cope with the problem of leaching, field test are implemented inside the rautenweg landfill where a drain leachage system has been willing. The possibility to exploit areas inside the rautenweg landfill is a solution to one of the most cited negative aspects of phytomining that is the intensive plantation of monoculture, these exotic species could reveal negative impacts on the ecosystem replacing endemic vegetation [11] but this drawback is avoided growing plants inside an enclosed area. The bio-ores from phytomining are virtually sulphur free and their smelting requires less energy than sulphide ores. While energy production and metal recovery are theoretically feasible, in practice this could only happen if phytomining occurs in proximity to existing energy conversion facilities or to infrastructure that is actively processing metal (for example a Ni smelter).

### 1.3 Mechanism of metal phytoaccumulation

The hypothesis on why determined plant species developed the necessity of storing high metal concentration in their biomass, which has attracted most attention, suggests that the high heavy metal concentrations in aerial tissues may function as a self-defence strategy evolved in hyperaccumulator plants against some natural enemies, such as herbivores and pathogens [12]. Phytoaccumulation process depends on different factors attributable to soil and to the plant itself.
1.3.1 Soil-associated factors

(1) Moisture levels. It is observed that high moisture level determines greater metal uptake, and plants also produce greater biomass at higher soil moisture levels [18].

(2) Soil pH affects the solubility of trace elements, and hence is a major factor influencing the bioavailability of elements in the soil for plant uptake. Usually, the concentrations of heavy metals in soil solution can be increased by decreasing the soil pH value because a large quantity of heavy metal ions are desorbed from the surface of colloids and clay mineral particles, and then enter into the soil. This aspect is particularly important working with substrates based of bottom ash in which pH in generally around 10 or even higher [14].

(3) High cation exchange capacity determines high sorption and immobilization of the metals. and competition between different ions for the same types of reactive surface sites.

(4) Biochemical processes determine the presence of organic and/or inorganic ligands that can inhibit or enhance sorption of a metal ion, and the presence of surface coatings such as biofilms that may block reactive sites and/or create new sorption sites.

1.3.2 Plant-associated factors

Plant-associated factors mainly involve the development of a deep root apparatus and of adequate rhizospheric processes. Rhizosphere zone is populated with large concentrations of microorganisms mainly consisting of bacteria and mycorrhizal fungi. These microorganisms can enhance biomass production and thus tolerance of the plants to heavy metals, and are considered to be an important component of phytoextraction technologies [19]. Microorganisms catalyze redox transformations and exudate organic acids, phytochelatins (PCs), amino acids, and enzymes which stimulate bioavailability of metals and nutrients; this interaction enhance either the potential of root absorption area and facilitate root absorption of metal ions [8]. Roots of many hyperaccumulator plants exudate organic acids, such as malic, malonic and oxalic acids, this exudation decrease the rhizosphere pH. Furthermore metal chelating compounds (phytosiderophores), and enzymes (reductase) are produced, these agents enhance metal desorption from soil increasing the bio-availability of metals in the soil solution and a greater accumulation in plants.
1.4 State of the art of phytomining and economics

Up to now there are no reports of commercial application of metals recovery from bio-ore but lot of investigation have been made especially concerning Ni. This metal is the most studied metal involved in phytoextraction process, because its abundance in crustal tailings (generally between 1000 and 7000 mg/kg), a concentration below the minimum Ni level required for modern mining technologies (<30,000 mg/kg) [27], but adequate to supply to Ni hyperaccumulators. Pioneer of phytomining is Rufus Chaney, who has worked for the US government and has planned in 1997, the recovery of metals from contaminated soils using plants [20] and created several patents that deal with various aspects concerning metals and plants. In an old patent, the entire process is described: for example the cultivation, fertilisation and burning of Alyssum species biomass in the normal substrate in order to use the ash in conventional Ni-metallurgical processes as ore. Few years later, the recovery of cadmium and zinc with Thlaspi caerulescens has been patented. Another patent describes the effect of the entry of bacteria into the soil, to increase the accumulation rate. The most recent patent of Chaney in this context deals with the use of pH regulators with Alyssum species (Ni and Cd hyper accumulators) for the recovery of metals [21]

Another important step in development of phytomining process occurred with field experiments carried out in Albania with the Hyperaccumulator plant Alyssum murale in which it was stated the possibility to obtain ca 120 kg Ni per hectare [22]

Other studies conducted over smaller areas achieved interesting results including those with Streptanthus polygaloides in California that yielded 100 kg Ni/ha [23], A. bertolonii in Italy, 72 kg Ni/ha [24], and Berkheya coddii in South Africa, 100 kg Ni/ha [25]. The first field trial for phytomining was reported by then US Bureau of the Mines, Reno, Nevada [20] [26] on a naturally occurring strain of S. polygaloides which is a species known to hyperaccumulate nickel. The serpentine soil at the site contained normal range of about 3500 mg/kg Ni. The total dry biomass obtained was 10,000 kg/ha, containing Ni 10,000 mg/kg. [11] Considering an average price for Ni of $12,000 per metric ton (Average 2015 value London Metal Exchange) it means a profitable income of 1200$/ha

Interesting results are related to recovery of Thallium. The growing demand of thallium coupled with the relative unavailability of this metal in nature, makes it the fourth most-precious metal on the earth from economic point of view after gold, platinum, and palladium. Reported results in field trials in New Zealand with a biomass of 10,000 kg/ha Iberis. The hyperaccumulator plant produced about 700 kg/ha of bio-ore containing 8 kg of Tl worth $US 2400 at that time world price of $US 300/kg. Nowadays Tl prices decreased a lot, US$60,000 London Metal Exchange 2015), this is an example of how much profitability of this technology
relied on market prices [27].

Gold has been suggested as a likely candidate for phytomining. Plants do not normally accumulate gold; the metal must be made soluble before uptake can occur. Various researches have shown that uptake of gold can be induced using lixiviants such as sodium cyanide, thiocyanate, thiosulphates. The highest individual gold concentration determined through analysis of selected biomass was 63 mg/kg (NaCN treatment of B. juncea) also predicted that a harvested crop of 10,000 kg/ha biomass (dry) with gold concentration of 100 mg/kg, which would yield 1 kg of gold/hectare could be economically viable with an average gold price US$ 1100 per ounce (London Metal Exchange).

In addition to price fluctuations, other factors must be taken in account: (1) total development and optimization costs through trial stages to full operation; (2) predicted annual costs, which include working costs and machinery costs of propagation, fertilization, irrigation (if needed), plant protection and harvesting; (3) land costs; (4) the value of alternative land uses [13]. Furthermore phytomining is feasible only if occurs in proximity to existing energy conversion facilities or to infrastructure that is actively processing metal (for example a Ni smelter), long-distance transport of the biomass would greatly reduce any profits. Another additional potential source of income from phytomining comes from the sale of carbon dioxide credits [28].

