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Présentée Par

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**Procédé hydrométallurgique pour la valorisation du nickel
contenu dans les plantes hyperaccumulatrices**

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and continued support from everyone.

Travis Barker

Develop an attitude of gratitude, and give thanks for everything

that happens to you, knowing that every step forward is

a step toward achieving something bigger

and better than your current situation.

Brian Tracy

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- 4) **Zhang X.**, Houzelot V., Plasari E., Barbaroux R., Laubie B., Mercier G., Blais J.F., Bani B., Echevarria G., Morel J.L., and Simonnot M.O. Phytomining: a win-win partnership between agronomy and chemical engineering to recycle metals dispersed in soils. *GPE – 4th International Congress on Green Process Engineering*, Sevilla (Spain), 7-10 April 2014.
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- 6) A. Bani, G. Echevarria, **X. Zhang**, J.L. Morel, M.O. Simonnot. New trends for nickel phytomining. (Communication orale) 2014. ICSE (Sabah, Malaysia)
- 7) G. Echevarria, AJM. Baker, A. Bani, M. Konstantinou, **X. Zhang**, E. Benizri, M.O. Simonnot, J.L. Morel. New trends for nickel phytomining. (Communication orale) 2014. ICSE (Sabah, Malaysia)
- 8) **Zhang X.**, Laubie B., Houzelot V., Plasari E., Bani B., Echevarria G., Morel J.L., Simonnot M.O. New information for phytoextraction and phytomining. 11th International Phytotechnologies Conference, Heraklion, Crete, Greece, Sept 30 – Oct 3, 2014.

Résumé

Certaines plantes, dites hyperaccumulatrices, ont la capacité de se développer sur des sols riches en métaux (sols naturels ou contaminés) et d'accumuler ces métaux à des concentrations élevées. Elles peuvent être utilisées en phytoextraction, pour la dépollution de sols contaminés en métaux et/ou la phytomine, c'est-à-dire pour la récupération et la valorisation des métaux contenus dans leur biomasse. Cette dernière application est particulièrement intéressante dans le cas du nickel puisque plus de 400 hyperaccumulateurs de nickel ont été identifiés et le nickel a une valeur marchande relativement élevée. L'incinération de la biomasse produit des cendres qui contiennent de 10 à 25 % en masse de nickel, teneurs plus élevées que certains minerais. Ce travail s'inscrit dans la continuité d'une recherche menée par l'équipe depuis plusieurs années, qui a donné lieu notamment à un brevet sur la production hydrométallurgique du sel double sulfate de nickel et d'ammonium hexahydraté (ANSH) à partir de la biomasse d'*Alyssum murale*. Il s'agit d'améliorer la voie de synthèse de ce sel en vue de transposer le procédé à l'échelle du pilote et d'explorer de nouvelles voies conduisant à de nouveaux produits.

Le manuscrit comprend d'abord une synthèse bibliographique sur la phytomine, allant des hyperaccumulateurs aux procédés de valorisation, essentiellement centrée sur le nickel. Ensuite, ont été comparées quinze plantes hyperaccumulatrices (des genres *Alyssum*, *Leptoplax* et *Bornmuellera*) provenant d'Albanie ou de Grèce, en vue de leur application pour la phytomine. Les teneurs en nickel ont été mesurées dans les différents organes des plantes et dans les cendres obtenues par combustion. Les trois genres ont de l'intérêt pour l'application, les plantes contiennent 1 à 3 % en masse de nickel et les cendres 15 à 20 %. Le procédé hydrométallurgique de production d'ANSH a été étudié étape par étape en vue d'optimiser chaque étape pour produire un sel très pur tout en économisant matière et énergie et

minimisant la production d'effluents et de déchets. Ce travail a conduit à l'amélioration du procédé de départ. Enfin, de nouvelles pistes ont été proposées pour conduire à de nouveaux procédés et produits du nickel. Les résultats obtenus et la dynamique actuelle autour de la phytomine montrent l'intérêt de cette approche et annoncent son développement imminent.

Abstract

Some plants, known as hyperaccumulators, are able to develop on metal containing soils (natural or contaminated soils) and to accumulate these metals at high concentrations in shoots. They can be used in phytoextraction to clean up contaminated soils and/or in phytomining to recycle the metals contained in their biomass. The latter technology is particularly interesting in the case of nickel since more than 400 nickel hyperaccumulators have been identified and nickel has a relatively high economic value. Biomass incineration leads to ash containing 10 to 25 wt % nickel, greater than in some mineral ores. This work follows a research that has been carried out by the team for several years, which has resulted in a patent on the hydrometallurgical production of the double salt ammonium and nickel hexahydrate (ANSH) from the biomass of *Alyssum murale*. It aims at improving the synthesis method of this salt in order to upscale it at the pilot scale and explore new methods leading to new products.

The manuscript begins with a bibliographic review on phytomining from hyperaccumulators to metal recycling processes, essentially focused on nickel. Then *ca* 15 hyperaccumulator plants (genus *Alyssum*, *Leptoplax* and *Bornmuellera*) collected in Greece or Albania have been compared, in the objective of phytomining. Nickel concentrations were measured in the plant organs and in the ashes after combustion. The three types of plants are of great interest for the technology, they contain 1 to 3 wt % of nickel and the ashes 15 to 20 %. The hydrometallurgical process of ANSH production was investigated step by step to optimize each step to produce a salt of high purity, to decrease materials and energy consumption and to minimize effluent and waste production. The process was thus improved. Eventually, new ideas have been tested for new processes and nickel products. The obtained results and the current dynamics prove the interest of phytomining and announce its imminent development.

List of Tables

Table 1. 1: Lower limit for hyperaccumulation of various metals, and number of known hyperaccumulators with their families (Baker et al., 2000)	9
Table 2. 1: List of Ni-hyperaccumulating taxa collected, locations and sampling period	33
Table 2. 2: Concentrations of the major elements (Ca, Fe, K, Mg) and Ni in the leaves, stems, flowers and seeds of <i>Alyssum</i> hyperaccumulator species ($\text{mg (g}_{\text{dry plant}})^{-1}$)	39
Table 2. 3: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in <i>Leptoplax</i> and <i>Bornmuellera</i> hyperaccumulator species ($\text{mg (g}_{\text{dry plant}})^{-1}$)	40
Table 2. 4: Concentration of the minor elements in the leaves, stems, flowers and seeds of <i>Alyssum</i> hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)	42
Table 2. 5: Concentration of the minor elements in the leaves, stems, flowers and seeds of <i>Leptoplax</i> and <i>Bornmuellera</i> hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)	43
Table 2. 6: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the whole 15 hyperaccumulator species, calculated from the concentrations and the weight ratios of the organs of the plants ($\text{mg (g}_{\text{dry plant}})^{-1}$)	45
Table 2. 7: Concentration ratios Ni:Ca, Ni:Mg and Ca:Mg in the 15 hyperaccumulators	45
Table 2. 8: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the ashes obtained from the different organs of the <i>Alyssum</i> hyperaccumulator species (except <i>A. murale</i> 6 for which the total biomass was mixed) ($\text{mg (g}_{\text{ashes}})^{-1}$).....	51
Table 2. 9: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the ashes of the leaves, stems, flowers and seeds of <i>Leptoplax</i> and <i>Bornmuellera</i> hyperaccumulator species ($\text{mg (g}_{\text{ashes}})^{-1}$)	51

Table 2. 10: Concentration of the minor elements in the whole hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)	52
Table 2. 11: Concentration of the minor elements in the ashes of the leaves, stems, flowers and seeds of <i>Alyssum</i> hyperaccumulators species ($\mu\text{g (g}_{\text{ashes}})^{-1}$)	53
Table 2. 12: Concentration of the minor elements in the ashes of the leaves, stems, flowers and seeds of <i>Leptoplax</i> and <i>Bornmuellera</i> hyperaccumulator species ($\mu\text{g (g}_{\text{ashes}})^{-1}$)	54
Table 3. 1: K mass balance and removal yields in three steps washing.....	68
Table 3. 2: K mass balance and recovery yields with method M#1, two steps.....	70
Table 3. 3: K mass balance and removal yields with method M#1, eight steps	71
Table 3. 4: K mass balance and removal yields with method M#2	72
Table 3. 5: Recovery rates of Mg and Ni from washed ashes (A_{12}) and raw ashes (A_{10})	80
Table 3. 6: Materials of selective leaching.....	81
Table 3. 7: Ni extraction yield from <i>A. murale</i> washed ashes	83
Table 3. 8: Mass balance of the whole process	89
Table 3. 9: Elemental concentrations in the powder crystals after the first (1 st) and second (2 nd) crystallization.....	98
Table 3. 10: Elemental concentrations in the big crystals after the first (1 st) and second (2 nd) crystallization.....	98
Table 3. 11: Amounts of plant, reactants and water needed to prepare 1 kg of ANSH. .	101
Table 4. 1: Solubility (expressed in g / 100g H ₂ O) of some common inorganic salts at 25 °C.....	105
Table 4. 2: Examples of prices of common nickel salts.....	106
Table 4.3: Elemental concentrations in ANSH crystals produced from the process of using Na ₂ S·9H ₂ O.....	111

Table 4.4: Amounts of reactants needed to prepare ANSH from <i>A. murale</i> ash with NiS as an intermediate.....	111
Table 4.5: Major element concentrations and extraction in the boiled solution for two different particle sizes	118
Table 4.6: Concentration of organic acids in the boiled solution from the crushed plants	119
Table 4.7: Formula, mass molar and pKa at 25 °C of acids in the boiled solution.....	120
Table 4.8: Metal extractions of boiling plants (2 mm) using hot plate & ultrasonic wave & autoclave heating.....	120
Table 4. 9: Mass balance of producing NiS from boiling plants (2 mm) in 0.05 M H ₂ SO ₄	123

List of Figures

Figure 1. 1: Photograph of contaminated soil (Guangzhou, China).....	7
Figure 1. 2: Mechanism of major processes proposed to be involved in heavy metal hyperaccumulation by plants (Yang et al., 2005).....	11
Figure 1. 3: A schematic illustrating the different categories of phytoremediation (source:bio349.biota.utoronto.ca)	13
Figure 1. 4: Phytomining scheme from already experimented process	15
Figure 1. 5: Distribution of the serpentine sites studied in Eurasia (Dilek and Flower, 2003).....	17
Figure 1. 6: Wild <i>A. murale</i> growing in Albania (a) and field of flowering <i>A. murale</i> and <i>A.corsicum</i> in USA (b)	22
Figure 1. 7: The scheme of electroplating of Ni	23
Figure 1. 8: Organic reaction studied with the catalyst derived from Ni-hyperaccumulator plants	24
Figure 1. 9: Simplified scheme to produce ANSH salt from <i>A.murale</i> biomass (Simonnot, private communication).....	25
Figure 1. 10: Diagram of producing nickel ammonium sulfate from ash of <i>A.murale</i> (Barbaroux et al., 2012).....	26
Figure 1. 11: Nickel market price value at the London Metal Exchange from 2007 to 2014.....	28
Figure 2. 1: Weight fractions of the leaves, stems, flowers and seeds of the 14 Ni-hyperaccumulators	37
Figure 2. 2: Comparison between the concentrations of the major elements in the biomass of <i>A.murale</i> 6 and the ashes obtained at 550 and 1,100 °C for 2 h. All the concentrations are relative to the mass of dry plants.	47

Figure 2. 3: Evolution of the mass reduction factor and of the Ni concentration in the ashes of the ashes of <i>A. murale</i> 6 during the combustion at 550 °C.....	49
Figure 2. 4: Appearance of the ashes of <i>A. murale</i> 6 after different durations of combustion at 550 °C	49
Figure 2. 5: XRD pattern of <i>A.murale</i> ashes	54
Figure 2. 6: Particle size distribution of the raw <i>A.murale</i> ashes.....	55
Figure 2. 7: SEM images of <i>A.murale</i> raw ashes	55
Figure 2. 8: (a,b,c) EDX of <i>A.murale</i> raw ashes at three different points.....	56
Figure 2. 9: Elemental weight percentage of the stem, leaf and flower ashes	57
Figure 3. 1: The representative photos of the existed hydrometallurgical process	60
Figure 3. 2: Diagram of the washing process following method M#1	63
Figure 3. 3: Process diagram of simulating counter-current washing following the 2 nd method.....	64
Figure 3. 4: Variation of K concentration in the supernatant with the agitation time.....	65
Figure 3. 5: Raw ashes and mixtures observed at different ash mass fractions:	66
Figure 3. 6: K concentrations evolution at different ash mass fractions	66
Figure 3. 7: K concentration evolution in the supernatant at 3 stirring speeds, 500, 1000 and 2000 rpm.....	67
Figure 3. 8: K concentrations in the supernatants PE1, PE2 and PE3, following method M#1	68
Figure 3. 9: XRD pattern of the raw and washed ashes (A10 & A12).....	69
Figure 3. 10: XRD pattern of the washed ashes (A11 & A12 & A13)	69
Figure 3. 11: K concentrations and pH change with the washing steps.....	70
Figure 3. 12: K concentrations in each first effluent of simulating counter-current washing M#2	72

Figure 3. 13: Evolution of Ca concentrations in PE1 and PE2	74
Figure 3. 14: Appearance of the ashes after filtration (the white spots are CaCO_3)	75
Figure 3. 15: Evolution of Mg concentrations in PE1 and PE2	76
Figure 3. 16: Mg and Ni concentrations and recovery rates at different pH.....	82
Figure 3. 17: XRD pattern of the ashes (A_{12} & SW_1)	84
Figure 3. 18: a) Photograph of Na_2SO_4 generated in crystallization process when using NaOH as neutral agent;	87
Figure 3. 19: XRD pattern of the solid waste (SW_2)	88
Figure 3. 20: Photograph of different colors of SW_2	90
Figure 3. 21: Chemical structure of HDMG and $\text{Ni}(\text{HDMG})_2$	95
Figure 3. 22: ANSH crystals obtained a) with stirring and b) without stirring.....	95
Figure 3. 23: a) X-ray diffraction patterns of ANSH crystals made under stirring and the commercial salt of high purity (salt#1 of 99.999 % purity); and b) Infra-red spectrum	97
Figure 3. 24: The new flow sheet of the process to produce ANSH salt from ashes of <i>A.</i> <i>murale</i>	100
Figure 3. 25: Photograph of the whole process	100
Figure 4. 1: Main steps of ANSH production with NiS as an intermediate product.....	109
Figure 4. 2: XRD pattern of NiS	110
Figure 4. 3: Diagram of producing ANSH salt from <i>A.murale</i> ash with NiS as intermediate product.....	112
Figure 4. 4: Comparison of the different solid/liquid ratios (4, 10 and 15 %).....	115
Figure 4. 5: Evolution of K, Mg, Ca and Ni concentration in the solution obtained by boiling	117

Figure 4. 6: Chromatogram of organic acids in the boiled solution with crushed plants (2 mm)	119
Figure 4. 7: Photos of adding $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ into the boiled solution.....	122
Figure 4. 8: Process of producing NiS from the <i>A.murale</i> plants	124

Contents

Introduction	1
Chapter 1: Bibliography study.....	6
1 Introduction	7
2 What are hyperaccumulating plants?	9
2.1 Definition of hyperaccumulator	9
2.2 Types of hyperaccumulators	9
2.3 Mechanism of metal hyperaccumulation	10
2.4 Methods of improving the ability of metal accumulation.....	11
3 Phytoextraction.....	13
3.1 Definition of phytoextraction.....	13
4 Phytomining	15
4.1 Nickel in soils: what are the potential resources for phytomining?	16
4.1.1 Serpentine soils containing nickel	16
4.1.2 Main sources of nickel pollution in soils	17
4.1.3 Ni speciation in soils.....	18
4.1.4 Health risks due to nickel contaminated soils	18
4.1.5 Nickel contaminated soil remediation	19
4.2 Agronomy of phytomining	20
4.3 Recovery of Ni from hyperaccumulator plants.....	23
4.3.1 Producing pure Ni from hyperaccumulators	23

4.3.2 Producing Lewis acid catalysts from hyperaccumulators	24
4.3.3 Producing nickel double salt from hyperaccumulators	24
4.3.4 Hydrothermal process of extracting nickel from the Ni-hyperaccumulators	27
4.4 Feasibility of nickel phytomining	27
5 Hypothesis and objectives	29
5.1 Hypothesis.....	29
5.2 Objectives	29
Chapter 2: Characterizations of plants and ashes from Ni-hyperaccumulators	31
1 Introduction	32
2 Materials and methods	32
2.1 Sampling and preparation of the plants	32
2.2 Sampling of the ashes	34
2.3 Mineralization and ICP-AES analysis of biomass and ashes	35
2.4 X-ray diffraction analysis	36
2.5 Particle size distribution, SEM and EDX analysis.....	36
3 Results and discussion.....	36
3.1 Mass distribution of biomass among stems, leaves, flowers and seeds of the different taxa.....	37
3.2 Elemental concentration of the hyperaccumulators	38
3.3 Optimization of the furnace treatment	46
3.3.1 Influence of temperature on ashes composition	47
3.3.2 Influence of combustion duration on ash composition.....	48

3.4 Concentrations in the ashes of the 14 hyperaccumulators	50
3.5 XRD of the ashes of the hyperaccumulators.....	54
3.6 Particle size distribution, SEM and EDX analysis.....	55
4 Conclusions	57
Chapter 3: Optimization of the process to produce ANSH (ammonium nickel sulfate hexahydrate) from ashes of <i>A. murale</i>	59
1 Introduction	60
2 Part I–Ash washing stage	61
2.1 Introduction.....	61
2.2 Materials and methods	61
2.2.1 Composition of the raw ashes.....	61
2.2.2 Influence of the washing duration	62
2.2.3 Influence of ash mass fraction	62
2.2.4 Influence of the stirring speed	62
2.2.5 Influence the washing methods	63
2.3 Results and discussion	65
2.3.1 Influence of the stirring duration	65
2.3.2 Influence of the ash mass fraction	65
2.3.3 Influence of the stirring speed	67
2.3.4 Influence of the washing methods	67
2.3.5 Dissolution equilibrium calculations in the washing step	73
2.4 Conclusions.....	76

3 Part II-Acid leaching	77
3.1 Introduction.....	77
3.2 Materials and methods	77
3.2.1 Experiments to test acid leaching at room temperature.....	77
3.2.2 Acid leaching at high temperature	78
3.3 Results and discussion	79
3.3.1 Controlling the pH	79
3.3.2 Recovery rates of Mg and Ni from A ₁₂	80
3.3.3 Selective leaching	81
3.3.4 Optimal parameters for acid leaching.....	83
3.4 Conclusion	84
4 Part III – Leachate purification before crystallization	85
4.1 Introduction.....	85
4.2 Materials and methods	85
4.2.1 Neutralization	85
4.2.2 Adding NaF to eliminate Mg.....	85
4.2.3 Evaporation.....	86
4.3 Results and discussion	86
4.3.1 Neutralization	86
4.3.2 NaF addition to remove Mg followed by evaporation	90
4.4 Conclusion	91
5 Part IV- Crystallization.....	92

5.1 Introduction.....	92
5.2 Materials and methods	93
5.2.1 Different methods of crystallization	93
5.3 Results and discussion	95
5.3.1 Crystals	95
5.4 Conclusion	99
6 Conclusion.....	100
Chapter 4: New ideas of producing nickel products from <i>A. murale</i> biomass	103
1 Introduction	104
2 Part I-Producing nickel products from <i>A. murale</i> ash	104
2.1 Introduction	104
2.2 Materials and methods	107
2.2.1 Preparing NiSO ₄ at pH between 4 and 5	107
2.2.2 Producing NiS, ANSH salt and other nickel products.....	107
2.3 Results and discussion.....	109
2.3.1 Production of ANSH with NiS as an intermediate product.....	109
2.4 Conclusion.....	112
3 Part II--Producing nickel products directly from <i>A. murale</i> plants.....	113
3.1. Introduction	113
3.2. Materials and methods	114
3.2.1 Extracting nickel from <i>A. murale</i> plants in deionized water at 100 °C.....	114

3.2.2 Ni extraction by heating with ultrasonic wave or autoclave	114
3.2.3 Producing nickel sulfide, nickel sulfate.....	115
3.3. Results and discussion.....	116
3.3.1 Extraction of the major elements.....	116
3.3.2 Organic acids in the boiled water	118
3.3.3 Comparison of different heating methods	120
3.3.4 Trial to prepare NiS from the aqueous solution	122
3.3.5 Ni extraction from the boiled solution at pH 1	123
3.4 Conclusion.....	124
4 Conclusion.....	125
Conclusion & perspectives.....	126
Résumé étendu en français	131
References:	141

Introduction

A goal is a dream with a deadline.

Napoleon Hill

Hyperaccumulators were fully discovered in the seventies (Jaffre et al., 1976). These plants have the ability to extract metals from soils and accumulate high amounts in their tissues by a process called **phytoextraction**. Firstly, these plants can be considered as indicators of the presence of metals in soils but immediately the idea came about of applying them to soil remediation (Chaney, 1983; Huang et al., 1996; Kumar et al., 1995; Macek et al., 2007; Robinson et al., 1999; Salt et al., 1998; Sarma, 2011; Schroeder et al., 2010). In a second stage, it was found that metals present in the plant biomass could be recovered, and this technique was called **phytomining** (Bani et al., 2007; Brooks et al., 1998; Brooks et al., 2001; Chaney et al., 1998; Chaney et al., 2007; Robinson et al., 1997a; Robinson et al., 1997b). Among the metals and metalloids that can be accumulated by plants (e.g. As, Au, Co, Cu, Cd, Ni, Pb, Zn), nickel (Ni) has a great interest since more than 400 nickel hyperaccumulators have been recorded worldwide and nickel has a high economic value (see London Metal Exchange).

At the beginning of Ni-phytomining development, Ni was recovered from the biomass of *Alyssum murale* by pyrometallurgy: after biomass incineration of the biomass, pure Ni was generated by smelter process from the ash, considered as a high grade Ni ore (Brooks et al., 2001; Chaney et al., 1998; Li et al., 2003a; Li et al., 2003b). In 2007, Ni reached prices of more than US\$ 40 per kg, which meant that a total value of at least US\$ 16 000 could be achieved per annual harvest (Chaney et al., 2007). Later on, hydrometallurgical extraction was investigated. It was proved that Ni could be extracted from the biomass of *A. murale* by acid leaching, without burning (Barbaroux et al., 2009; Barbaroux et al., 2011). The acid leachate could be processed to obtain pure Ni after extraction stages and electrowining (Barbaroux et al., 2011).

Other contributions have aimed at obtaining Ni products from the hyperaccumulator ashes. Pure Ni was obtained from the ash of Ni hyperaccumulators (*Hybanthus enneaspermus* (violacea)) by electrochemical process (Tennakone et al., 2007). Lewis catalysts were also produced from the ashes of *P. douarrei* and *G. pruinosa* grown in New Caledonia (Losfeld et al., 2012a; Losfeld et al., 2012b). And a process was designed and patented by our team to produce a nickel salt, **ammonium nickel sulfate hexahydrate (ANSH)**, from the ashes of *A. murale* grown in Albania (Barbaroux, 2010; Barbaroux et al., 2012; Mercier et al., 2012). This research was achieved in the context of an interdisciplinary and international partnership between Laboratoire Sols et Environnement (Université de Lorraine, INRA, France), Laboratoire Réactions et Génie des Procédés (Université de Lorraine, CNRS, France), Institut National de la Recherche Eau Terre Environnement (Québec, Canada) and the Agricultural University of Tirana (Albania).

At the moment, there is a growing interest worldwide for phytomining development. A current issue concerning this development is the regular supply of high amounts of hyperaccumulator biomass containing high metal concentrations. This issue has been investigated for years by the Laboratoire Sols & Environnement and the Agricultural University of Tirana. The implementation of agronomic approach has enabled us to grow *A. murale* in Albania and produce more than 100 kg Ni per hectare (Bani, 2009; Bani et al., 2014; Bani et al., 2009; Bani et al., 2007). Then phytomining must be considered as the combination between agronomic process and pyro or hydrometallurgical process. To emphasize this aspect, we have recently introduced the term “**agromining**”.

Currently, very wide scientific and applied issues are remaining, although more and more teams are involved in research on agromining. The scientific topics can be organized as follows:

1. **Hyperaccumulator plants:** research needs to be done on the mechanisms of hyperaccumulation, on plant ecophysiology, on genetics to select the best individuals and possibly isolate the genes responsible for hyperaccumulation;
2. **Soils and agronomy for hyperaccumulator cropping:** the agronomic processes, as the one established for *A. murale* in Albania, should be extended to different plants, soils and climates worldwide. There is also a need of understanding the parameters governing metal availability in soils. Moreover plant cultivation can be combined to other aims, e.g. soil restoration (in mining areas), valorization of derelict land (brownfields, constructed soils) etc.
3. **Metal processing:** the existing process should be improved and upscaled; they should be extended to other plants and/or metals to produce different types of products;
4. **Evaluation of the agromining chain:** from the economic and environmental points of view and comparison with conventional techniques.

Among these topics, we have focused on the scientific objectives presented thereafter to answer the following questions:

1. **Selection of the best hyperaccumulators** grown in the Balkans: Ni hyperaccumulating plants have different homeostasis properties and might show different abilities for Ni recovery. Among the main Ni hyperaccumulators found in the Balkans, which are the species with the most interesting properties for Ni recovery?
2. **Optimization of the ANSH production process** in view of upscaling: this process has been designed at the bench scale and has brought the evidence of the possibility of

making ANSH. However, is it possible to improve its effectiveness and optimize it in terms of water, reactant, energy saving? And also to increase salt purity?

3. **Investigating other methods to make Ni products** from the plant biomass: it is possible to make other products, and possibly with other extraction methods?

To answer these questions, this manuscript is organized in four chapters. The first chapter presents a bibliographic survey on nickel phyto- and agromining. It presents the state of the art on phytomining, Ni hyperaccumulator plants and metal processing methods.

The second chapter deals with the selection of Ni hyperaccumulators from the Balkans. A series of plants belonging to three genera had been sampled in Albania and Greece. We analyzed the major elements, including Ni, in the organs of the plants, to find the best individuals. Then we have studied biomass incineration in order to determine the best conditions to avoid Ni loss, we have determined the ash composition and established mass balances between biomass and ash.

The third chapter focuses on the improvement of the ANSH process. We have studied each step of the process to bring better understanding of the mechanisms. This work has enabled us to improve the process by modifying some steps and globally save matter and energy and increase salt purity.

In the fourth chapter, we propose methods to obtain other Ni products, such as nickel sulfide and nickel sulfate from *A. murale*. Also we have investigated the feasibility of Ni extraction directly from the biomass with water. This method opens new possibilities to produce other Ni compounds.

Chapter 1: Bibliography study

A journey of a thousand miles

starts with a single step.

Lao Zi

1 Introduction

With the expanding economics, rapid population growth and disarrayed industrialization, environmental pollution is becoming more and more serious (Fig.1.1). Especially, soil contamination by heavy metals threatens ecosystems functioning and may seriously affect human and animal health (Dankoub et al., 2012; Maisto et al., 2011; Zhao et al., 2012).



Figure 1. 1: Photograph of contaminated soil (YX Wu, Sun Yat-sen University)

Mankind has to deal with two main issues regarding heavy metals. The first one is soil contamination due to heavy metal dissemination from many sources. Metals present in soils are persistent and may be transferred to organisms, including edible plants, soil fauna, and threaten human health (Chaney, 1983; Grasmuck and Scholz, 2005; Jadia and Fulekar, 2009; Morel, 1997). The second issue is the expected lack of metals regarding the limited global stock and the increasing economic demand for metals, which will eventually exceed the current production (Yellishetty et al., 2011). For example, some sources put that Ni shortage is expected in a few decades (<http://minerals.usgs.gov/>), even if Ni is not in the most critical metals. Hence, Ni production from low grade ores or from secondary sources has become a major challenge. The increasing concern for the recycling of raw materials in the European

Union (especially strategic metals) has enhanced many research programmes¹ on secondary resources that would be available locally. According to this new paradigm, low grade ores, mineralized soils, man-contaminated soils and urban and industrial wastes could become potential metal resources for the metal industry.

The use of metal hyperaccumulator plants may be a great help to face both issues (Chaney et al., 2007; Barbaroux et al., 2012; Tang et al., 2012). Therefore, phytoextraction (using hyperaccumulator plants to recover the metals from the substrate) was considered as a green in-situ technology with the advantages of clean, simple, cost-effective, and non-environmentally disruptive to resolve some of heavy metal contamination problems (Macek et al., 2007; Marmioli et al., 2006; Mohanty et al., 2004; Salt et al., 1998) and the economic secondary resource of strategic metals (Li et al., 2003; Chaney et al., 2007).

Phytomining has concentrated on the economic recovery of metals (mainly Ni) from hyperaccumulators belonging to the *Brassicaceae* family and more precisely from the *Alyssum* genus. Work has mainly focused on the *A. murale* species and secondarily on *A. corsicum* (Bani et al., 2009; Bani et al., 2007; Barbaroux et al., 2011; Barbaroux et al., 2012; Cecchi et al., 2010; Losfeld et al., 2012a; Tennakone et al., 2007; Zhang et al., 2014).

In this bibliographic study, firstly, hyperaccumulation and hyperaccumulator plants are presented. Secondly, phytoextraction and phytomining concepts are described, as well as the use of hyperaccumulator plants in phytomining research. Then, the potential soil targets for phytomining are discussed according to the specificity and properties of Ni-rich soils (either man-contaminated or natural serpentine/peridotite derived soils). Finally, the processes for the recovery of nickel from Ni-hyperaccumulator biomass are described.

¹ http://ec.europa.eu/enterprise/policies/raw-materials/files/docs/eip-sip-part1_en.pdf
http://www.sipri.org/research/conflict/pko/PKO_archive/resources/RCSfull1206.pdf.

2 What are hyperaccumulating plants?

2.1 Definition of hyperaccumulator

The term “hyperaccumulator” was firstly defined by Jaffré and co-workers who described the spectacular accumulation of Ni in the plant *Sebertia accuminata* (Jaffre et al., 1976). The threshold concentrations for hyperaccumulators are in Table 1.1, such as 1,000 mg metals kg⁻¹ (0.1 %) dry mass for As, Co, Ni, Cu, Pb and U, except for Mg and Zn, which it is 10,000 mg kg⁻¹, Ag and Au is 1 mg kg⁻¹, and Cd, Se and TI of 100 mg kg⁻¹ (Baker et al., 2000; Brooks, 1998; Sheoran et al., 2009).

Table 1. 1: Lower limit for hyperaccumulation of various metals, and number of known hyperaccumulators with their families (Baker et al., 2000)

Element	Lower limit for hyperaccumulation (mg kg ⁻¹)	No. of hyperaccumulators	Families of hyperaccumulators
Arsenic	1,000	5	<i>Pteridaceae</i>
Cadmium	100	2	<i>Brassicaceae, asteraceae, chenopodiaceae</i>
Cobalt	1,000	30	<i>Lamiaceae, scrophulariaceae</i>
Copper	1,000	34	<i>Cyperaceae, Lamiaceae, Brassicaceae, poacea, scrophulariaceae</i>
Gold*	1	-	<i>Brassicaceae</i>
Lead*	1,000	14	<i>Compositae, Brassicaceae,</i>
Manganese	10,000	11	<i>Apocynaceae, Cunoniaceae, Proteaceae</i>
Nickel	1,000	320	<i>Brassicaceae, Cunoniaceae, Flacortiaceae, Violaceae, Euphorbiaceae,</i>
Selenium	100	20	<i>Fabaceae, Brassicaceae</i>
Silver*	1	-	<i>Brassicaceae</i>
Thallium	100	1	<i>Brassicaceae</i>
Uranium*	1,000	-	<i>Brassicaceae</i>
Zinc	10,000	16	<i>Brassicaceae, Crassulaceae, Leguminosae,</i>

* For induced hyperaccumulation.

2.2 Types of hyperaccumulators

Up to now, there are over 500 plant species (approximately 0.2 % of all known species) that were reported with the abilities to hyperaccumulate metals (As, Cd, Co, Cu, Mn, Ni, Pb, Sb,

Se, Ti, Zn) (Kramer, 2010; Sarma, 2011). Most of these plants (about 70 %) are Ni-hyperaccumulating plants, particularly from the genus of *Alyssum*. Among Ni-hyperaccumulators, hypernickelophores have the ability to store more than 10,000 $\mu\text{g Ni g}^{-1}$ of dry matter in their tissues without heavy metal toxicity symptoms (Baker and Brooks, 1989; Brooks, 1977, Van der Ent et al., 2013).