1.5 Principal technologies developed for metal recovery from bio-ore

The first example of metal recovery from a bio-ore was made by Anglo-American Platinum Corporation (Amplats) at Rustenberg, S. Africa, that in 1996 commissioned a project to investigate the feasibility of using B. coddii to phytoremediate the area next to the company that was contaminated with nickel. The biomass of B. coddii was collected and incinerated to produce a bio-ore and smelted. The crude metal was then refined and cast into small ingots containing predominantly nickel. In carrying out this process, the Amplats team was the first in the world to show that metal from a hyperaccumulator crop could effectively be recovered in a relatively pure form. Amplats feed the dry biomass directly in metal smelter. In this way the resulting bio-ore was incorporated into bulk metal ore and contaminating metal from outside the refinery turned into a valuable product [27]. Another trial to recover metals was done at the Inco Ltd., smelter complex (Sudbury, Ontario, Canada), in which 500 kg of ash, coming from ashing of A. Murale, were placed in a “revert bag” and added to an electric arc
Principal technologies developed for metal recovery from bio-ore

furnace, Ni was very readily recovered from the ash. Ni concentration in the biomass of *A. Murale* was as high as 22,000 mg/kg with a production of 20,000 kg/ha [29].

Principal options, according to recent researches, which are mainly focused in Ni recovery, include a pyrometallurgical first step of ashing that is obtained with a pyrolytic process. Pyrolysis decomposes material under anaerobic conditions and moderate temperatures. The process destroys organic matter, releasing metals, mainly as oxides. The liberated metals are entrained in the slag or released to the effluent gases. Modern flue gas-cleaning technology assures effective capture of the metal-containing dust Sas-Nowosielska. The final products are pyrolytic gas and coke breeze with high levels of heavy metals concentration, more than 98.5% of the metal in the bio-ore is present in the ash [30] that recovers the metals in the form of a metal concentrate that may be processed using commercially available metallurgical operations. Bio-ore must be dried at least at 30% of moisture content before feeding the pyrolysis [31].

Second step is direct smelting of the plants ashes that are compatible with traditional metal smelting facilities, especially because bio-ore contain higher enrichment levels of metals than raw ores, furthermore they present lower density and the absence of sulfur [26] [14].

Figure 1.3 Flow sheet of Ni bio-ore processing steps. Major inputs, intermediate products and wastes are indicated. Approximate Ni concentrations of the biomass, bio-ore and products are also indicated [13].

Another nuov hydrometallurgical option can be applied to the incineration slag. Existing hydrometallurgical operations could integrate bio-ore into their feed; for example, pressure acid leaching where concentrated H$_2$SO$_4$ is injected into an autoclave at 250 °C would likely
solubilize the Ni [32]. Hydrometallurgical processes are flexible concerning product and by-product recovery options. Once present in aqueous solution at sufficient concentration and purity, a Ni product can be recovered in numerous forms such as in the metallic state by electrochemical reduction to plates, rondelles, powders, or as a compound by chemical or evaporative precipitation. Another simple option would be to chemically precipitate Ni as a hydroxide consisting of \( \approx 40 \) wt % Ni or crystallizing pure Ni salts, such as \((\text{NH}_4)_2\text{Ni(SO}_4)_2\cdot 6\text{H}_2\text{O}\) (ANSH). An alternative way of retrieve metal–derivatives from bio-ore is the recently designed and patented a method for the synthesis of a nickel salt, ammonium nickel sulfate hexahydrate (ANSH: \(\text{Ni(}\text{NH}_4)_2\text{(SO}_4)_2\cdot 6\text{H}_2\text{O}\)), from the biomass of the hyperaccumulator plant \textit{Alyssum murale}, grown in the Balkans. The economic feasibility has been proven [33] and the process is currently upscaled to the pilot scale. A life cycle assessment is ongoing to evaluate the environmental impacts of the phytomining process, from the plant cultivation to the ANSH production.
Alongside with Bergwerk Pflanze project aims, the primary aspect to consider is the choice of the most suitable substrate, among the different bottom ashes coming from Vienna incineration plants. In the following test are evaluated bottom ash coming from 2 different incineration facilities. In order to make these evaluations a germination test is set down on the selected substrates after a proper conditioning of the bottom ash. In particular it was tested in which way the addition of three different inorganic amendments (two different zeolites and ground bricks) improves the characteristics of the ash-based substrate, e.g. via a reduction of pH or the salinity content. Furthermore, also the effect of compost, from mechanical biological treatment of organic wastes, on the bioavailability of targeted elements was measured. The different substrates generated by the combination of two different bottom ashes, compost and amendments were evaluated through a germination test in which the capacity of seeds to grow in different environments was evaluated. Bioavailability fraction of targeted metals was evaluated with ammonium nitrate extraction and mass spectrometry analysis.

2.1 Bottom ash sampling and characterisation

Bottom ashes tested were chosen as representatives of 2 different incineration plants for municipal solid waste incineration. The first bottom ash was the result of an incineration process from a rotary kiln which operates at temperatures of up to 1200 °C, with a residence time of the waste in the incinerator of about half an hour. This material will be referred to as DRO from the german world Drehrohrofen (rotary kiln). The second kind of bottom ash considered in these tests comes from a burning process in the grate furnace incinerator of Flötzersteig that is the first incineration plant installed in the city of Vienna in the year 1963. This treatment plant operates at temperatures of around 850 °C, with a
residence time of the waste of about half an hour. This material resulting from this incineration process will be referred to FLÖ. Bottom ash samples were taken from different points of stockpiles of freshly quenched material. Samples of each bottom ash type were manually homogenised by mixing and subsamples of about 1 kg were obtained. These subsamples were air-dried, sieved to 5 mm and stored in closed plastic bags until further analyses [34].

Characterisation of the metal concentration in the 2 different slags were previously made through the use of inductively coupled plasma mass spectrometry (ICP-MS) that is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations in the lower ppb range. This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions. The analysis were conducted with the machinery Perkin Elmer Elan 9000 DRCe. Blanks and certified reference materials were used for quality control, in-house reference materials were used where certified materials were not available. In table 2.1 the summary of element concentrations in the ashes. EC and pH for the two bottom ash are summarized in table 2.2.