2.3 Mechanism of metal hyperaccumulation

Metal hyperaccumulation by plants is achieved through the coordination of several processes: bio-activation in the rhizosphere, root absorption and compartmentation, xylem transport (metal uptake by shoot) and distribution and sequestration (Clemens et al., 2002; Krämer, 2010; Verbruggen et al., 2009; Zhao and McGrath, 2009).

The major processes involved in mechanism of heavy metal hyperaccumulation by plants are displayed in Fig.1.2. Most heavy metals are largely immobile and their bioavailability to plant root is restricted. Internal and external factors affected the bioavailability of heavy metals for plant uptaking and biomass increasing were investigated, such as soil pH, fertilizers, chelator-buffered nutrient solution (Bani et al., 2007; Centofanti et al., 2011; Chaney et al., 2007; Li et al., 2003a; Qui et al., 2008; Smolinska and Cedzynska, 2007). Some scientists tried to develop transgenic hyperaccumulators to obtain phytoextraction (Chaney et al., 2007; Cherian and Oliveira, 2005; Clemens et al., 2002).

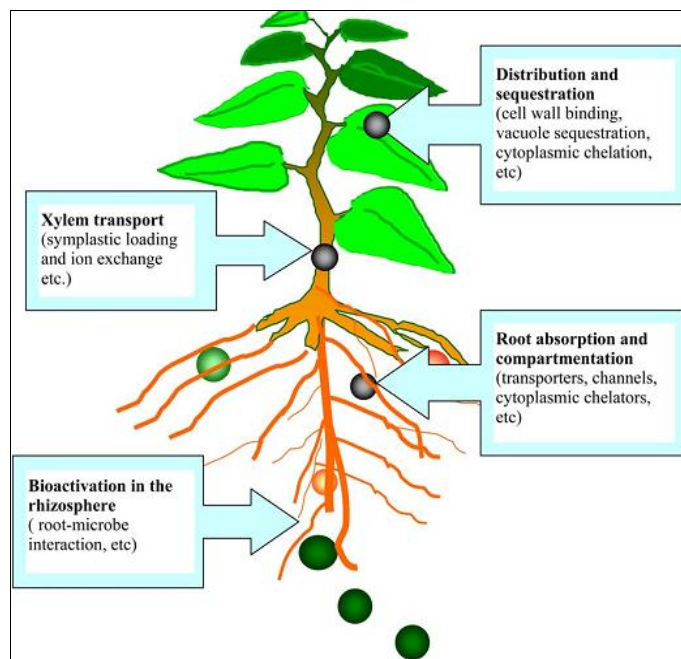


Figure 1. 2: Mechanism of major processes proposed to be involved in heavy metal hyperaccumulation by plants

(Yang et al., 2005)

2.4 Methods of improving the ability of metal accumulation

Although hyperaccumulation ability seems to act whatever the edaphic conditions are, many factors can improve metal phytoextraction. Therefore, there has been a growing interest in investigating the associated factors improving the accumulation abilities of the hyperaccumulating plants (Bhargava et al., 2012; Chaney et al., 2008; Chaney et al., 2000; Hsiao et al., 2007; Li et al., 2003a; Reeves and Adiguzel, 2008; Sheoran et al., 2011). The major factors affecting heavy metal accumulation ability are as follows:

❖ Soil pH

Soil pH affects not only the types of soils (acid or alkaline), and also the bioavailable metal cations quantity in the soils. The group of Chaney R.L. (USDA) investigated the pH effect on metal accumulation in two *Alyssum* species. pH treated by 0.01 M $\text{Sr}(\text{NO}_3)_2$ in the Quarry and Welland (Typic Epiaquoll; Canadian classification, Terric Mesisol) lead to a decline of extractable Ni with the increase in pH ($\text{Sr}(\text{NO}_3)_2$ -extractable of Ni is 4.85 % at pH of 5.6 but 2.07 % at pH of 7.3 (Kukier et al., 2004; Li et al., 2003b). And pH of about 6 was the best pH

to have the maximum Ni phytoextraction from *A. murale* and *A.corsicum* grown on serpentine soil (Qui et al., 2008).

❖ Use of fertilizers

Fertilizers are often used to increase the crop production, such as phosphorus (P) was used to enhance the vegetable-rice crop and potato production (Alam and Ladha, 2004; Rosen et al., 2014). Based on the same idea, inorganic and organic nitrogen, phosphorus (such as N, NP and NPK etc.) were studied to increase the metal up taking capacity of the hyperaccumulators (Bani, 2009; Bani et al., 2007; Chaney et al., 2007; Chaney et al., 2005; Li et al., 2012). For instance, NH_4^+ -N was the best fertilizer for *P. vittata* as it can phytoextract maximum of As from the soil (Liao et al., 2007).

❖ Chelator-buffered nutrient solution

Using chelating agents to enhance metal uptake by hyperaccumulator plants has been studied (Chaney et al., 1998; Chaney et al., 2007; Karami and Shamsuddin, 2010; Leštan et al., 2008). For example, EDTA (ethylenediamine tetraacetic acid) was known as the best chelate to improve certain metals (Cr, Cd, Ni, Hg etc.) uptake by hyperaccumulators, especially for Pb (Leštan et al., 2008; Smolinska and Cedzynska, 2007). EDTA and DTPA (diethylenetriamine pentaacetate) have shown their effectiveness to increase Cr and Ni levels in the soil solution (Bani et al., 2014; Bani et al., 2009; Hsiao et al., 2007). However, chelating agents may cause unacceptable contaminant leaching. As a consequence, we should have to balance the positive and negative sides of using chelating agents (Centofanti et al., 2011; Chaney et al., 2007; Smolinska and Cedzynska, 2007).

❖ Tailoring rhizosphere microorganisms communities to improve metal phytoextraction

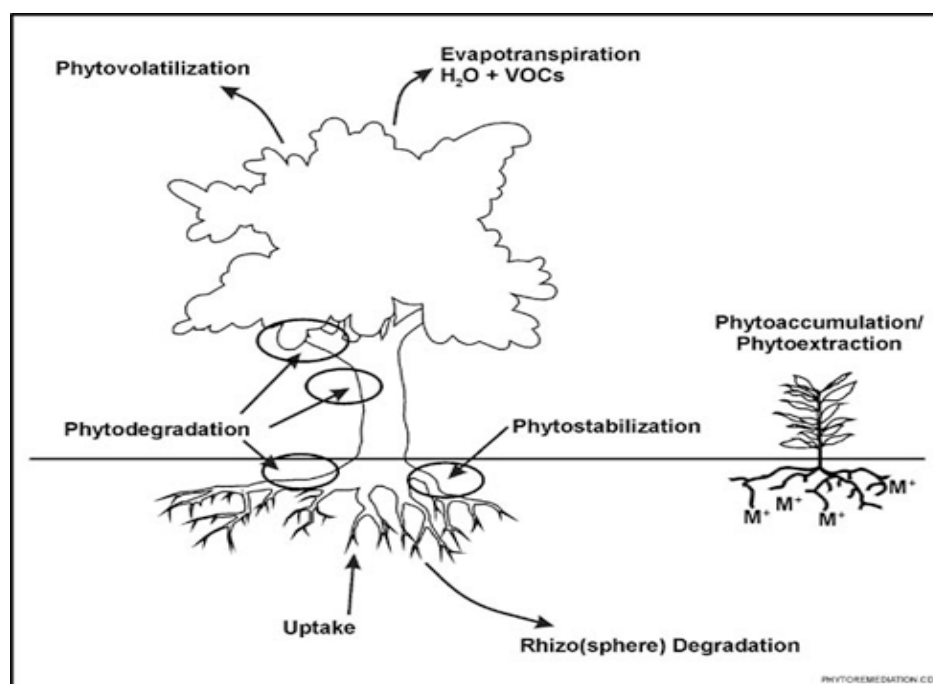
Rhizobacteria may affect hyperaccumulator plants growth and metal phytoextraction, then work on tailoring rhizosphere microorganisms communities to improve metal phytoextraction was carried out (Cabello-Conejo et al., 2014; Lucisine et al., 2014). The conclusion was that

the underground rhizosphere microbial communities could improve the values of biomass and Ni phytoextraction, such as a strain of *Arthrobacter nicotinovorans* SA40.

3 Phytoextraction

3.1 Phytoextraction and the other phytoremediation techniques

Phytoextraction is one of the techniques of phytoremediation. **Phytoremediation** consists in using plants to absorb, accumulate, degrade, volatilize or immobilize soil pollutants (Macek et al., 2007; Salt et al., 1998; Sarma, 2011; Schroeder et al., 2010; Wendy Ann Peer et al., 2005). As illustrated in Fig.1.3, the different types of phytoremediation are phytostabilization, phytodegradation, phytovolatilization (evapotranspiration), and phytoextraction (Macek et al., 2007; Marques et al., 2009; Nwoko, 2010; Sarma, 2011; Schroeder et al., 2010).



A schematic illustrating the different categories of phytoremediation.
Reprinted with permission from <https://portal.navy.mil/pls/portal/docs/1/3106018.JPG>

Figure 1. 3: A schematic illustrating the different categories of phytoremediation (source:bio349.biota.utoronto.ca)

Phytostabilization is a sub-process of phytoremediation in which the plant roots immobilize the contaminants by binding them to soil particles, then pollutants can be stabilized in the

rhizosphere (Cheraghi et al., 2011; Salt et al., 1995). For instance, plants can prevent water erosion, reduce wind erosion, and stabilize and restrain mine tailings (Mendez and Maier, 2008).

Phytodegradation consists in using metabolic activities within plant tissues or enzymes released from roots to break down the contaminants (Jiang et al., 2010; Macek et al., 2007; Marmiroli et al., 2006). For example, plants of Brassicaceae, *Brassica napus* (rape) and *Brassica oleracea* (cabbage), and a grass, *Festuca rubra* (red fescue) were cultivated on a sediment to study (Caille et al., 2005). And also many plants are able to break down organic compounds such as polycyclic aromatic hydrocarbons (PAH) using a biodegradation process (Biache et al., 2013; Cébron et al., 2013; Joner et al., 2004).

Phytovolatilization aims at using plants to take up contaminants from soil or water to the leaves, and evaporate, or volatilize to release them directly or modify the form of pollutants into the atmosphere (Macek et al., 2007; Salt et al., 1998). For example, poplar trees can volatilize 90 % of trichloroethylene (TCE) by converting it to chlorinated acetates and CO₂. Phytovolatilization can be used to remediate selenium polluted soils and waters by plants (Orchard et al., 2000; Zayed et al., 2000).

Phytoextraction consists in using plants to remove dangerous contaminants, metals or metalloids, from soils or water; pollutants are transferred to the aerial plant tissue, and the pollution level can be lowered by harvesting the biomass (Chaney et al., 2000; Garbisu and Alkorta, 2001; Robinson et al., 2003; Tang et al., 2012).

Phytoextraction is a continuous process based on the capacity of hyperaccumulator plants to gradually accumulate metals into their biomass. Metals present in soils, or more precisely, the available fraction of these metals are taken up by plant roots to be translocated to the aerial parts. It depends on several key factors: the extent of soil contamination, metal availability for uptake by roots (bioavailability), ability of selected plants to grow and accumulate metals

under the specific climatic and soil conditions of the site being remediated, etc. (Angle et al., 2001; Bani, 2009; Chardot et al., 2005; Do Nascimento and Xing, 2006; Li et al., 2003b; Mahmood, 2010).

4 Phytomining

Among phytotechnologies, phytomining combines the cropping of hyperaccumulating plants and the recycling of the metals stored by the biomass. Then it can be viewed (Fig. 1.4) as the combination of (1) an agronomic process aiming at producing high metal yield and (2) a pyro or hydrometallurgical process to produce metal or metal salts (Barbaroux et al., 2012; Boominathan et al., 2004; Brooks et al., 1998; Chaney et al., 2007).

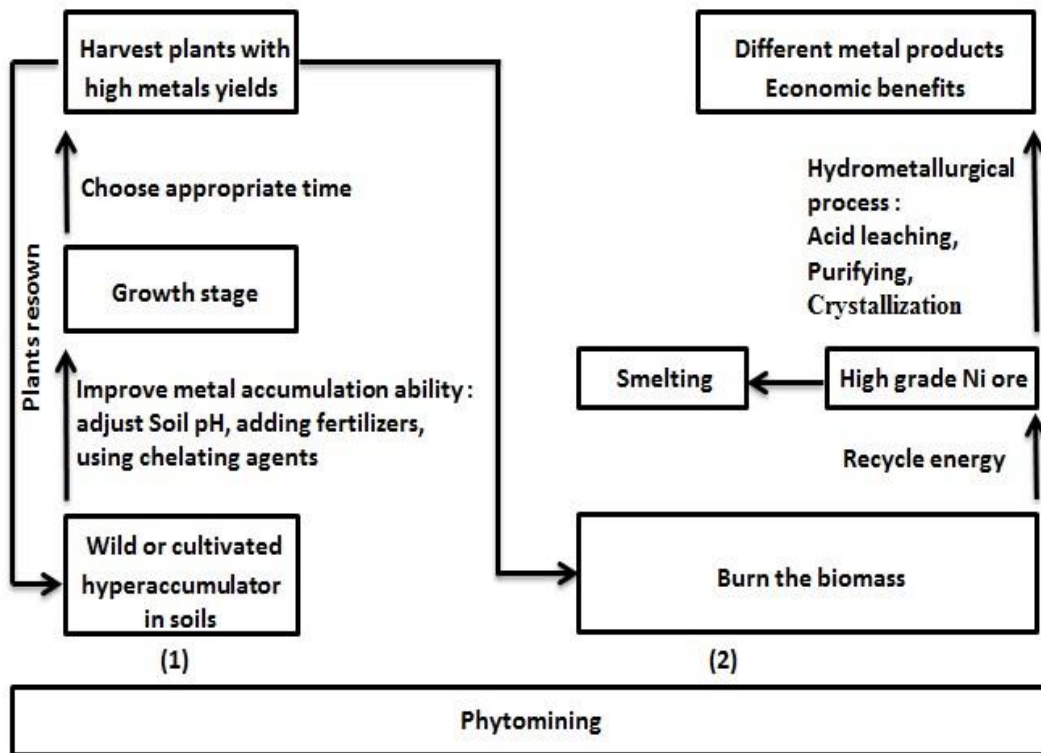


Figure 1. 4: Phytomining scheme from already experimented process

Phytomining has an interest if the economic value of the metal is high enough. It has been mainly applied to Ni (Barbaroux et al., 2012; Brooks et al., 2001; Chaney et al., 1998; Harris

et al., 2009; Mercier et al., 2012; Robinson et al., 1997b; Sheoran et al., 2009) and in a lesser extent to Au (Anderson et al., 1999; Harris et al., 2009; Sheoran et al., 2013; Wilson-Corral et al., 2012). In this review, we focus on Ni phytomining and present the agronomic process, Ni recycling and give some elements on feasibility.

4.1 Nickel in soils: what are the potential resources for phytomining?

This section gives an overview of the different situations in which Ni can be hyperaccumulated (present in natural or contaminated soils), the associated risks for human health, the regulations and the possible remediation technologies.

4.1.1 *Serpentine soils containing nickel*

Ultramafic soils are extensively present in many regions worldwide (Cuba, New Caledonia, Australia, Turkey, Brazil, China etc.). In Europe, they are mainly found in the Balkans (Fig.1.5: Albania, Greece, Bosnia and Serbia etc.) (Bani et al., 2010). Serpentine soil is a specific kind of ultramafic soils – deriving from serpentinite rocks – which are usually shallow, coarse, and have special physical and chemical properties (Rajakaruna and Bohm, 2002), *e.g.* low nutrient status, cation imbalances, moisture stress, soil instability, high surface temperature effects, and high metal concentrations. Iron (Fe), magnesium (Mg), and silicon (Si) as well as nickel (Ni), chromium (Cr) and cobalt (Co) often occur in large amounts but such for nitrogen (N), phosphorus (P), potassium (K) and boron (B) are usually deficient (Shallari et al., 1998). In serpentine soils, Ni and Cr concentrations are usually a few g kg⁻¹ and can reach more than 10 g kg⁻¹ (Alves et al., 2011; Brooks, 1987; Cheng et al., 2011; Proctor, 1999). In Albania, values of more than 3.0 g kg⁻¹ of Ni are often recorded on the major ultramafic soil types (Bani, 2009).

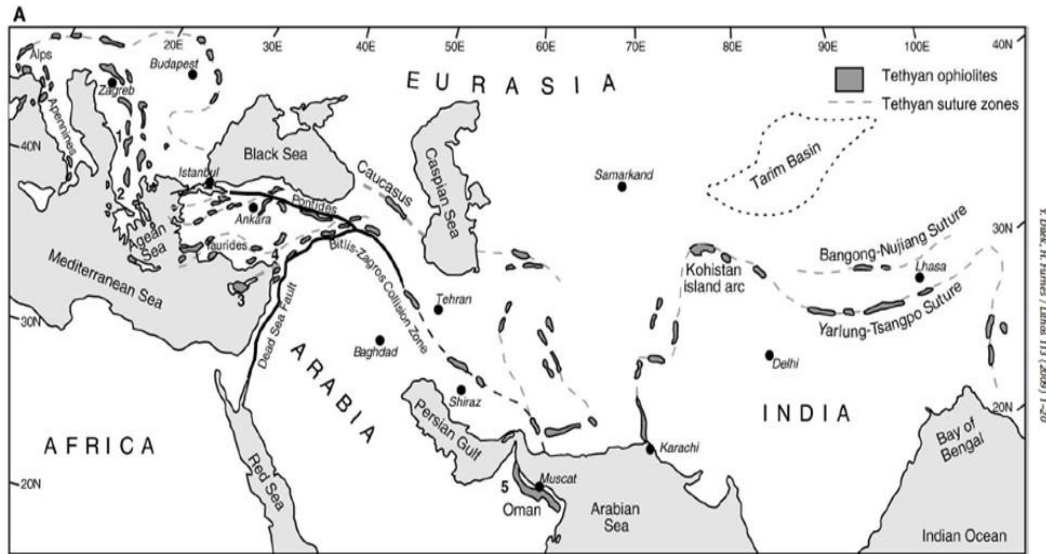


Figure 1. 5: Distribution of the serpentine sites studied in Eurasia (Dilek and Flower, 2003)

4.1.2 Main sources of nickel pollution in soils

Besides natural soils, other soils may contain Ni as a consequence of pollution. At first, Ni is present in soils at the vicinity of Ni mining areas. For example, in China, studies of ten polluted soil site samples in the renowned "Nickel City"-Jinchang showed: Ni concentration ranges from $139 \mu\text{g g}^{-1}$ to $1099 \mu\text{g g}^{-1}$ and the mean total content is $300 \mu\text{g g}^{-1}$. This outstrips the Ni limit of grade II (GB15168-1995) for soil environmental quality standards in China (Wang et al., 2009). Also, values of 0.2 to 5.2 % Ni have been recorded from 201 samples of saprolite and laterite in New Caledonia and the Ni concentration is $69\text{--}4764 \mu\text{g g}^{-1}$ (0.0 to 0.5 %) in Levisa Bay, Cuba ((González and Ramírez, 1995; Quiniou and Laperche, 2014).

More generally, Ni can be found in a bunch of contaminated soils from various industries since Ni is produced and used in many sectors (Ni, steel and alloys production, chemical industry, battery production, electrolytic surface treatment etc.) (Details in Section: 1.5).

The emissions of effluents and wastes in the Ni production process and the improper disposal of these wastes are the main source of Ni pollution (Wuana and Okieimen, 2011). For nickel, generally, a maximum permissible value of $100 \mu\text{g g}^{-1}$ in agricultural land soil is accepted in

Poland (Oleszek et al., 2003). In France there are no limit values for metal concentrations in soils although $50 \mu\text{g}\cdot\text{g}^{-1}$ implies restrictions in applying wastes in agricultural soils. Usually, concentrations are compared to the geochemical background. In Canada, the limit value is $50 \mu\text{g}\cdot\text{g}^{-1}$.

4.1.3 Ni speciation in soils

Nickel exists in soils in forms of inorganic crystalline minerals or precipitates, complexed or adsorbed on organic cation surfaces or on inorganic cation exchange surfaces, water soluble, free-ion or chelated metal complexes in soil solution (Cempel and Nikel, 2006).

Ultramafic soils undergo a particular pedogenesis, under moderate weathering conditions, cambisols are formed with significant amounts of Ni-enriched amorphous metal oxides and secondary clays which hold large pools of available Ni. High-exchange clays (e.g. smectites) may form and are another source of available Ni (Bani et al., 2014). In tropical climates Fe-oxides concentrate in soils with a higher degree of crystallinity and contain unavailable forms of Ni included in their crystal lattice. Nickel is lost at each dehydration step. Further dehydration of goethite into hematite in ferricrete decreases Ni content with strictly unavailable forms (Becquer et al., 2006; Massoura et al., 2006, Raous et al., 2013). In poor draining conditions (lowlands) in warm-temperate to tropical climates, Mg-rich clays may accumulate in soils and become the main bearing phases for available Ni (Bani et al., 2014; Raous et al., 2013).

4.1.4 Health risks due to nickel contaminated soils

Human-beings may be affected by heavy metal pollutants through the food chain: including direct ingestion or contacting with contaminated soil, soil-plant-human or soil-plant-animal-human, drinking of contaminated ground water, reduction in food quality, reduction in land

usability for agricultural production and land tenure problems (Anwar et al., 2009; Kierczak et al., 2008; Salt et al., 1995).

Nickel is an essential element for all the life in our biosphere. However, it should be only at very low levels, generally nickel daily intake from food range from 100 to 300 µg per person per day in most nations (Grandjean, 1984). In France, value estimated for the total population of nickel from wine consumption is based on 66 L per year per person, 30.6 µg per week, 4.4 µg per day (Cempel and Nickel, 2006). In Danish, on average, the value of nickel intake is estimated as 150 µg per person per day (Flyvholm et al., 1984). If not, it will be quite dangerous when we contact and intake excessive Ni and Ni salts. Especially, too much Ni absorbed from the respiratory tract into the body will hurt the lungs, cause pulmonary edema, acute lung inflammation, and various kinds of cancer, such as larynx cancer, lung cancer, prostate cancer and nose cancer (Andersen et al., 1996; Goldberg et al., 1994; Grasmuck and Scholz, 2005; Khodadoust et al., 2004; Lessard et al., 1978; Marwa et al., 2012; Weber et al., 2001).

Also some studies showed that excessive Ni release from mobile phones which we use every day, excessive Ni release from cheap jewellerys (hair clasps, earrings, necklaces, etc.) would cause Ni skin allergy, inflammation, eczema and atopic dermatitis (Cempel and Nickel, 2006; Jensen et al., 2011; Thyssen et al., 2011).

4.1.5 Nickel contaminated soil remediation

The conventional restoration methods for treating Ni contaminated soils are the same approaches as for treating the heavy metal polluted sites. They mainly include physical (rowing soil, soil replacement, changing the topsoil, deep plowing, etc.) and chemical methods (chemical fixation and chemical leaching) (Hamby, 1996; Wuana and Okieimen, 2011; Yao et al., 2012).

About current technologies, in 1983, the idea was put forward of using hyperaccumulating plants to reduce the content of heavy metals in soils (Chaney, 1983). This idea is promising since it is a clean, simple, cost-effective, non-environmentally disruptive, long-lasting, green and also aesthetically pleasing technology (Ali et al., 2013; Dobson et al., 1997; Macek et al., 2007; Marmiroli et al., 2006; McIntyre, 2003; Nwoko, 2010; Qui et al., 2008; Salt et al., 1995; Sheoran et al., 2009; Suresh and Ravishankar, 2004; Wei et al., 2004).

Moreover, in the particular case of phytoextraction, soil remediation could be combined with metal recycling to valorize the metals that have been transferred from the soil to the plant biomass (Bani, 2009; Chardot et al., 2005; Do Nascimento and Xing, 2006; Mahmood, 2010; Robinson et al., 2003; Van Nevel et al., 2007).

4.2 Agronomy of phytomining

Much work of using agronomic practice to improve crop productivity and also to enhance phytoextraction was done (Claus et al., 2007; Hdider et al., 2007; Hilton et al., 2009; Mahapatra et al., 2013; Reicks et al., 2009; Tang et al., 2012). As most Ni-phytomining work is carried out with hyperaccumulators from *Alyssum* genera (Barbaroux et al., 2012; Brooks et al., 2001; Robinson et al., 1997b), the agronomic process in this part mainly involves choosing the best hyperaccumulating plants, selective breeding of *A. murale* and enhancing its growing soil conditions to produce as much as possible biomass with high Ni yields.

About choosing the best Ni-hyperaccumulating plants, firstly, metal-hyperaccumulators are distinguished from related non-hyperaccumulating plants. There are three mainly hallmarks: a strong ability to take up heavy metals, a capacity to translocate the heavy metal from root to shoot rapidly, and a great capacity to detoxify heavy metals and keep them in plants (Rascio and Navari-Izzo, 2011). Moreover, these special plants should have good growth ability, grow well in the particular environment at large scale and have a lot of biomass.

Secondly, Ni-hyperaccumulators are selected from all these heavy metal hyperaccumulating plants. Approximate 400 plants have been identified as Ni-hyperaccumulators, such as *Streptanthus polygaloides* (on Ni rich soils at Red Hills near Chinese Camp, California, USA); *Sebertia* (=Pycnandra) *acuminata* (on serpentine soils, New Caledonia); *Berkheya coddii* (Asteraceae, on serpentine soils, Southern Africa); *Alyssum bertolonii* (*A. bertolonii*) (on serpentine soils, Tuscany and Albania); *Alyssum argenteum* (*A. argenteum*) Burt. (over ultramafic outcrops, the northwest of Italy); *Alyssum serpyllifolium* ssp. *lusitanicum* (on serpentine soil, in the Tras-os-Montes region of Northeast Portugal and in Galicia, Spain); *Centaurea thracica* and *Viola vourinensis* (serpentine flora in Greece); *Eichhornia crassipes* (water hyacinth, native of South America and also abundantly found in South Asia); *Hybanthus floribundus* (in the Eastern Goldfields area of Western Australia); *Stackhousia tryonii* (endemic to ultramafic soils of central Queensland, Australia); *A. murale* (native to Mediterranean serpentine soils) etc. (Bhatia et al., 2005a; Brooks et al., 1998; Hussain et al., 2010; Psaras and Constantinidis, 2009; Rascio and Navari-Izzo, 2011). And recently, nine new species of *Timonius* (Rubiaceae) were found from Kinabalu Park, Borneo for phytomining (Chen et al., 2014; Van der Ent, 2013).

A. murale is chosen for Ni-phytomining at the lab and pilot scales because it has more biomass production than most of reported Ni-hyperaccumulators from temperate regions and because it is easily grown. The average height of *A. murale* from individual growing system is 83-85 cm, the highest height is 98-99 cm, and some lowest is 70-73 cm. The dry weight of one single plant can reach 1.1 kg. Further, it is easily propagated from seeds, and it can be grown in dry, well-drained soils under full sunlight.

Then the climate (temperature and rainfall) and soil conditions (pH, clay, OM, Ca:Mg, fertility as available N, P, K, etc.) to grow the plants, planting density, the harvest methods and schedule are studied to improve Ni accumulation (Bani, 2009; Bani et al., 2014; Bani et

al., 2007; Bhargava et al., 2012; Broadhurst et al., 2004; Chaney et al., 2000; Topi et al., 2012).

Lots of research were carried out to do traditional crop breeding and use genetic engineering to improve high Ni accumulation from hyperaccumulators near Cave Junction, Oregon, USA (Angle et al., 2001; Cabello-Conejo et al., 2013; Chaney et al., 1998; Chaney et al., 2007; Chaney et al., 2008; Li et al., 2003a; Li et al., 2003b). In Chaney's group, the crop yield can reach at least 20 t ha⁻¹ (400 Kg Ni ha⁻¹) with appropriate fertilizers (N+K+P) and management (weeding, irrigation, etc.) (Chaney et al., 2007). Wild *A. murale* growing in Albania is in Fig. 1.6 (a) and (b) is field of flowering *A. murale* and *A. corsicum* in Oregon, USA.

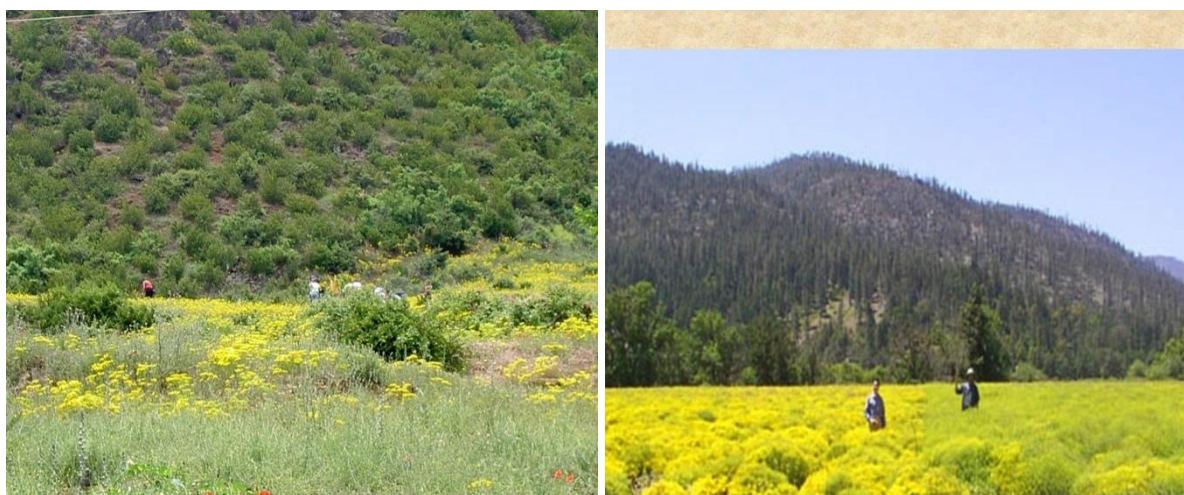


Figure 1. 6: Wild *A. murale* growing in Albania (a) and field of flowering *A. murale* and *A. corsicum* in USA (b).

Photographs by G. Echevarria (a) and R. Chaney (b)

Also cloning special genes needed for hyperaccumulators to obtain transgenic plants to improve high-biomass yields was reported (Cherian and Oliveira, 2005; Clemens et al., 2002). At last, for agronomy in Ni-phytomining, there is still much work to investigate, such as continuing to improve the ability for plant breeding; increase of the biomass amount; methods to enhance Ni accumulations; appropriate management, and choose the best Ni-hyperaccumulating plants etc.

4.3 Recovery of Ni from hyperaccumulator plants

4.3.1 Producing pure Ni from hyperaccumulators

From 1997 to recent years, Ni recycling from hyperaccumulators has been carried out to produce different Ni products. After biomass incineration, pure Ni was generated by smelter process from the high grade Ni ore (Brooks et al., 2001; Chaney et al., 1998; Li et al., 2003a; Li et al., 2003b). In 2007, Ni was sold at the price of \$ 40 per kg and then a value of \$16 000 ha⁻¹ could be planned per annual harvest (Chaney et al., 2007). Chemical leaching to extract Ni from the seeds of *A. murale* was studied in detail. 0.5 M H₂SO₄ solution to leach crushed *A. murale* flowers and seeds at 90 °C with a 15 % solid, for 120 min. Results displayed that 72.4 ± 6.4 % of the whole Ni was recovered from *A. murale* (Barbaroux et al., 2011; Barbaroux et al., 2009). In addition, pure Ni can be successfully extracted electrolytically from the original leachate of sulfuric acid with ashes from Ni-hyperaccumulating plants (Barbaroux et al., 2011; Tennakone et al., 2007). A conducting tin oxide glass cathode and a carbon counter electrode are used to extract Ni in his process (Fig.1.7).