Table 2.1 Concentrations of selected elements in bottom ash determined with *acqua regia* extraction

<table>
<thead>
<tr>
<th>Element</th>
<th>Unit</th>
<th>FLÖ mean</th>
<th>SD</th>
<th>DRO mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>mg/kg</td>
<td>261.1</td>
<td>20.3</td>
<td>545.4</td>
<td>45.9</td>
</tr>
<tr>
<td>Ba</td>
<td>mg/kg</td>
<td>774.1</td>
<td>157.1</td>
<td>1160.6</td>
<td>456.3</td>
</tr>
<tr>
<td>Co</td>
<td>mg/kg</td>
<td>Nd</td>
<td>Nd</td>
<td>55.1</td>
<td>12.2</td>
</tr>
<tr>
<td>Cr</td>
<td>mg/kg</td>
<td>143.6</td>
<td>8.9</td>
<td>468.7</td>
<td>35.9</td>
</tr>
<tr>
<td>Cu</td>
<td>mg/kg</td>
<td>1698.1</td>
<td>283.8</td>
<td>2388.8</td>
<td>189.3</td>
</tr>
<tr>
<td>Li</td>
<td>mg/kg</td>
<td>17.1</td>
<td>1.1</td>
<td>48.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Mn</td>
<td>mg/kg</td>
<td>859.6</td>
<td>247.3</td>
<td>3200.2</td>
<td>364.4</td>
</tr>
<tr>
<td>Mo</td>
<td>mg/kg</td>
<td>Nd</td>
<td>Nd</td>
<td>67.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Ni</td>
<td>mg/kg</td>
<td>66.7</td>
<td>7.5</td>
<td>261.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Pb</td>
<td>mg/kg</td>
<td>2338.3</td>
<td>629.1</td>
<td>311.8</td>
<td>55.6</td>
</tr>
<tr>
<td>Sr</td>
<td>mg/kg</td>
<td>159.1</td>
<td>11.0</td>
<td>194.2</td>
<td>43.84</td>
</tr>
<tr>
<td>V</td>
<td>mg/kg</td>
<td>20.4</td>
<td>3.4</td>
<td>116.2</td>
<td>16.4</td>
</tr>
<tr>
<td>Zn</td>
<td>mg/kg</td>
<td>2042.7</td>
<td>107.9</td>
<td>3732.5</td>
<td>583.8</td>
</tr>
<tr>
<td>P</td>
<td>mg/kg</td>
<td>6402.4</td>
<td>661.7</td>
<td>2146.9</td>
<td>774.9</td>
</tr>
<tr>
<td>B</td>
<td>mg/kg</td>
<td>261.1</td>
<td>20.3</td>
<td>545.4</td>
<td>45.9</td>
</tr>
</tbody>
</table>
The electrical conductivity of 23.1 µS/cm reported for an Austrian ultramafic soil [35] was considerably lower compared to values reported for the bottom ash, even after a decrease in salinity with the pre-treatment of the material. Salinity can be seriously detrimental to plants, especially salt sensitive plants, causing water stress and toxicity symptoms.

### 2.2 Substrate preparation

The fresh bottom ash used in this experiment was characterised by an extremely alkaline pH, high salinity (table 2.2) and soluble Cu concentrations likely toxic to plants. To improve plant growth on this material these characteristics had to be altered (rosenk). Fresh bottom ash was incubated for 72 h in plastic buckets of 10 L capacity with 0.912 mol L\(^{-1}\) HNO\(_3\) at the same ratio of 1:2.5 (w/w) for the 2 different ashes. After 72 h the suspension was carefully decanted. After the acid treatment the material was leached twice with deionised water at a ratio of 1:10 (w/v), the water was decanted and the substrate dried at 50 °C for 24 h. The intention of the acid incubation, water leaching and amending of the fresh bottom ash was to decrease the pH, decrease the ionic strength and introduce organic material to the substrate in order to improve chemical and physical conditions for plant growth [34]. The pH was lowered after the acid incubation to 7.6 in both bottom ash materials. The substrate used in these experiments are the results of conditioning of bottom ash plus the addition of different percentage of compost coming from a mechanical biological treatment (MBT) obtained from Rautenweg landfill. The compost was added in 2 different concentrations: 15% (w/w) and 30% (w/w) based on dry weight, thus the first step was to measure the water content of the 2 ashes and compost. Three samples of each kind of ash and compost were weighted and placed in oven at 105°C for 24h. The successive weighting of the samples permitted to identify the water content of ash and compost, in this way it was possible to prepare substrates with the desired % compost dry weight. Addition of composts have been investigated in terms of their effects on TE immobilization [36]. Compost composition in table 2.3. With the goal of providing multiple benefits including soil improvement and increasing crop productivity while promoting long-term carbon (C) sequestration. After mixing, the substrates were placed in plastic bags in order
to maintain the moisture level. In the table below is represented the element composition the compost (MBT material) determined after *aqua regia* extraction (*aqua regia* extraction, HCl:HNO3 3:1, 3 h at 160 °C). Values are reported in g/kg and mg/kg with relative standard deviation in percentage in Table 2.3 [34].

Table 2.3 Compost composition

<table>
<thead>
<tr>
<th>Element</th>
<th>Unit</th>
<th>Value</th>
<th>Element</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>g/kg</td>
<td>11.1 ± 11%</td>
<td>As</td>
<td>mg/kg</td>
<td>18.5 ± 13%</td>
</tr>
<tr>
<td>Ca</td>
<td>g/kg</td>
<td>106 ± 2%</td>
<td>Ba</td>
<td>mg/kg</td>
<td>516 ± 3%</td>
</tr>
<tr>
<td>Fe</td>
<td>g/kg</td>
<td>25.1 ± 9%</td>
<td>Cd</td>
<td>mg/kg</td>
<td>5.4 ± 11%</td>
</tr>
<tr>
<td>K</td>
<td>g/kg</td>
<td>25.1 ± 9%</td>
<td>Co</td>
<td>mg/kg</td>
<td>16.9 ± 12%</td>
</tr>
<tr>
<td>Mg</td>
<td>g/kg</td>
<td>14.0 ± 4%</td>
<td>Cr</td>
<td>mg/kg</td>
<td>132 ± 1%</td>
</tr>
<tr>
<td>Na</td>
<td>g/kg</td>
<td>1.42 ± 7%</td>
<td>Cu</td>
<td>mg/kg</td>
<td>926 ± 2%</td>
</tr>
<tr>
<td>P</td>
<td>g/kg</td>
<td>1.15 ± 6%</td>
<td>Mn</td>
<td>mg/kg</td>
<td>58.2 ± 39%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mo</td>
<td>mg/kg</td>
<td>112 ± 1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ni</td>
<td>mg/kg</td>
<td>450 ± 3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pb</td>
<td>mg/kg</td>
<td>197 ± 7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sr</td>
<td>mg/kg</td>
<td>1662 ± 7%</td>
</tr>
</tbody>
</table>