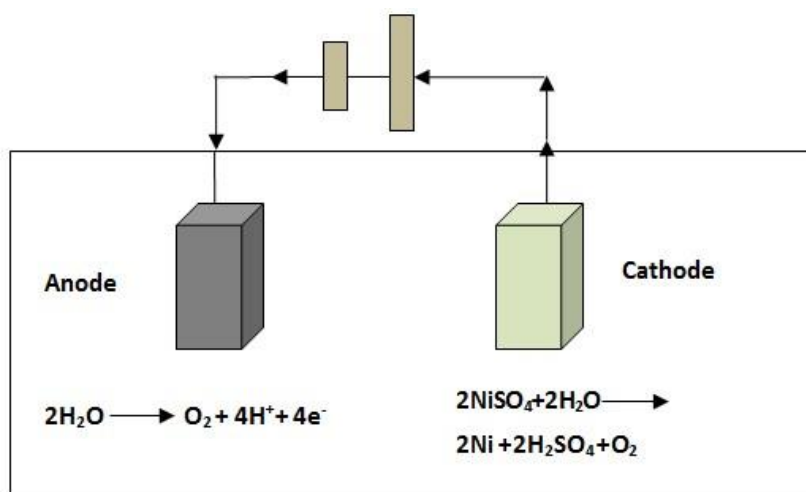


Figure 1. 7: The scheme of electroplating of Ni

However, this pathway was abandoned because the Ni recovery rate is low and Ni concentration in ashes is tenfold to twentyfold higher than that in plants.

The idea of recycling Ni from *A. murale* ashes is supported by many authors (Barbaroux et al., 2012; Boominathan et al., 2004; Keller et al., 2005; Machado et al., 2010; Sas-Nowosielska et al., 2004). Moreover, many nickel products sold in the market are generated from pure nickel reacted with acids to get the nickel leachates. Then if nickel leachates could be obtained directly from *A. murale* ash leaching, it would be more interesting.

4.3.2 Producing Lewis acid catalysts from hyperaccumulators

Moreover, metal ions as Ni^{2+} derived from hyperaccumulators (*P. douarrei* and *G. pruinosa*) of New Caledonia were used as Lewis acid catalysts in organic chemistry (Losfeld et al., 2012a; Losfeld et al., 2012b). The reaction (Fig.1.8) was extensively investigated and the catalysts extracted from *P. douarrei* and *G. pruinosa* have interesting properties, such as thermal stability, high activity after recycling etc.

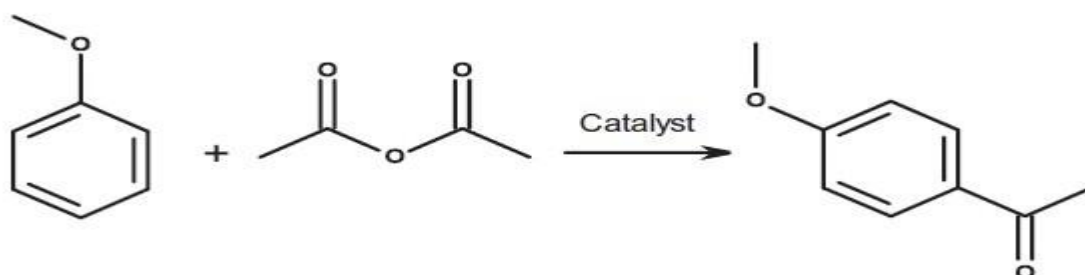


Figure 1. 8: Organic reaction studied with the catalyst derived from Ni-hyperaccumulator plants

4.3.3 Producing nickel double salt from hyperaccumulators

The hydrometallurgical process of producing ammonium nickel sulfate hexahydrate (ANSH) crystals from *A. murale* ashes has been designed and patented (Barbaroux, 2010; Barbaroux et al., 2012; Mercier et al., 2012). This process includes three main steps: 1) biomass incineration, ash washing and acid leaching, 2) ANSH crystallization and 3) ANSH purification, as displayed by the simplified scheme (Fig. 1.9).

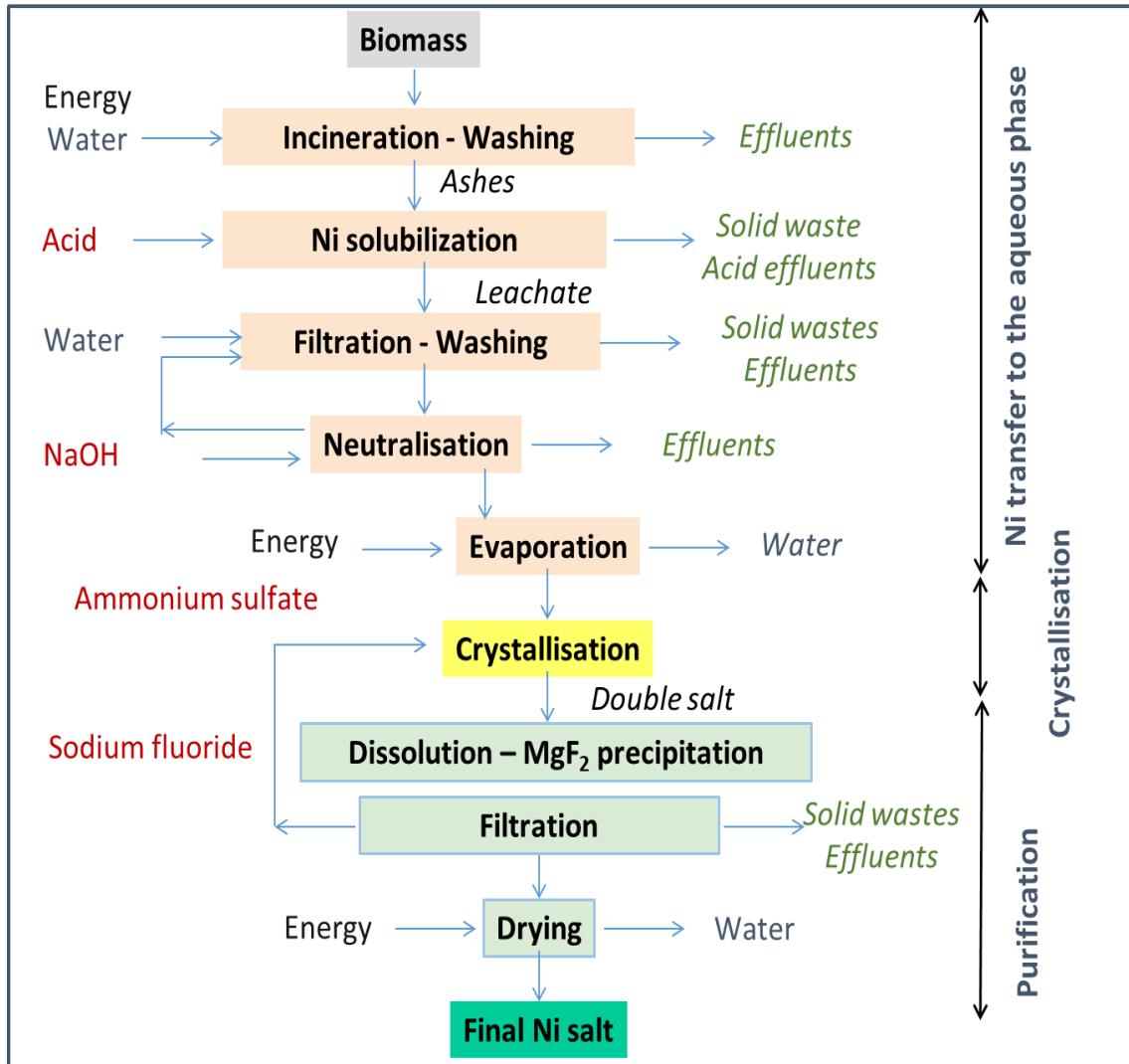


Figure 1. 9: Simplified scheme to produce ANSH salt from *A.murale* biomass (Simonnot, private communication)

The overall process with the accurate conditions of reactant and energy consumption as well as effluent and waste production was studied by R. Barbaroux (Fig. 1.10).

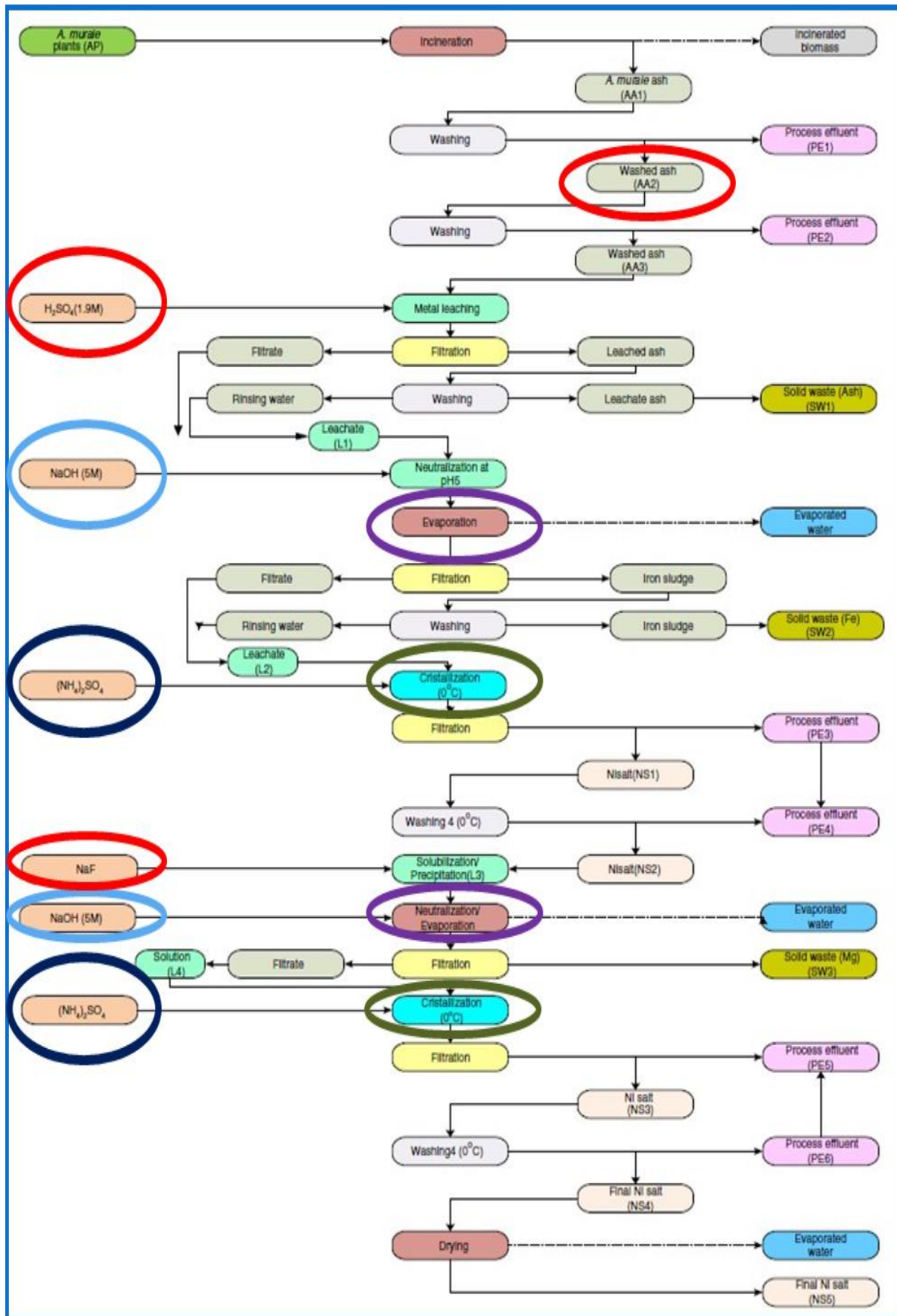


Figure 1. 10: Diagram of producing nickel ammonium sulfate from ash of *A. murale* (Barbaroux et al., 2012)

This process has been designed at the bench scale; it enables us to produce the salt with a purity of 88.8 %. Moreover, an economic study, based on assumptions, has shown that this process makes profit because of the economic value of the salt (Barbaroux, 2010).

However, improvements could be done: the process needs to be investigated in details to search possible improvements in terms of 1) reduction in the consumption of mass and energy, 2) decrease in waste and effluent rejection, and 3) increasing salt purity in the objective of upscaling to the pilot and industrial scales.

4.3.4 Hydrothermal process of extracting nickel from the Ni-hyperaccumulators

The feasibility of extracting metals (Ni, Zn, Pb and Cu) directly from hyperaccumulators by hydrothermal processes was investigated (le Clercq et al., 2001; Yang et al., 2009a; Yang et al., 2009b). Le Clercq et al. (2001) studied the experiments of Ni-hyperaccumulator (*Berkheya coddii*) with hot compressed water between 200 and 375 °C and 25 MPa using a molten salt bath. This method is very simple since it uses only deionized water to boil the plants. Yang et al. (2009) demonstrated the feasibility to recover Zn, Pb and Cu from a hyperaccumulator (*Sedum alfredii Hance*) using an autoclave at 370 °C and 22.1 MPa.

Nevertheless, envisioned ideas could be tried: 1) hyperaccumulator (*A. murale*) was used by this hydrothermal method, 2) the heating way of boiling the plants at 100 °C was tried to reduce the economic cost, and 3) various Ni-products were produced by this approach.

4.4 Feasibility of nickel phytomining

Some studies have shown that Ni-phytomining was on the verge of becoming a business (Anderson et al., 1999; Harris et al., 2009; Li et al., 2003a; Sheoran et al., 2009).

For instance, *A. murale* Waldst & Kit and *Alyssum corsicum* Duby grown on serpentine soils with minimal addition of fertilizers could accumulate more than 20 g Ni kg⁻¹ dry biomass with no

evidence of toxicity. Then, the yields reached 400 kg Ni ha⁻¹ (Li et al., 2003a), which generates a high economic profit (Chaney et al., 2007; Chaney et al., 2005; Li et al., 2003a).

An economic study was done after Barbaroux thesis (Barbaroux, 2010) with the following assumptions: production of 1 t ha⁻¹ of biomass on ultramafic soil, with a field of 21 600 ha (15 km x 15 km), and a treatment of 120 t d⁻¹, 180 d yr⁻¹. In these conditions, and with Canadian investment and operating costs (2012), the benefit would be higher than 8 million CAN \$ per year, that is 6 million €.

One must be aware that Ni prices may vary strongly (Fig.1.11), but Ni products could be stored until a higher price.

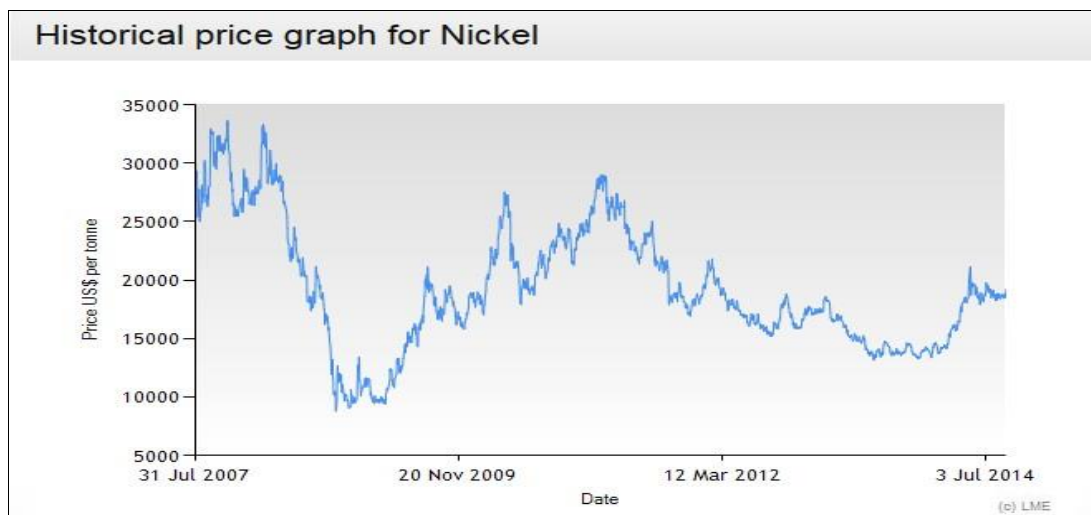


Figure 1. 11: Nickel market price value at the London Metal Exchange from 2007 to 2014

<http://www.lme.com/en-gb/metals/non-ferrous/nickel/#tab2>

These elements clearly show that Ni phytomining is promising and that business is on the verge of emerging.

5 Hypothesis and objectives

This bibliographic survey enabled us to point out the following hypothesis and objectives that are the guidelines of the work.

5.1 Hypothesis

Hyp.1 Several Ni hyperaccumulator plants can be used for Ni phytomining.

The previous work (Barbaroux, 2010; Barbaroux et al., 2011; Barbaroux et al., 2009; Barbaroux et al., 2012) was done with the hyperaccumulator plant *A. murale* grown in Albania. Other plants could be used for the same target.

Hyp.2 The production process of ANSH from ashes of A. murale can be improved.

It is possible to optimize the ANSH production process (Fig.1.11) to obtain ANSH salt at higher purity from *A. murale* ashes while saving mass and energy. This step is of importance before upscaling to the pilot scale.

Hyp.3 Other Ni products can be prepared and new methods can be used to extract Ni from hyperaccumulator biomass.

Other Ni salt can be produced from hyperaccumulator ash and also as shown by previous studies (le Clercq et al., 2001; Yang et al., 2009a; Yang et al., 2009b), other methods can be proposed to extract metals from the hyperaccumulators.

5.2 Objectives

According to the previous hypothesis, the objectives of this research are given thereafter. They will be the subjects of the three following chapters.

1. Determine to which extent other hyperaccumulator plants from the Balkan could be used, which species could be more interesting and determine the better conditions to

burn the biomass without Ni loss to obtain a Ni rich bio-ore for metal processing.

2. Investigate the ANSH production process step by step to analyze each operation and propose possible improvements. Increase salt purity and characterize it thoroughly. Study every step of the existed process (Fig.1.10) in detail and try to get high Ni purity
3. Elaborate other scenarios for the production of Ni salts from the ashes and search to extract Ni from the biomass without burning the plants.

Chapter 2: Characterizations of plants and ashes from Ni-hyperaccumulators

Science is built up of facts, as a house is built of stones;
but an accumulation of facts is no more a science
than a heap of stones is a house.

Henri Poincaré

1 Introduction

The selection of the best hyperaccumulating plants is one of the key issues of phytomining. In this chapter, we studied 15 hyperaccumulators of 5 taxa of the *Brassicacea* family collected in the Balkans (Albania and Greece) in the past years.

The first objective was to determine which plant and/or which organ was the best candidate for phytomining. We measured the distribution and the composition of the organs of all the plants to determine which plant/organ had the highest Ni content and also to figure out which other elements were present that could be a drawback for further Ni product synthesis.

Then, given that in the ANSH process (Barbaroux et al., 2012; Mercier et al., 2012) as in other ones (Barbaroux et al., 2012; Boominathan et al., 2004; Keller et al., 2005; Losfeld et al., 2012a; Machado et al., 2010; Sas-Nowosielska et al., 2004), plants are burnt to produce ashes, the second objective was to study and optimize the incineration step. Experiments were run in different conditions of temperature and duration to determine the optimal conditions, particularly the temperature range required to avoid Ni loss.

This part of the work was published in International Journal of Phytoremediation (Zhang et al., 2014).

2 Materials and methods

2.1 Sampling and preparation of the plants

Samples of 15 hyperaccumulators from 5 taxa were collected in Greece and Albania at different locations with multiple origins (Table 2.1) (Bani, 2009; Bani et al., 2009). Samples were collected on several occasions from 2008 to 2012 between May and July, according to plant phenological stage. It has been previously proved that flowering stage was the best period for harvest and after flowering stage, all the element concentrations decrease in shoots (Bani, 2009).

Table 2. 1: List of Ni-hyperaccumulating taxa collected, locations and sampling period

Name	Species	Country	Location	GPS coordinates	Soil type	Date and Phenological stage
<i>A. markgrafii</i>	<i>A. markgrafii</i> O.E. Schulz ex Markgraf	Albania	Gjegjan	41°55'47''N;20°00'09''E	Cambisol	May 2011, flowerbuds
<i>A. bertolonii</i>	<i>A. bertolonii</i> subsp. <i>Scutarinum</i> Nyár	Greece	QafëShtamë	41°52'17''N;19°89'53''E	Vertisol	May 2011, flowerbuds
<i>A. murale1</i>	<i>A. murale</i> Waldst. & Kit	Albania	Pojškë	40°59'55''N;20°38'55''E	Vertisol	May 2011, flowering
<i>A. murale2</i>	<i>A. murale</i> Waldst. & Kit	Albania	Prrenjas	41°04'13''N;20°33'53''E	Vertisol	May 2011, flowering
<i>A. murale3</i>	<i>A. murale</i> Waldst. & Kit	Greece	KataraPass	39°47'46''N; 21°13'44''E	Cambisol	July 2011, flowering-seeding
<i>A. murale4</i>	<i>A. murale</i> Waldst. & Kit	Albania	Pojškë	40°59'55"N;20°38'55"E	Vertisol	May 2012, flowering
<i>A. murale5</i>	<i>A. murale</i> Waldst. & Kit	Albania	Pojškë	40°59'55"N;20°38'55"E	Vertisol	May 2011, flowering
<i>A. murale6</i>	<i>A. murale</i> Waldst. & Kit	Albania	Pojškë	40°59'55"N;20°38'55"E	Vertisol	June 2008, flowering
<i>A. murale7</i>	<i>A. murale</i> Waldst. & Kit	Albania	Gjegjan	41°55'47''N;20°00'09''E	Cambisol	May 2011, flowerbuds
<i>A. murale8</i>	<i>A. murale</i> Waldst. & Kit	Albania	Pojške	40°59'55"N,20°38'55"E	cambisol	June 2012 flowering-
<i>Leptoplax1</i>	<i>Leptoplax emarginata</i> (Boiss.) O.E. Schulz	Greece	KataraPass	39°47'46''N; 21°13'44''E	Cambisol	July 2011, vegetative stage
<i>Leptoplax 2</i>	<i>Leptoplax emarginata</i> (Boiss.) O.E. Schulz	Greece	KataraPass	39°47'46''N; 21°13'44''E	Cambisol	July 2011, seeding
<i>Bornmuellera1</i>	<i>B.tymphaea</i> (Hausskn.)Hausskn.	Greece	KataraPass	39°47'46''N; 21°13'44''E	Cambisol	July 2011, vegetative stage
<i>Bornmuellera2</i>	<i>B.tymphaea</i> (Hausskn.)Hausskn.	Greece	KataraPass	39°47'46''N; 21°13'44''E	Cambisol	July 2011, seeding
<i>Bornmuellera3</i>	<i>B.tymphaea</i> (Hausskn.)Hausskn.	Greece	Vouvoussa	39°52'07''N;21°02'59''E	Cambisol	July 2011, seeding

Plants were collected manually, air dried, and kept at ambient temperature (20 ± 2 °C) until experiments. Flowers, seeds and leaves were separated from the stems and dried in thermostat oven (Memmert Edelstahl) at 70 °C for 24 h. All plant parts were weighed; crushed and finely ground using a model Prep'line 850 grinder. Elemental concentrations in the dried flowers, seeds, leaves and stems of all these plants were determined.

2.2 Sampling of the ashes

Incineration trials were firstly run with the most common Ni-hyperaccumulator: *A. murale*. About 20 g of finely ground dried plants were deposited on three stainless steel plates (10 cm × 15 cm) that were placed in furnace (model 1400 furnace, Barnstead Thermolyne). Combustion was done at two “extreme” temperatures: a low value, 550 °C and a high value 1,100 °C. The first one was high enough to burn organic carbon and had been already used in previous contributions (Barbaroux et al., 2012; Mercier et al., 2012). The second one was close to one used in other studies (1,200 °C) (Barbaroux et al., 2012; Boominathan et al., 2004; Keller et al., 2005; Machado et al., 2010; Sas-Nowosielska et al., 2004). It is the maximum possible temperature range, knowing that metals may be volatilized at higher temperatures. Moreover, at 550 °C, we tested incineration durations from 1 to 6 h to choose the best combustion time cost-effective and hyperaccumulators were completely combusted). Thereafter, the temperature and duration of furnace treatment were set constant: 550 °C - 3 h. The ashes were removed from the furnace immediately after the combustion of the plants was complete. They were weighed and maintained at room temperature before analysis. At last, the other 16 different Ni-hyperaccumulators (were incinerated in the same conditions (550 °C - 3 h). Another experiment consisted in burning the stems, leaves and flowers separately to compare the Ni concentration, and elemental concentrations in the ashes of these organs.

From October 2012, a new larger furnace (Barnstead Thermolyne 1400 Furnace) was bought. With this new device, samples of 300-400 g of plants can be completely combusted using the following procedure: During 10 min the temperature increases from 20 °C (room temperature) to 550 °C and then, the biomass is heated during 2 h at 550 °C.

2.3 Mineralization and ICP-AES analysis of biomass and ashes

Aliquots of fine powdered plants samples (0.1- 0.2 g) or ashes samples were deposited in PTFE vessels with 8.5 mL HNO₃ ($\geq 65\%$) and 1.5 mL H₂O₂ (50 %) before microwave digestion (Milestone StartD Microwave Digestion System). Mineralization was achieved as follows: increase of temperature during 20 min from 20 to 200 °C and stabilization of temperature at 200 °C for 40 min. All the samples were transferred into tubes of 50 mL and volume was completed with deionized water after temperature decreased to the room temperature. Solution samples were filtered with a filter syringe (Cellulose nitrate, 25 mm disks, pore size 0.45 μ m) and then 10 mL samples of the filtrate were prepared for the ICP-AES analysis.

Metal concentrations were measured in triplicate by plasma emission spectroscopy by ICP-AES (Thermo iCAP 6000 Series ICP scientific Emission Spectrometer). The peristaltic pump, nebulizer, spray chamber, torch configuration chamber and gas of Argon were used. A wide wavelength range of 166 to 847 nm could be detected. The iTEVA (iCAP 6000 series) software with powerful tools was used. A multi-element solution (SCP sciences: Trace elements (As, Co, Cr, Cd, Pd, Pt, Si, Cu, Mn, Zn and Ni) of 1000 ppm and major elements (Ca, K, Mg and Na) of 1000 ppm with 5 % HNO₃) of 0.01, 0.1, 0.5, 1, and 10 ppm for the trace elements, 0.1, 1, 5, 10 and 100 ppm for the major elements and maximum of Ni 100 ppm were used for standardization.

2.4 X-ray diffraction analysis

Ash composition was determined by X-ray diffraction analysis with a Rigaku – Miniflex II diffractometer, with a scan range from 20 to 90 ° at a scanning speed of 1.0 ° min⁻¹ (LRGP).

We also used a D8 Bruker diffractometer equipped with a (theta, 2theta) goniometer using Co K α 1 radiation (wavelength: 1.7902 Å) (LIEC laboratory). X-ray diffractograms were collected on powder samples in ambient conditions, within the 2theta range [3, 65°], with 0.036° step and 3 s collecting time in LIEC (Laboratoire Interdisciplinaire des Environnements Continentaux, 15 Avenue du Charmois, 54500 Vandoeuvre-lès-Nancy).

2.5 Particle size distribution, SEM and EDX analysis

Particle size distribution of raw ashes, washed ashes and ashes after leaching were analyzed, as well as Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) with the help of JEOL JSM-6490LV instrument (LRGP).

3 Results and discussion

In the first part (section 3.1 to 3.4), we present the results and discussion concerning the first series. We compared the biomass distribution of the 15 taxa, studied the composition of the different plant organs and investigated the conditions of incineration and the composition of the ashes. The last section is devoted to the comparison of *A. murale* populations grown on the same site at the same time but in different agronomic conditions, one was taken from a group of plants as the other one has grown individually.

3.1 Mass distribution of biomass among stems, leaves, flowers and seeds of the different taxa

A series of 15 taxa of three Ni-hyperaccumulators genera of the *Brassicaceae* family in the Balkans, including 3 *Alyssum* species (*A. murale*; *A. markgrafii*; *A. bertolonii* subsp. *scutarinum*), *L. emarginata* (Boiss.), and *B. tymphaea* (Hauskn.) were analyzed (Table 2.1).

Biomass distribution of flowers, seeds, leaves and stems among species and ecotypes at harvest were plotted (Fig.2.1). For all the plants but *Bornmuellera* 2, stems represented the largest proportion of the biomass weight (48-78 %), followed by leaves (12-42 %) whilst flowers and seeds accounted for less than 25%. For *Bornmuellera* 2, we observed more leaves (64 %) than stems (36 %). In general, *Alyssum* and *Bornmuellera* species showed a higher leaf proportion than *L. emarginata* (Boiss.) which can be explained by the greater size of stems and plants for the latter species. Seeds also accounted for a high proportion of the biomass (37 %) in the case of *Leptoplax emarginata* (Boiss.) at seeding stage, leaves had probably fallen to the ground and had not been totally collected.

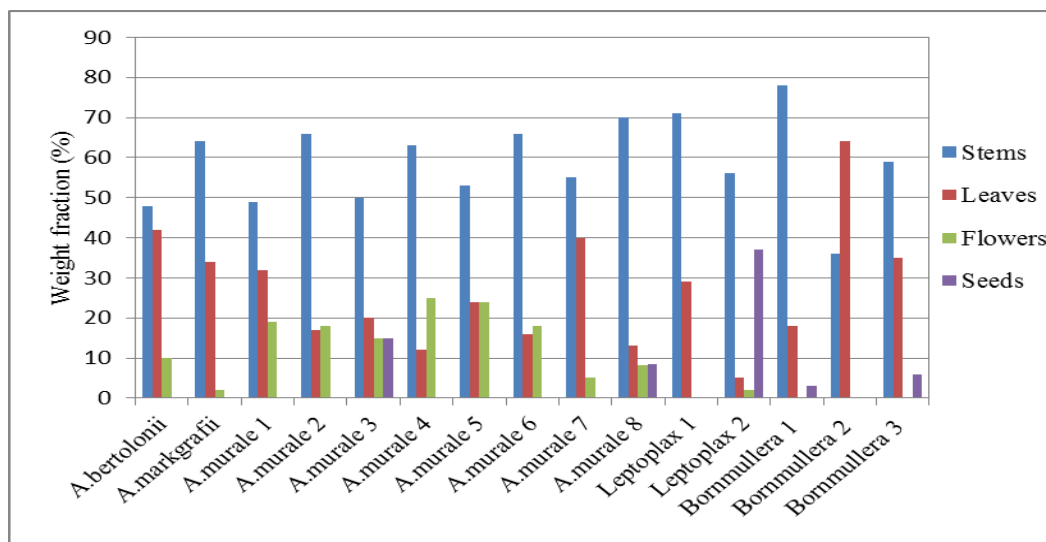


Figure 2. 1: Weight fractions of the leaves, stems, flowers and seeds of the 14 Ni-hyperaccumulators

3.2 Elemental concentration of the hyperaccumulators

Nickel

The concentration of major elements in the aerial tissues of the plants of *Alyssum* species, *L. emarginata* and *B. tymphaea* showed a high variability (Tables 2.2 & 2.3). However, some general trends could be noted. For all the plants, the highest Ni concentrations were recorded in leaves: they were three to four times higher than in stems. They reached 0.8-1.6 % in *Alyssum* species, 1.4-2.2 % in *B. tymphaea* and 3.2-3.4 % in *L. emarginata*. Ni concentrations in *L. emarginata* leaves were three to four times higher than in *Alyssum* leaves, and nearly twice as high as in *B. tymphaea* leaves.

In the stems, Ni concentrations were almost the same in the nine *Alyssum* hyperaccumulators ranging from 0.2 to 0.5 %; they were slightly higher in *Bornmuellera* plants (from 0.5 to 0.7 %) and much higher in *Leptoplax* plants (0.8 to 1.1 %). Flowers and seeds showed no specific trends among species and individuals: Ni concentrations ranged from 0.6 to 1.5 % for flowers and from 0.9 to 1.2 % for seeds.