2.3 Pots preparation

Aim of this study was to test possible amendments to condition the substrate in substitution of biochar, that it was proved to generate good conditioning results in bottom ash substrate especially in reference to immobilisation of potentially toxic elements present at high level such as Cu and Pb, but in at the same time it may have affected also the bioavailability of targeted metals like Ni [34]. From economic perspective the cost of certified biochar is quite high and it can develop a negative impact on the economic evaluation of the process. In this sense were evaluated two cheap inorganic amendments were evaluated: milled ground bricks and milled pomice zeolite, both these material are charachterised by high porosity, high specific surface area (SSA) that can produce a good sorption rate in order to reduce high EC levels of the two bottom ash. A third amendment tested was natural zeolite that was proved to have high affinity to sorb and to complex trace elements, particularly heavy metals immobilizing them and preventing their accumulation in agricultural plants. Differently from bricks, zeolite materials are very expensive.
The 3 different amendments were added to the starting substrates in an extent of 5% (w/w) and 10% (w/w). The scheme below shows the configuration of the different samples that are set down.

![Figure 2.1 Scheme of the possible combination of substrates, compost addition and amendments addition](image)

Togheter there were 2 different bottom ashes, 2 percentage of compost and 3 amendments in 2 different weight percentage, plus a control without any amendments, thus $2 \times 2 \times 7 = 28$ different combination to test. For each different combination 3 replicates were set down, for a total amount of 84 pots to set up. Each one of the different combination replicates was prepared in amount of 80g of dry weight per pot. The water content of the 4 substrates was already calculated, but it was necessary to know the water content of each amendment in order to add the proper amount because these additions are made in reference to dry weight. Thus 3 samples of each amendment where weight and put in oven at 105 °C for 24h in order to completely remove the water, than by subtraction it was possible to determine the moisture level of the amendments and consequently determine the weight in normal condition correspondent to dry conditions.

At the end of data analysis process it was decided to focus the attention only on four elements that appeared to be more significant in relation to the content in the starting substrates, the bioavailability and the phytoaccumulation made by the plants, and in the end to the phytomining process itself. These elements are: Cu, Mn, Sr, Zn.
Statistical analysis was carried out using SPSS 21 (IBM). One-way analysis of variance (ANOVA) and were performed for identification of significant differences between the individual treatments.

2.4 Germination results

A germination test aims to verify if the characteristics of a substrate wheater they are favourable for plant growth. The Brassica napus species proved to be more tolerant to high salinity and Cu concentrations of the ash and produced considerably higher biomass than other plants tested in previous experiments [34], that’s why seeds of Brassica napus were used for the germination test. These certified seeds, obtained from Templiner Kräutergarten (Germany), were stored in a refrigerated room inside the Soil Science department maintained at adequate condition in order to ensure high seed quality. For this test 20 seeds, randomly taken, are placed in each pots. To guarantee proper condition to let the germination happen, the pots were maintained in a moisture condition of 80% of WHC (water holding capacity). Firstly it was necessary to identify which was the WHC for each substrate. 2 samples from each different substrate were saturated till equilibrium, weighted, and put in oven at 105°C for 24h, then weighted again to evaluate the dry weight. WHC represents the water held by a certain substrate in saturation condition. Knowing the moisture content present in the 4 substrates from previous evaluation (substrates are maintained close in plastic bags to prevent evapotraspiration) and the corresponding WHC, it was possible to calculate the exactly amount of water to add in order to reach a moisture level corresponding to 80% of the WHC. This moisture level was maintained for the subsequent days watering regularly the pots that in the meanwhile were cover with plastic film, properly drilled to let the natural evapotranspiration happen, and exposed to natural sunlight. These conditions were kept until the uprising of the first sprouts, than plastic film was removed and the sprouts were let grow up for a couple of days in order to evaluate the quality of the plant growth. Sprouts from DRO slag made their appearance after 5 days whereas sprouts from FLÖ slag after 6 days and showed worst development condition. In the table 2.4 is summarized the number of germinated seed for each different amendment combination:
Analysis of variance of the results showed that generally higher compost content slightly reduced the number of germinated seeds for both the substrates. Furthermore FLÖ substrate in average developed lower germination rate that DRO, where sprouts germinated more homogeneously. This is probably due to higher salinity in FLÖ ash. It must be also said that sprouts in FLÖ slags germinated with 2 days of delay compared to DRO ones, developing less biomass. Concerning amendments, no relevance of enhancement in germination rate can be noticed, but these analysis are not so robust because dimension of sample is very low and even if seeds were stored in controlled condition there is possibility the some seed can be flawed.

Table 2.4 Number of germinated seed

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Compost</th>
<th>treatment</th>
<th>mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLÖ</td>
<td>15%</td>
<td>CONTROL</td>
<td>12</td>
<td>4</td>
</tr>
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<td></td>
<td></td>
<td>ZEOLITE 5%</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZEOLITE%10</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRICKS 5%</td>
<td>16</td>
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<td>15</td>
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<td>POMICE 5%</td>
<td>15</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>POMICE 10%</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>FLÖ</td>
<td>30%</td>
<td>CONTROL</td>
<td>10</td>
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<td></td>
<td></td>
<td>POMICE 10%</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>DRO</td>
<td>15%</td>
<td>CONTROL</td>
<td>19</td>
<td>1</td>
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<td></td>
<td></td>
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<tr>
<td>DRO</td>
<td>30%</td>
<td>CONTROL</td>
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<td></td>
<td></td>
<td>POMICE 10%</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>
2.4.1 EC and pH evaluations

At the end of the germination test the substrate was recovered for further evaluations, shoots and roots were collected by hand and properly disposed in the hazardous waste as well as the first layer of substrate present in the pots, in which oxidation phenomena due to air contact and degradation due to roots exudate may be happened. The substrates thus recovered were close in plastic bags and labelled in order to maintain the same conditions, after a new evaluation of the moisture content according to the criterion previously descripted. To understand the role of amendments on the substrates firstly were investigated pH and EC according to OENORM 1999 (Austrian standards). To make these evaluations a portion correspondent to 10g of dry weight of each different amended substrate was put in a 50 ml vial. Vials were previously let rest in a nitric acid bath for at least 6 h. Than 25 ml of deionised water was added, vials were closed and properly shaken and let to rest. After 2 h resting time, according to Austrian standards pH an EC were easured. In the table below the results of this analysis are shown.