It clearly appeared that whatever the species, leaves and reproductive organs (flowers, seeds) were the main storage locations for Ni. Stems were probably transport organs and only stored Ni in their epidermis. These results are in agreement with the literature, which mentioned that Ni is highly concentrated in epidermal cell vacuoles, and that leaves have the greatest Ni concentrations among all organs of Ni-hyperaccumulators (Bani, 2009; Broadhurst et al., 2004). It was also shown previously that seeds may contain high concentrations of Ni depending on the harvesting stage (Barbaroux et al., 2011; Barbaroux et al., 2009).

Table 2. 2: Concentrations of the major elements (Ca, Fe, K, Mg) and Ni in the leaves, stems, flowers and seeds of *Alyssum* hyperaccumulator species (mg (g dry plant)⁻¹)

	A.markgrafii			A.bertolonii			A. murale 1			A. murale 2			A. murale 3			A. murale 4			A. murale 5			A. murale 6			A. murale 7		
	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower
Ca	54.0 ±4.7	6.2 ±0.5	46.9 ±1.7	35.0 ±0.8	10.4 ±1.2	31.0 ±3.5	39.3 ±5.4	2.8 ±0.2	29.1 ±1.8	35.1 ±0.3	5.6 ±3.9	31.3 ±3.5	35.8 ±1.2	8.5 ±0.8	21.0 ±0.4	36.5 ±3.5	1.1 ±0.7	14.3 ±0.7	19.4 ±1.1	5.8 ±0.9	21.2 ±1.2	39.8 ±1.7	1.97 ±3.3	29.0 ±6.1	30.2 ±0.6	6.4 ±1.9	33.6 ±0.6
Fe	0.6 ±0.1	0.1 ±0.0	0.2 ±0.0	1.4 ±0.2	0.6 ±0.0	0.7 ±0.0	0.7 ±0.0	0.2 ±0.0	0.3 ±0.0	0.5 ±0.0	0.0 ±0.0	0.2 ±0.0	0.3 ±0.0	0.1 ±0.0	0.1 ±0.0	0.7 ±0.1	0.1 ±0.0	0.1 ±0.0	1.1 ±0.8	0.1 ±0.0	0.1 ±0.0	0.9 ±0.4	0.4 ±0.1	0.4 ±0.2	0.5 ±0.1	0.1 ±0.0	0.2 ±0.0
K	14.9 ±0.6	11.9 ±0.4	11.9 ±0.5	10.3 ±0.1	13.9 ±1.6	12.0 ±0.1	14.3 ±1.7	13.1 ±0.1	16.5 ±0.7	14.9 ±0.4	12.3 ±1.3	16.4 ±1.8	17.1 ±0.9	16.0 ±1.2	19.4 ±1.1	18.1 ±1.7	11.0 ±1.1	18.2 ±1.0	13.9 ±0.4	11.1 ±2.7	16.8 ±0.9	9.5 ±0.5	14.6 ±2.1	17.7 ±1.6	18.2 ±0.3	16.0 ±0.9	13.0 ±0.4
Mg	3.5 ±0.2	1.2 ±0.0	3.4 ±0.2	10.4 ±0.7	4.4 ±0.6	8.3 ±0.4	7.1 ±0.8	2.5 ±0.4	7.4 ±0.4	5.2 ±0.4	2.1 ±0.5	5.3 ±0.6	5.4 ±0.3	1.7 ±0.1	5.7 ±0.6	5.7 ±0.5	1.3 ±0.1	3.1 ±0.2	7.4 ±0.4	2.4 ±0.1	5.5 ±0.4	6.4 ±0.5	2.9 ±0.6	5.4 ±0.6	5.9 ±0.1	1.6 ±0.0	7.0 ±0.2
Ni	11.5 ±0.5	3.0 ±0.3	10.1 ±0.5	8.7 ±0.3	4.3 ±0.5	6.3 ±0.5	14.5 ±1.5	5.0 ±0.7	9.5 ±0.4	13.8 ±0.2	3.7 ±1.0	9.2 ±0.8	11.8 ±0.6	3.3 ±0.3	11.1 ±0.8	15.5 ±1.0	4.0 ±0.2	10.2 ±0.6	14.8 ±0.1	5.1 ±1.2	11.4 ±0.4	12.2 ±0.4	5.0 ±1.6	9.6 ±0.5	8.2 ±0.2	2.3 ±0.0	7.6 ±0.2

Table 2. 3: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in *Leptoplax* and *Bornmuellera* hyperaccumulator species (mg (g_{dry plant})⁻¹)

	<i>Leptoplax1</i>		<i>Leptoplax2</i>				<i>Bornmuellera1</i>		<i>Bornmuellera2</i>			<i>Bornmuellera3</i>		
	leaf	stem	leaf	stem	flower	seed	leaf	stem	leaf	stem	seed	leaf	stem	seed
Ca	23.6 ±5.2	0.3 ±0.2	18.5 ±0.1	0.6 ±0.4	7.2 ±0.0	6.4 ±0.0	13.3 ±2.4	0.8 ±0.8	28.6 ±0.0	7.8 ±0.0	7.1 ±0.0	24.2 ±0.0	6.0 ±0.1	6.7 ±0.0
Fe	0.2 ±0.0	0.4 ±0.1	0.2 ±0.5	0.1 ±0.0	0.1 ±1.1	0.1 ±0.8	0.2 ±0.0	0.1 ±0.0	0.2 ±1.8	0.6 ±1.3	0.1 ±0.3	0.2 ±0.8	0.5 ±0.5	0.2 ±1.3
K	21.4 ±2.8	18.8 ±1.6	12.5 ±1.1	16.7 ±1.2	16.5 ±3.8	10.2 ±0.5	19.3 ±1.1	26.3 ±0.9	16.1 ±1.3	10.3 ±1.3	11.7 ±0.0	14.7 ±1.2	9.4 ±0.7	13.3 ±0.8
Mg	6.2 ±0.6	3.7 ±0.5	12.0 ±0.6	3.9 ±0.4	5.6 ±1.0	4.3 ±0.5	5.0 ±0.2	2.4 ±0.1	5.2 ±0.1	2.0 ±0.1	2.7 ±0.2	7.9 ±0.3	2.9 ±0.4	3.0 ±0.6
Ni	31.9 ±3.1	10.8 ±2.1	34.3 ±0.7	7.7 ±0.9	14.8 ±2.6	10.1 ±1.1	20.5 ±2.1	7.1 ±0.6	13.9 ±0.5	4.5 ±0.5	9.4 ±0.4	21.8 ±1.1	4.7 ±0.4	11.6 ±0.7

Calcium

Calcium is in general highly accumulated by all plants. As for Ni it is mainly stored in leaves and reproductive parts (flowers and seeds) while stems contain much lower concentrations. The concentrations in leaves were higher for *Alyssum* species (1.9 to 5.4 %), followed by *Bornmuellera* and *Leptoplax* (1.3 to 2.9 %). The highest concentration of Ca was recorded in the leaves of *A. markgrafii*, ($54.0 \pm 4.7 \text{ mg g}^{-1}_{\text{DM}}$). Stems of *L. emarginata* exhibited particularly low Ca contents with respect to the other species. This result was assigned to the lack of epidermis of the stems in this particular species. It has been noted previously that Ca and Ni may be antagonists in hyperaccumulator shoots (Bani, 2009; Bani et al., 2009). The higher Ca contents in *Alyssum* leaves may explain the fact that Ni concentrations were lower for this species than in leaves of *L. emarginata* and *B. tymphaea* which both had Ca concentrations lower than in *A. murale* leaves. The quantity of Ca taken up by plants was high considering that these plants were grown on Ca-depleted (ultramafic) soils. As a consequence, phytomining of Ni with such plants will probably require Ca-rich amendments over the long term.

Magnesium, potassium and other elements

Although it is the most abundant cation in the cation exchange capacity (CEC) of ultramafic soils, Mg was not really accumulated by these species in comparison to Ni and Ca. The highest concentration of Mg recorded in the leaves of *Alyssum* (*A. bertolonii*) was $10.4 \pm 0.7 \text{ mg g}^{-1}_{\text{DM}}$ (2-3 times higher than that in stems). Otherwise, Mg concentrations in leaves were usually lower than 0.74% for *Alyssum* species. For *L. emarginata* and *B. tymphaea*, they reached the same values in leaves, with one exception (*L. emarginata* 2: $12.0 \pm 0.6 \text{ mg g}^{-1}_{\text{DM}}$). The same translocation rules seemed to apply for Mg as for Ni as stems were roughly three times less concentrated than leaves or flowers.

Regarding K and Fe, no significant concentration difference was found between leaves, flowers, stems and seeds (Tables 2.2 & 2.3). Potassium is a key nutrient in the plants whose most important role is osmoregulation. Potassium is also low in the CEC of ultramafic soils and yet fairly accumulated by all the hyperaccumulator plants. It was the dominant cation found in stems (up to 2.6 % in *B. tymphaea* 1). It was also accumulated in leaves (0.9 to 2.1 %) and reproductive organs. As a consequence, potassium fertilization is also an issue for long term phytomining.

Iron is also essential for many plant functions since it is a constituent of certain enzymes and proteins and plays a role in energy transfer within the plant. No trend in Fe accumulation and distribution was recorded among the studied species.

Regarding trace elements (Tables 2.4 & 2.5), their concentrations were relatively lower in the plants but could not be ignored. We found that there was much more Co, Cr, Cu, Mn, Pb and Zn in leaves than in flowers and stems for all these plants including *Alyssum* species, *L. emarginata* and *B. tymphaea*. While, the concentration of Se in stems was higher than in leaves and flowers; the concentration of Mo in the flowers and leaves was much higher than in stems.

Table 2. 4: Concentration of the minor elements in the leaves, stems, flowers and seeds of *Alyssum* hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)

	<i>A.markgrafii</i>			<i>A.bertolonii</i>			<i>A. murale 1</i>			<i>A. murale 2</i>			<i>A. murale 3</i>			<i>A. murale 4</i>			<i>A. murale 5</i>			<i>A. murale 6</i>			<i>A. murale 7</i>		
	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower
Co	9.3 ±0.4	0.7 ±0.0	3.2 ±0.0	22.8 ±1.7	5.2 ±0.4	7.4 ±0.4	20 ±0.2	2.2 ±0.3	7.8 ±0.5	53.2 ±2.8	3.6 ±1.0	11.2 ±0.1	13.9 ±0.7	1.1 ±0.1	6.5 ±1.2	32.1 ±0.5	2.1 ±0.1	7.5 ±0.6	19.1 ±1.5	1.3 ±0.4	3.9 ±0.5	22.4 ±2.0	2.6 ±0.7	4.7 ±0.1	8.6 ±0.0	0.9 ±0.0	3.1 ±0.1
Cr	7.5 ±2.1	1.3 ±0.2	1.9 ±0.1	21.1 ±3.9	9.3 ±0.5	11.4 ±1.9	6.1 ±2.1	1.3 ±0.6	3.5 ±3.0	3.5 ±0.8	0.5 ±0.2	1.3 ±0.6	1.6 ±0.2	0.4 ±0.2	0.8 ±0.1	4.9 ±0.5	0.9 ±0.2	1.0 ±0.2	6.8 ±4.9	0.5 ±0.2	0.5 ±0.0	14.4 ±16	3.5 ±0.8	4.7 ±5.6	4.0 ±0.7	0.8 ±0.2	1.6 ±0.1
Cu	12.8 ±0.2	7.3 ±0.0	8.4 ±0.3	3.4 ±1.0	3.1 ±0.2	3.5 ±0.2	3.6 ±0.0	2.6 ±0.2	3.0 ±0.1	5.5 ±0.3	2.2 ±0.0	3.7 ±0.1	2.9 ±0.1	2.0 ±0.2	2.8 ±0.1	3.6 ±0.1	1.9 ±0.1	2.7 ±0.2	2.6 ±0.1	3.2 ±2.5	2.9 ±0.2	2.7 ±0.4	3.3 ±0.1	2.2 ±0.1	6.3 ±0.2	2.8 ±0.0	6.0 ±0.2
Mn	59.9 ±1.8	9.2 ±0.7	57.2 ±1.4	101 ±6.0	38.6 ±2.0	83.9 ±4.7	73.6 ±2.0	20 ±2.5	61 ±1.2	113 ±4.4	21.3 ±5.8	79.1 ±2.9	27.4 ±0.9	9.3 ±0.4	24 ±0.9	71.7 ±3.0	15.7 ±0.6	49.1 ±4.3	46.2 ±8.9	10.8 ±3.8	26.7 ±0.9	42.4 ±1.0	23.8 ±7.4	38.8 ±1.6	44.8 ±0.6	8.3 ±0.4	52.4 ±1.2
Mo	0.4 ±0.0	0.4 ±0.0	0.8 ±0.0	0.2 ±0.3	0.3 ±0.3	0.9 ±0.0	0.4 ±0.5	1.4 ±2.0	0.5 ±0.1	0.7 ±0.2	0.0 ±0.0	0.7 ±0.0	0.3 ±0.5	0.0 ±0.0	1.0 ±0.1	0.4 ±0.0	0.4 ±0.2	0.7 ±0.1	0.5 ±0.2	0.3 ±0.1	0.8 ±0.1	0.3 ±0.5	0.2 ±0.0	0.4 ±0.4	0.5 ±0.0	0.4 ±0.1	0.8 ±0.1
Pb	2.8 ±0.1	0.8 ±0.2	2.1 ±0.2	2.6 ±0.2	1.5 ±0.3	1.7 ±0.1	2.7 ±0.1	0.9 ±0.3	1.8 ±0.2	3.3 ±0.2	0.8 ±0.3	2.0 ±0.1	2.1 ±0.3	0.5 ±0.1	2.0 ±0.1	2.8 ±0.2	0.5 ±0.1	1.3 ±0.3	2.9 ±0.1	1.0 ±0.5	2.1 ±0.2	3.5 ±0.2	1.3 ±0.3	2.1 ±0.1	2.1 ±0.2	0.9 ±0.3	1.7 ±0.0
Se	0.6 ±0.5	1.1 ±0.1	0.7 ±0.4	0.2 ±0.3	0.5 ±0.5	0.6 ±0.6	0.5 ±0.5	0.6 ±0.2	0.7 ±0.7	0.9 ±0.2	0.9 ±0.3	1.1 ±0.3	1.0 ±0.6	1.2 ±0.2	0.5 ±0.6	0.4 ±0.3	0.2 ±0.3	0.1 ±0.2	0.9 ±0.4	1.5 ±0.3	1.2 ±0.2	0.9 ±0.3	2.6 ±0.4	0.7 ±0.3	0.8 ±0.3	0.9 ±0.4	1.2 ±0.1
Zn	249 ±3.3	211 ±14	197 ±1.7	88.7 ±3.2	83.3 ±9.2	81.2 ±4.4	111 ±4.0	60.6 ±6.7	93.3 ±1.2	107 ±3.5	36.5 ±9.2	88.3 ±3.1	92.5 ±5.5	57.6 ±7.1	104 ±4.1	120 ±3.5	53.0 ±2.3	97.3 ±5.9	112 ±9.4	55.3 ±17.8	97.1 ±5.3	91.7 ±0.8	75.1 ±17	81.0 ±1.4	95.3 ±1.7	75.5 ±5.3	117 ±3.1

Table 2. 5: Concentration of the minor elements in the leaves, stems, flowers and seeds of *Leptoplax* and *Bornmuellera* hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)