Table 2.5 Results of EC measurement

<table>
<thead>
<tr>
<th>Amendment</th>
<th>FLÖ +15 % COMPOST [mS/cm]</th>
<th>SD</th>
<th>FLÖ +30 % COMPOST [mS/cm]</th>
<th>SD</th>
<th>DRO +15 % COMPOST [µS/cm]</th>
<th>SD</th>
<th>DRO +30 % COMPOST [µS/cm]</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>3.48</td>
<td>0.08</td>
<td>3.41</td>
<td>0.02</td>
<td>1648.67</td>
<td>108.26</td>
<td>1926.00</td>
<td>39.95</td>
</tr>
<tr>
<td>ZEOLITE 5%</td>
<td>3.16</td>
<td>0.20</td>
<td>2.87</td>
<td>0.10</td>
<td>1563.00</td>
<td>48.51</td>
<td>1720.00</td>
<td>77.50</td>
</tr>
<tr>
<td>ZEOLITE 10%</td>
<td>2.92</td>
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<td>2.17</td>
<td>0.11</td>
<td>1430.00</td>
<td>30.81</td>
<td>1714.67</td>
<td>76.87</td>
</tr>
<tr>
<td>BRICKS 5%</td>
<td>3.48</td>
<td>0.12</td>
<td>3.31</td>
<td>0.03</td>
<td>1629.33</td>
<td>56.05</td>
<td>1870.00</td>
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<tr>
<td>BRICKS 10%</td>
<td>3.34</td>
<td>0.09</td>
<td>3.41</td>
<td>0.19</td>
<td>1677.33</td>
<td>43.98</td>
<td>2015.67</td>
<td>126.82</td>
</tr>
<tr>
<td>POMICE 5%</td>
<td>3.04</td>
<td>0.25</td>
<td>3.20</td>
<td>0.05</td>
<td>1534.67</td>
<td>56.15</td>
<td>1664.00</td>
<td>72.96</td>
</tr>
<tr>
<td>POMICE 10%</td>
<td>3.22</td>
<td>0.12</td>
<td>3.22</td>
<td>0.28</td>
<td>1588.33</td>
<td>190.13</td>
<td>1599.00</td>
<td>123.92</td>
</tr>
</tbody>
</table>

In reference to both the two substrates it can be said that bricks didn’t affect the EC; zeolite on the other hand reduced slightly the salinity in extent of 10 % on average. Higher reduction can be noticed in the combination FLÖ + 30% compost + bricks in 10%, here EC was reduced in extent of 30%. In relation to DRO in both percentage of compost no effect can be noticed in EC reduction.
Table 2.6 results of pH measurement

<table>
<thead>
<tr>
<th>Amendment</th>
<th>FLÖ +15 % COMPOST [mS/cm]</th>
<th>SD</th>
<th>FLÖ +30 % COMPOST [mS/cm]</th>
<th>SD</th>
<th>DRO +15 % COMPOST [µS/cm]</th>
<th>SD</th>
<th>DRO +30 % COMPOST [µS/cm]</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>8,13</td>
<td>0,04</td>
<td>8,12</td>
<td>0,06</td>
<td>8,42</td>
<td>0,02</td>
<td>8,13</td>
<td>0,02</td>
</tr>
<tr>
<td>ZEOLITE 5%</td>
<td>8,10</td>
<td>0,01</td>
<td>8,05</td>
<td>0,02</td>
<td>8,20</td>
<td>0,04</td>
<td>8,10</td>
<td>0,04</td>
</tr>
<tr>
<td>ZEOLITE 10%</td>
<td>8,03</td>
<td>0,01</td>
<td>8,05</td>
<td>0,02</td>
<td>8,21</td>
<td>0,06</td>
<td>8,10</td>
<td>0,06</td>
</tr>
<tr>
<td>BRICKS 5%</td>
<td>8,09</td>
<td>0,02</td>
<td>8,07</td>
<td>0,05</td>
<td>8,36</td>
<td>0,02</td>
<td>8,13</td>
<td>0,03</td>
</tr>
<tr>
<td>BRICKS 10%</td>
<td>8,14</td>
<td>0,03</td>
<td>8,01</td>
<td>0,03</td>
<td>8,37</td>
<td>0,02</td>
<td>8,07</td>
<td>0,02</td>
</tr>
<tr>
<td>POMICE 5%</td>
<td>8,05</td>
<td>0,03</td>
<td>8,01</td>
<td>0,03</td>
<td>8,25</td>
<td>0,06</td>
<td>8,12</td>
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<tr>
<td>POMICE 10%</td>
<td>8,11</td>
<td>0,01</td>
<td>7,94</td>
<td>0,02</td>
<td>8,29</td>
<td>0,02</td>
<td>8,10</td>
<td>0,02</td>
</tr>
</tbody>
</table>

Statistical analysis showed no relevant changes in pH level. Higher pH decrease can be noticed in the substrate made of DRO ash + 15% of compost + zeolite 5% in which the reduction was in extent of 2.6%. In DRO substrate can be noticed how the higher compost content (30%) resulted in reducing pH, this was due to the lower pH of compost and also because of the ionic exchange capacity.

### 2.5 Labile metal fraction assessment

Labile metal fractions in the substrates samples were determined using the ammonium nitrate (NH₄NO₃) extraction. Therefor 20g on dry weight basis, sieved at 2mm, were weighted for each amendment combination, with the same procedure as for previous evaluations, and added to 100 ml plastic bottle together with 50 ml of 1 M NH₄NO₃–solution (solution ratio of 1:2.5 (w/v)). Bottle were previously leaned in 5% nitric acid bath for at least 6 h. Such bottles were shaken for 2 hours at 20 revolutions per minute in an end-over-end shaker at room temperature. After shaking, the samples were filtered through filter paper (Munktell folded filters) and acidified for storage using HNO₃. In house reference soil samples and method-blanks were included in the procedure for quality control. Substrate extraction with ammoniumnitrate NH₄NO₃ allows to estimate the plant potentially available fraction of metal content in the substrate. (Analysis on the labile fraction of the metal content was conducted on ICP- MS (Elan 9000 DRCe, Perkin Elmer) using Indium as internal standard. Quality controls and blanks were included in the measuring procedure. Below are represented values that appeared to be most interesting for his research.
2.6 Results

Firstly the role of compost in bioavailability of metals and TE was investigated, in particular focusing on the elements that appeared to be more interesting in relation to the recovery in the phytomining process, i.e. Cu, Mn, Sr, Zn. Mixing FLÖ ash with 15% of compost maintained higher bioavailable fractions for Cu, Sr and Zn. In general, concentrations (mg of targeted metal per kg of dry weight substrate) of target metals were lower in DRO ash compared to FLÖ. (Figure 2.2)

![Concentration of labile fraction elements](image)

Figure 2.2 Concentration [mg/kg] of the targeted metals in the labile fraction after ammoniumnitrate (NH4NO3) extraction. Columns represent the mean values, error bars represent standard deviation

Statistical analysis for the targeted elements stated that in reference to FLÖ substrate inorganic amendments hadn’t any significant effect on labile concentration of Cu and Mn; it can be notice a slightly decrease on Zn labile fraction for all the inorganic amendments tested. Different results were found for Sr: for the substrate FLÖ + 15% of compost the addition of pomice in 5% and 10% dry weight respectively enhanced Sr extractability by 28% and 76%; as far as concerns the substrate FLÖ + 30% it can be noticed that the addition
of pomice in 10% enhanced Sr availability of 84% in average. In the substrate made of DRO ash + 15% compost Sr bioavailability was enhanced by pomice 5%, pomice 10% and zeolith 10% respectively in a rate of 74%, 179% and 136%. Figure 2.3 represents the labile metal concentration in FLÖ-based substrate (15% and 30% compost).

Figure 2.3 Concentrations of the targeted metals in FLÖ-based substrates. Columns indicate mean values, error bars indicate standard deviation.
In the substrate made of DRO ash + 15% compost Sr bioavailability was enhanced by pomice 5%, pomice 10% and zeolith 10% respectively in a rate of 74%, 179% and 136%. For the substrate DRO +30%, Sr was enhanced by 62% and 100% respectively for pomice 5% and pomice 10%. No enhancement in the labile fractions of the other targeted metals can be seen for all the amendments addition. Figure 2.4 represent the labile metal concentration in DRO-based substrate (15% and 30% compost).

Figura 2.4 Concentrations of the targeted metals in DRO-based substrates. Columns indicate mean values, error bars indicate standard deviation.
2.6 Results

A special mention needs to be done concerning Ni. Most part of researches showed that Ni is one of the best candidates for a phytomining process, because ultramafic soils are abundant on terrestrial crust and most part of metal accumulator plant studied up to now are classified as Ni-accumulators. It is reported a table showing labile concentration for the substrates analysed. In this case values are particularly low but this depending on low concentration in the bottom ash, both for FLÖ and DRO. This means that Ni can’t be considered as a candidate for a phytomining process base on this bottom ashes substrates. In particular, none of the tested amendments resulted in enhancing significantly Ni availability after ANOVA analysis.

![Figura 2.5 Concentrations of Ni in the different substrates combination. Columns indicate mean values, error bars indicate standard deviation.](image-url)
A pot experiment was set up in an experimental greenhouse in May 2015. The test consisted of a full factorial design involving four plant species (Brassica napus, B. juncea, Heliantus annuus and Salix smithiana), an unplanted control and two substrate mixtures (FLÖ and DRO). The aim of the experiment was to test the efficiency of EDTA to increase phytoextraction of TE from the substrates. The substrates used in this test were composed of the same bottom ash used for the first experiment but moisture level was valuated again because two weeks passed since the beginning of the first experiment. The substrates mixtures were all conditioned with 20% (w/w) of compost from MBT obtained from Rautenweg landfill and 10% biochar (w/w) dry weight. Water content of compost and biochar was determined drying samples for 24h in oven at 105°C. The biochar was made from mixed woodchips slowly pyrolyzed in a rotary furnace (residence time: 60 min, highest treatment temperature: 525 °C; woodchip dimensions: 2 × 2 × 2 cm). Important characteristics of wcBC were an ash content of 15.2%, pH (in CaCl2) of 8.9, EC of 1.6 mS cm−1, CEC of 93.0 mmolc kg−1. [37]. Previous studies indicate that soils amended with biochar showed decreased nitrate leaching and enhanced P bioavailability [38]. Positive effects of BC application on the improvement of physicochemical soil properties are: provision organic carbon source, and elevated concentrations of certain TEs without biological function such as the heavy metals Pb and Cd may lead to persistent soil contamination and pose a threat to plants, the food chain as well as water resources [39] increase in saturated hydraulic conductivity, water use efficiency and total C and N [39], furthermore its high porosity, pH, specific surface area (SSA), and cation exchange capacity (CEC) [39], determine high soil sorption capacity of metal cation and ions.

The effect of the chelating agent was tested on 4 different plant species: Brassica napus and Brassica iuncea (from Templerin Kräutergarten, Germany) were already tested in
previous experiment on bottom ash substrate. These plants showed good results concerning growing capacity and biomass development in the harsh salinity and pH condition of the bottom ash [34]. Together with the Brassicaceae there were tested Heliantus annuus and Salix smithiana. None of these species, as well as the Brassicaceae, are considered metal hyperaccumulator but they were widely applied in metal phytoaccumulation researches (cfr references) and were reported to be resistant to substrates characterised by high metals concentration and wide pH range. These species show high growing rates and develop a large biomass, that’s why they appear interesting as species in which hyperaccumulation process could be induced by the addition of EDTA. Salix smithiana as well is a tall plant with a completely higher biomass development compared to other tested species that can be considered shrub plants.

Statistical analysis was carried out using SPSS 21 (IBM). One-way analysis of variance (ANOVA) and were performed

3.1 Pots prepration

For this test 80 pots were set up. Pots (ø 12 cm; height 9 cm) were filled with moist substrate, equivalent to 300 g dry substrate, mixed with cited percentages of compost and biochar. B. NApus, B. Juncea and H. annuus were grown from the seeds, using 10 seeds per pot for B. napus and B. juncea and 5 seeds per pot for H. Annuus. Differently Salix wasn’t planted by seeds because it is a tree species that can be more easily and quickly propagated from cuttings than from seeds. That’s why cutting of Salix, with approximately 1 cm diameter where placed in water until roots had developed. Once salix cuttings developed a proper root system, together with the other seeds were planted. For each plant species 8 pots were prepared with FLÖ substrate and 8 pots with DRO substrate, plus 8 controls with FLÖ and DRO, for a total of 80 pots. Pots were placed in the greenhouse of UFT BOKU University where light, temperature and relative humidity condition were automatically controlled (14-15 hours light; approx. 15/25 °C night/day temperature). Irrigation was carried out with an automatic drip irrigation system, keeping pots moist but avoiding excessive watering. Plants were harvested 8 weeks after germination.
3.2 EDTA addition and harvesting

The chelating agent solution EDTA was prepared from ethylenedinitrilotetraacetic acid disodium salt dihydrate commercially known as Titriplex III. It was decided to test a concentration of 0.8 g EDTA per 1000 g of substrate on the basis of previous application of EDTA in phytoextraction process \[35\], split in 2 applications, in reference to the tested pots of 300g dry weight it was added 0.24g EDTA per pot, splitteed in 2 applications of 0.12g EDTA. The amount of EDTA salt per pot was diluted in 100ml of Milli-Q water. First application was done after 6 weeks from the starting of germination, the second application one week later.

After 8 weeks from beginning of germination, plants were harvested. Shoots were cut at the substrate surface, rinsed with deionised water to avoid contamination with substrate particles and dried with tissue paper. The biomass produced was weighted after 24 h in oven at 65°C to identify in which condition plants developed higher biomass (Figure 3.1).

![Average values of biomass production (dry basis)](image)

Figure 3.1 Differences in biomass production for the tested plants in relation to the different substrates, on dry weight basis. Columns represent the mean values, error bars represent standard deviation

In general results showed that higher biomass production was achieved in FLÖ-based substrate. This can be explaining by a lower concentration of phytotoxic metals in this ash, especially in reference to Cu and Cr. Higher content of Pb in FLÖ compared to DRO, didn’t affect the plant growing thanks to the immobilisation effect of the biochar. Considering the
role of EDTA, statistical analysis proved no effect on biomass development for H. annuus and S. smithiana, on the other hand it was proved a lower biomass production in extent of 25% on average, for the two brassicaceae in both the two substrates.

- **Brassicaceae:**

Previous experiment applied on different bottom ash substrates showed that B. napus and B juncea looked healthy throughout the course of the experiment with high biomass production rate [34]. Same results were obtained in this experiment for both the substrates used. It was noticed the appearance of purple spots in the leaves of both of brassicaceae species planted in DRO substrate, more noticeable in B. juncea than in B. napus. This was determined by the higher concentration of Cu in DRO substrate (Table 2.1). According to Austrian legislation, NH4NO3-extractable Cu concentrations exceeding 1.5 mg kg\(^{-1}\) in agricultural soils are considered disturbing for plant growth (Austrian Standards, 2004), which is exceeded by far in the bottom ash. In Figure 3.1 are shown the differences in biomass development in relation to the two substrates. After statistical evaluation results demonstrated that the species of B. napus, B. Juncea have respectively demonstrated a biomass increase of 62%, 57% in substrate made of FLÖ bottom ash. In reference to EDTA addition B. napus showed 25% higher biomass development in FLÖ ash not treated with the chelating agent, whether in DRO no effect on EDTA addition could be noticed. In B. juncea the addition of EDTA reduced biomass development in a rate of 20% for FLÖ substrate and for 43% in DRO one.

- **Helianthus annuus**

Plants grew very healthy, and developed a particularly high biomass. No sign of intoxication or deficiency was noticed. After statistical evaluation results demonstrated plants from FLO ash developed on average 32% higher biomass than DRO bottom ash. Higher biomass development in FL In reference to EDTA addition B. napus showed 25% higher biomass development in FLÖ ash not treated with the chelating agent, whether in DRO no effect on EDTA addition could be noticed. In B. juncea the addition of EDTA reduced biomass development in a rate of 20% for FLÖ substrate and for 43% in DRO one. In reference to EDTA addition no significant difference was noticed for this species comparing biomass development in the two substrates.

- **Salix smithiana**

Even in this case plants grew very healthy, without any noticeable sign of intoxication or
deficiency but didn’t developed a particularly high biomass, the reason is because this species is a tall tree and needs longer time period to develop its biomass. After statistical evaluation is possible to state that no differences in biomass development could be noticed as far as concerns the use the two different substrates or the addition of EDTA, in relation to stems and leaves. It was not possible to achieve extensive informations regarding this plants because of its long growing period. Potentially good aspects of this species need to be further indagated with a longer time-scale plantation experiment.

### 3.3 EC and pH evaluation

After analysis of variance EDTA showed to have no effects on pH level of the two substrates, only sample of H. annuus in FIO substrate demontraded a low enhancement in pH after EDTA additio. Common aspects for the two substrates was the evidence of pH decrease induced by H. annuus. In literature is are reported this potential effect of this plant, expecially because this plant is proved to grow up optimally in acidic condition [42] The values of pH in this test are lower than the values found in the germination test although the bottom ashes used are the same and also the compost is the same. This is due to rhizosphere activity and partly because of after eight weeks, the water content in the soil togheter with the cation exchange capacity of the soil determined exchange of cations like Ca+, Mg+, Al+ with H+ in the water. [14]. Addition of biochar could also enhance short-term translocatioion of anionic TEs towards the groundwater at high leaching rates[36]. In Figure 3.2 pH values measured in the substrates.
Figure 3.2 Comparison of the mean pH values for the two substrates, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.

Statistically for DRO samples no changes in EC can be noticed after EDTA addition. In FLÖ can be noticed an increase of EC probably due to plant exudates that enhanced the availability of cations Na⁺, K⁺, Ca²⁺, Mg [16].
Figure 3.3 Comparison of the mean EC values for the two substrates, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation

### 3.4 Labile metal fraction assessment

Labile metal fractions in the substrates samples were determined using the ammonium nitrate (NH$_4$NO$_3$) extraction. Therefore after separating the roots, 20 g substrate on dry weight basis, sieved at 2mm, are weighted for each amendment combination, added to 100
ml plastic bottle together with 50 ml of 1 M NH₄NO₃–solution (solution ratio of 1:2.5 (w/v)). Bottle were previously let rest in nitric acid bath for at least 6 h. Such bottles were shaken for 2 hours at 20 revolutions per minute in an end-over-end shaker at room temperature. After shaking, the samples were filtered (Munktell folded filters) and acidified for storage using HNO₃. In house reference soil samples and method-blanks were included in the procedure for quality control. Analysis on the labile fraction of the metal content was conducted on ICP- MS (Elan 9000 DRCe, Perkin Elmer) using Indium as internal standard. Quality controls and Blanks were included in the measuring procedure. After statistical analysis of variance, and considering the implementation in a phytoming process, in relation with metal accumulation data that are showed in the next chapter, for the labile fraction in the substrate, attention is focused on Cu, Ni, Sr and Zn, Mn was excluded because of its low concentration in substrates treated with biochar [38].

General consideration: as far as concern bioavailability of Sr, EDTA didn’t show any effect each substrates no effect for each plat species both in DRO and FLÖ substrates. Nickel was present in both substrates at very low percentage, but in each combination it was an increase after EDTA addition for all the tested plants. [35] [40] [41]. B. napus in DRO showed a particularly increase in Ni labile fraction, from 0.06 mg/kg to 1.51. These results can be considered interesting if the focus is posed on the increased percentage of bioavailability, thus suggest that more investigations have to be done in this conditions but in Ni-richer substrate. Similar consideration regarding Ni can be done also for the other plants in the two substrates combinations. Zn enhancement was particularly high: 68-fold increase in FLÖ and 55-fold in DRO. Remarkable enhancement can be seen also for Cu: respectively of 20-fold and 10-fold in DRO and FLÖ, this because of higher content of Cu in DRO ash; differently Zn labile availability was increased at a higher extent in FLÖ -based ash. Figures 3.4, 3.5, 3.6, 3.7, show results of the EDTA addition on the different substrates.
Figure 3.4 Comparison of the mean values of the targeted metals the two substrates planted with B. napus, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.

Figure 3.5 Comparison of the mean values of the targeted metals the two substrates planted with B. juncea, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.
Figure 3.6 Comparison of the mean values of the targeted metals the two substrates planted with H. annuus, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.

Figure 3.7 Comparison of the mean values of the targeted metals the two substrates planted with S. smithiana, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.
The dried biomass was completely milled and subsamples of 0.2 g were digested in a mixture of concentrated HNO3 (65%, 5 mL) and H2O2 (30%, 1 mL) using an automated digestion block. Plant digestions were analysed for TE and nutrient concentrations using ICP-MS. Among elements analysed by mass spectrometry, attention was focused on Ni, Cu and Zn, in which it was noticed a strong enhancement in the amount phytoaccumulated by the plants after EDTA addition.

In general results showed that higher metal bioaccumulation levels of Cu and Ni were reached in DRO-based substrate while higher metal bioaccumulation of Zn were reached in FLÖ-based ash. This is in accordance with the labile composition found in the substrates and the starting composition of the two ashes. As expected Ni accumulation is particularly low, in accordance with the low labile concentration. Interesting results were found for Zn with B. juncea in FLÖ substrate reaching a mean value of 694.3 mg/kg (Figure 3.9). This result in the end, compared to literature foundings was not particularly high because values up to 12,000 mg/kg was reported in a pot experiment on Zn/Cd contaminated soils [53].
Figure 3.8 Comparison of the mean values of the targeted metals bioaccumulated in the biomass of B. napus for the two, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.

Figure 3.9 Comparison of the mean values of the targeted metals bioaccumulated in the biomass of B. juncea for the two, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation.
H. annuus showed results in accordance with literature in reference to Ni uptake, developing a 2-fold higher uptake in EDTA treated plants [44], respectively 3.2 mg Ni/kg and 1.6 mg Ni/kg. These values are far from profitable. Application of EDTA didn’t show any effect in FLÖ substrate.

**Figure 3.10** Comparison of the mean values of the targeted metals bioaccumulated in the biomass of H. annuus for the two, considering samples treated with EDTA and samples not treated. Error bars represent standard deviation.
Figure 3.12 Comparison of the mean values of the targeted metals bioaccumulated in the biomass of salix stems for the two, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation

Figure 3.11 Comparison of the mean values of the targeted metals bioaccumulated in the biomass of salix leaves for the two, considering samples treated with EDTA and samples not treated. Error bars represents standard deviation
The results of the first test on inorganic amendments showed no evidence in enhancement of germination rate in B.napus seeds. In general FLÖ-based substrate developed lower germination rate, this probably due to higher salinity levels and higher metal content in ash. Combination. in the second test results was the opposite because plants developed higher biomass in FLÖ substrate, this is due to the limitations of bioavailability of toxic metals like Cu and Pb made by use of biochar. In particular pomice addition seems to determine a decrease in germination rate as far as concern DRO + 30 % compost but this evaluation can’t be considered very reliable because test were made only with 3 replicates. Focusing After analysis of variance clearly appear that the substrate made of FLÖ ash plus an addition of 15% of compost guarantee higher bioavailable fractions for the selected elements. The different amendment didn’t reduce the salinity of the slags. The only evidence noticed regards zeolite at reduced slightly salinity in the case of 15% addition, a better result can be noticed in the combination 30% compost + bricks in 10%, here EC was reduced almost 1,5 fold. In reference to pH no amendments determined any change. Inorganic amendments were proved to have no effect on labile concentration of Cu and Mn. Different results can be shown in reference to Sr: for the substrate FLÖ + 15% of compost in which the addition of pomice in 5% and 10% dry weight respectively enhanced Sr content of 28 % and 76. In the end the tested amendments didn’t show any interesting perspective as conditioning amendments.

Results of second experiment demonstrated that plants were able to grow without any sign of intoxication in both the 2 ash-based substrates. Brassicaceae showed small sign of Cu intoxication but this didn’t affect the growing rate. The role of substrate was important, in fact FLÖ samples developed higher biomass, this is remarkable in reference to a phytomining
efficient process. The results of EDTA addition on the phytoaccumulation was particularly noticeable and was proved to determine enhancement in accumulation rate for the targeted metals. Increasing rate were lower in FLÖ substrate in extent from 2 to 3-fold the increasing level noticed in DRO.

EDTA effect were more noticeable in Brassicaceae were enhancement levels up to 9-fold were identified for Cu, 8-fold for Ni and 5-fold for Zn. Thus in the end the most impressive result was shown by Zn phytoaccumulation of Brassicaceae, especially from B. juncea in FLÖ substrate with a level of nearly 700 mg/kg that can be considere particularly noticeable result for developing the phytomining process especially because plants grew up healthy and undangered by high salinity, pH and levels of the metals. Regardless of the low price for Zn in its metallic form, the metal rich biomass containing Zn can be of greater value, e.g. catalysts prepared from hyperaccumulator biomass [45]. Findings of these researches demonstrated also that this treatment of bottom ash substrate allow growing different plants very healthy despite high Cu levels that are generally toxic for most of the species [46]. EDTA is confirmed to generate high enhancement level in bioavailability of metals without any endangering for plant growth in this condition. Other test should be set down in order to identify new plants with potential higher phytoaccumulation capacities for the targeted metals; in particular species like Salix smithiana need longer time scale test to prove the real potential of this plants. and also on other bottom ashes coming from the different incineration plants of Vienna.
Literature


[3] Österreichische Forschungsförderungsgesellschaft mbH (FFG)


https://www.wienenergie.at/eportal3/ep/channelView.do/pageTypeId/67831/channelId/-49065

[6] VIENNA GOVERNMENT ENVIRONMENTAL & CLIMATE PROTECTION
https://www.wien.gv.at/umwelt/ma48/entsorgung/abfallbehandlungsanlagen/deponie.htm1


Literature


[38] Luke Beesley, Eduardo Moreno-Jiménez, Jose L. Gomez-Eyles, Eva Harris, Brett Robinson, Tom Sizmur. A review of biochars’ potential role in the remediation, revegetation and restoration of contaminated soils, Environmental Pollution, Volume 159, Issue 12, December 2011, Pages 3269–3282


[40] Bernd Nowack, Rainer Schulin, And Brett H. Critical Assessment of Chelant-Enhanced Metal Phytoextraction Robinson Institute of Terrestrial Ecosystems, ETH Zurich Universitaetstrasse 16, 8092 Zu ”rich, Switzerland