	<i>Leptoplax1</i>		<i>Leptoplax2</i>				<i>Bornmuellera1</i>		<i>Bornmuellera2</i>			<i>Bornmuellera3</i>		
	leaf	stem	leaf	stem	flower	seed	leaf	stem	leaf	stem	seed	leaf	stem	seed
Co	18.7 ±1.2	2.5 ±0.5	18.9 ±2.2	1.6 ±0.1	4.6 ±0.9	2.2 ±0.2	9.6 ±2.1	1.5 ±0.1	6.2 ±0.4	1.6 ±0.2	4.7 ±0.3	15.2 ±0.2	2.2 ±0.3	7.3 ±0.6
Cr	1.0 ±0.3	2.9 ±0.8	1.6 ±0.3	0.0 ±0.0	1.3 ±0.3	0.6 ±0.1	0.6 ±0.6	0.3 ±0.3	1.5 ±0.3	2.9 ±0.4	0.9 ±0.4	1.1 ±0.1	2.7 ±0.6	0.8 ±0.3
Cu	1.9 ±0.1	1.4 ±0.1	3.6 ±0.5	1.4 ±0.1	4.2 ±0.3	1.9 ±0.2	2.0 ±0.1	1.6 ±0.1	1.9 ±0.0	2.7 ±0.3	2.2 ±0.1	2.2 ±0.2	2.3 ±0.1	3.0 ±0.3
Mn	33.1 ±3.6	15.2 ±3.0	35.5 ±6.7	9.2 ±0.5	16.4 ±30	10.7 ±3.5	26.0 ±2.0	13.4 ±0.6	35.4 ±12	25.1 ±77	12.5 ±45	57.9 ±20	26.7 ±85	24.3 ±57
Mo	1.8 ±0.4	1.4 ±0.3	1.2 ±3.4	0.3 ±0.3	1.0 ±3.5	0.8 ±0.8	0.5 ±0.5	0.3 ±0.2	0.0 ±0.0	0.2 ±2.0	0.3 ±1.6	0.0 ±0.8	0.1 ±2.8	0.2 ±4.0
Pb	4.5 ±0.2	1.7 ±0.3	5.1 ±0.8	1.1 ±0.2	2.5 ±0.3	1.6 ±0.1	2.9 ±0.4	1.1 ±0.0	2.3 ±0.0	1.2 ±0.3	1.6 ±0.4	3.4 ±0.0	1.4 ±0.2	1.8 ±0.4
Se	0.6 ±0.2	0.9 ±0.5	1.0 ±0.2	1.2 ±0.4	1.7 ±0.2	0.6 ±0.2	1.0 ±0.4	1.2 ±0.3	1.0 ±0.1	1.5 ±0.1	0.9 ±0.2	1.4 ±0.2	1.6 ±0.0	1.6 ±0.1
Zn	190 ±4.6	126 ±20	241 ±0.4	80.5 ±7.8	118 ±0.4	77.0 ±0.1	138 ±10	97.3 ±5.3	110 ±0.4	65.1 ±0.4	75.4 ±0.5	147 ±0.2	53.5 ±0.6	95.7 ±0.2

In order to calculate mass balances between plants and ashes, we calculated the average concentration of each element in the whole plants (including flowers, leaves, stems and seeds) according equation 2.1, using the data in Fig. 2.1, Tables 2.2 and 2.3.

$$C_{i,t} = \frac{C_{i,f} m_f + C_{i,l} m_l + C_{i,s1} m_{s1} + C_{i,s2} m_{s2}}{m_t} \quad (\text{Eq 2.1})$$

$C_{i,t}$: concentration of element i in the total plant ($\text{mg} \cdot \text{g}^{-1}_{\text{DM}}$); m_t : mass of total plant (g);

$C_{i,f}$: concentration of element i in the flowers ($\text{mg} \cdot \text{g}^{-1}_{\text{DM}}$); m_f : mass of flowers (g);

$C_{i,l}$: concentration of element i in the leaves ($\text{mg} \cdot \text{g}^{-1}_{\text{DM}}$); m_l : mass of leaves (g);

$C_{i,s1}$: concentration of element i in the stem ($\text{mg} \cdot \text{g}^{-1}_{\text{DM}}$); m_{s1} : mass of stems (g);

$C_{i,s2}$: concentration of element i in the seeds ($\text{mg} \cdot \text{g}^{-1}_{\text{DM}}$); m_{s2} : mass of seeds (g)

The results (Table 2.6) clearly showed that the highest average concentration of Ni was found in *Leptoplax* 1 ($16.8 \pm 0.9 \text{ mg g}^{-1}_{\text{DM}}$), followed by *Bornmuellera* 1 ($15.6 \pm 1.2 \text{ mg g}^{-1}_{\text{DM}}$) and *Leptoplax* 2 ($10.0 \pm 5.8 \text{ mg g}^{-1}_{\text{DM}}$). All the *Alyssum* species exhibited lower average Ni contents. This result is in agreement with the literature (Chardot et al., 2005). Basically, these concentrations were lower than those reported by previous surveys in which Ni concentration in *A. murale* ranged between 8.4 and 9.7 $\text{g Ni kg}^{-1}_{\text{DM}}$ (Bani et al., 2007; Barbaroux et al., 2012; Shallari et al., 1998). The composition of bulk biomass differed from one specimen to another: for example, Ni concentration in the leaves of *A. murale* 4 was higher than in that of *A. murale* 5, but there were more stems and less leaves in *A. murale* 4 than in *A. murale* 5. Hence the average concentration of Ni was higher in *A. murale* 5 than in *A. murale* 4. It cannot be compared to the specimen reported in the literature for which the weight fraction of leaves was not recorded.

Table 2. 6: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the whole 15 hyperaccumulator species, calculated from the concentrations and the weight ratios of the organs of the plants (mg (g_{dry plant})⁻¹)

	<i>A.markgrafii</i>	<i>A.bertolonii</i>	<i>A. murale</i> 1	<i>A. murale</i> 2	<i>A. murale</i> 3	<i>A. murale</i> 4	<i>A. murale</i> 5	<i>A. murale</i> 6	<i>A. murale</i> 7	<i>A. murale</i> 8	<i>Leptoplax1</i>	<i>Leptoplax2</i>	<i>Bornmuellera1</i>	<i>Bornmuellera2</i>	<i>Bornmuellera3</i>
Ca	23.6 ±2.4	22.8 ±1.7	19.5 ±2.8	15.1 ±2.1	17.7±0.5	8.4 ±1.7	12.7±0.5	12.2 ±2.4	17.3 ±0.9	9.4 ±1.6	7.0 ±3.6	3.8 ±3.2	8.7 ±1.2	15.1 ±0.0	9.4 ±0.0
Fe	0.3 ±0.0	1.0 ±0.1	0.4 ±0.0	0.2 ±0.0	0.2±0.0	0.2 ±0.0	0.3±0.4	0.3 ±0.2	0.2 ±0.1	0.1 ±0.0	0.3 ±0.0	0.1 ±0.0	0.2 ±0.0	0.4 ±0.9	0.5 ±0.5
K	12.9 ±0.2	12.2 ±0.6	14.2 ±0.6	13.5 ±0.8	17.2±0.3	14.2 ±0.5	13.1±1.3	9.2 ±0.9	16.7 ±0.4	7.9 ±0.2	19.6 ±1.0	14.1 ±7.3	21.9 ±0.3	12.4 ±0.9	10.5 ±0.4
Mg	2.0 ±0.1	7.3 ±0.2	4.9 ±0.3	3.2 ±0.1	3.6±0.3	2.4 ±0.2	4.3±0.2	2.8 ±0.1	3.6 ±0.1	2.2 ±0.2	4.4 ±0.1	4.5 ±2.6	4.1 ±0.1	3.2 ±0.1	3.8 ±0.2
Ni	6.1 ±0.2	6.4 ±0.2	8.9 ±0.6	6.3 ±0.5	7.3±0.3	6.9 ±0.5	8.9±0.5	5.2 ±0.7	4.9 ±0.0	8.6 ±0.8	16.8 ±0.9	10.0 ±5.8	15.6 ±1.2	8.1 ±0.1	8.1 ±0.4

Table 2. 7: Concentration ratios Ni:Ca, Ni:Mg and Ca:Mg in the 15 hyperaccumulators

Ratio	<i>A.markgrafii</i>	<i>A.bertolonii</i>	<i>A. murale</i> 1	<i>A. murale</i> 2	<i>A. murale</i> 3	<i>A. murale</i> 4	<i>A. murale</i> 5	<i>A. murale</i> 6	<i>A. murale</i> 7	<i>A. murale</i> 8	<i>Leptoplax1</i>	<i>Leptoplax2</i>	<i>Bornmuellera1</i>	<i>Bornmuellera2</i>	<i>Bornmuellera3</i>
Ni : Ca	0.2	0.2	0.5	0.4	0.4	0.8	0.7	0.4	0.3	0.9	2.4	2.7	1.8	0.6	0.9
Ni : Mg	3.0	0.8	1.8	2.0	2.0	3.0	2.1	1.9	1.3	4.0	3.8	2.2	3.9	2.6	2.1
Ca : Mg	13.1	3.4	3.9	4.6	5.1	5.0	2.9	4.4	4.8	4.4	1.6	0.8	1.9	4.8	2.5

Regarding the other major elements, the concentration of Ca was the highest in the genus *Alyssum*. It was twice higher than in the genus *Bornmuellera*. It was two to seven times higher than in the genus *Leptoplax*. The concentrations of Fe and K were approximately equal in these plants. The concentration ratios Ni:Ca, Ni:Mg and Ca:Mg in all plants are listed in Table 2.7.

For all *Alyssum* and *Bornmuellera* hyperaccumulators plants, the ratio Ni:Ca was lower than unity as it was higher for *Leptoplax* plants. And for all the plants except *A. bertolonii* species, the ratio Ni:Mg was higher than unity. Calcium hyperaccumulation by *Alyssum* specimens is probably an adaptive trait of this Genus (section *Odontarrhenae*) to Ca deficiency of ultramafic soils. The two other species also accumulate efficiently this element and all three species store Ca massively in seeds. Biogeochemical recycling of Ca maybe ecologically sound for these species so that they provide Ca supply to the seeds and thus to future seedlings. Magnesium is usually excreted by Ni-hyperaccumulator plant roots to ensure electroneutrality. The usually low Ni:Mg and Ca:Mg ratios in *Alyssum*, *Leptoplax* and *Bornmuellera* species are opposed to the equivalent ratios in the soil CEC (Bani et al., 2007).

3.3 Optimization of the furnace treatment

In most cases of Ni recycling from hyperaccumulator biomass, combustion is the first step (Barbaroux et al., 2012; Boominathan et al., 2004; Keller et al., 2005; Machado et al., 2010; Sas-Nowosielska et al., 2004). Our objective was to bring a better understanding of the process of incineration. The influences of combustion temperature and duration on ashes composition were investigated.

3.3.1 Influence of temperature on ashes composition

The concentrations of the major elements (Ca, Fe, K, Mg and Ni) in the biomass and in the ashes obtained at 550 and 1,100 °C after 2 h are plotted in Fig.2.2. To allow a straightforward comparison, we expressed all the concentrations relatively to the mass of dry plants. Then, in Fig.2.2, the concentrations in the biomass before combustion directly resulted from analysis. We notice that the initial biomass samples (1 and 2) had different compositions since they were obtained from the grinding of two lots. The concentrations in the ashes were calculated by multiplying the concentrations expressed per mass of ashes by the ratio (mass of ashes):(mass of biomass). This ratio was equal to 6.6 at 550 °C (4.7 g ashes from 30.5 g biomass) and to 18.9 at 1,100 °C (2.4 g ashes from 44.5 g biomass).

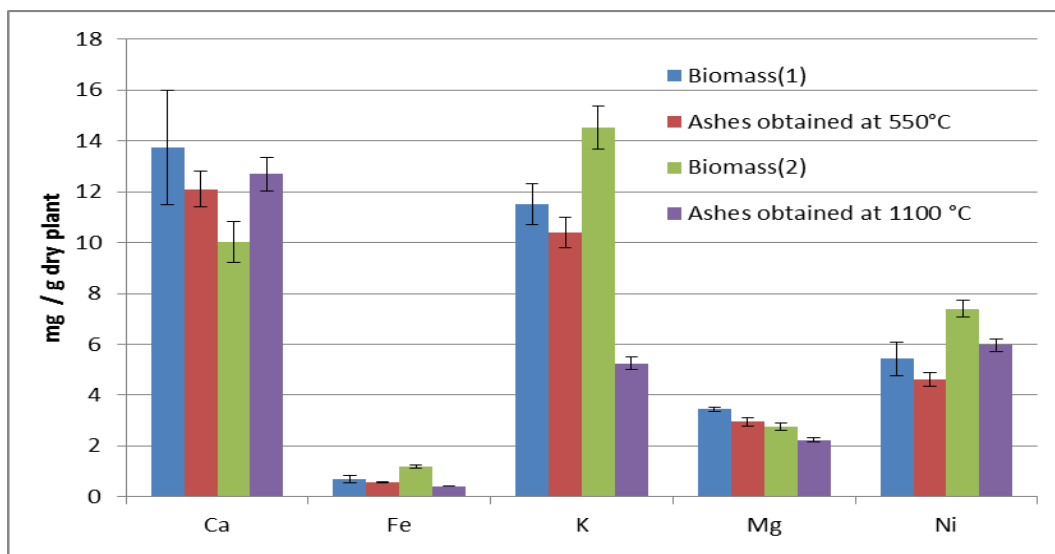


Figure 2. 2: Comparison between the concentrations of the major elements in the biomass of *A.murale* 6 and the ashes obtained at 550 and 1,100 °C for 2 h. All the concentrations are relative to the mass of dry plants.

To meet the mass balance for each element, the bars should be of same height between biomass (1) and ashes at 550 °C on the one hand and biomass (2) and ashes at 1,100 °C on the other hand. Obviously, Fig. 2.2 illustrated that it was the case for biomass (1) and the ashes obtained at 550 °C: the differences between the bars relative to biomass and ashes never exceeded 10 %. This result was expected since K, Ca and Mg are not volatilized below 600 °C (Mahendra et al., 1993). Besides, for the minor elements, the recovery rates of Cu, Pb and Zn were of 91 %, 86 % and 93 %, respectively.

At 1,100 °C, a significant loss of K was observed, which was assigned to volatilization above 900 °C (Mahendra et al., 1993). The enrichment in Ca was not expected but we explain it by a pollution problem met with the ICP-AES for Ca and Cu. A loss of Ni was recorded as well as losses of minor elements. Despite the uncertainties recorded for the ashes obtained at 1,100 °C, these results clearly demonstrated that a combustion temperature as low as possible must be chosen (i) to minimize energy consumption and ensure the feasibility of the whole process, (ii) to avoid the loss of elements and (iii) to avoid air pollution.

3.3.2 Influence of combustion duration on ash composition

We investigated the influence of combustion duration at 550 °C on mass reduction and Ni concentration in the ashes. The results presented in Fig.2.3 showed that the ratio (mass of biomass): (mass of ashes) sharply increased at the beginning to reach a plateau after 3 h: its value was then permanently equal to 14. The same behavior was recorded for Ni concentration, which reached $60 \text{ mg g}_{\text{ashes}}^{-1}$ after 3 h. Then combustion could be considered as complete at 3 h in this experimental system.

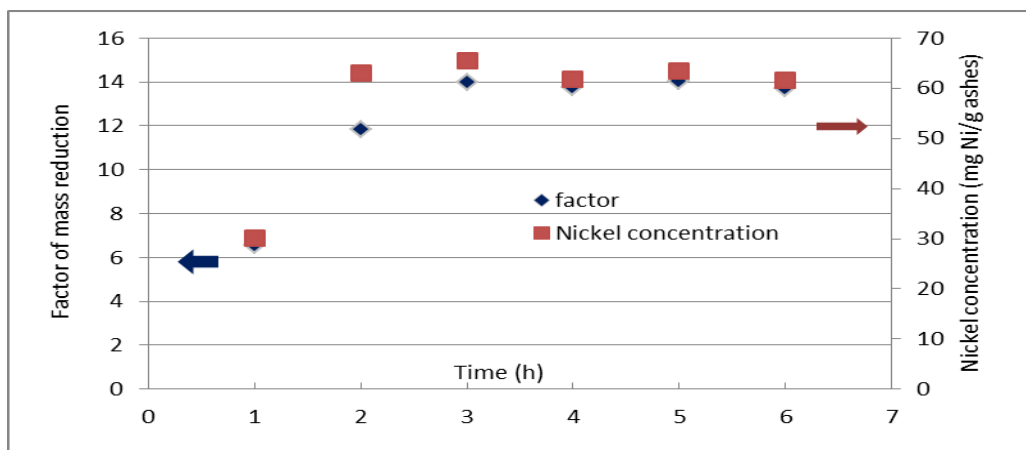


Figure 2. 3: Evolution of the mass reduction factor and of the Ni concentration in the ashes of the ashes of *A. murale* 6 during the combustion at 550 °C.

The photographs of the ashes at different times showed their evolution (Fig.2.4). After 2 h, black spots were observed, corresponding to remaining transition organic compounds. These spots were significantly decreased at 3 h and had totally disappeared at 4 h. These observations confirmed that combustion was almost complete after 3 h.

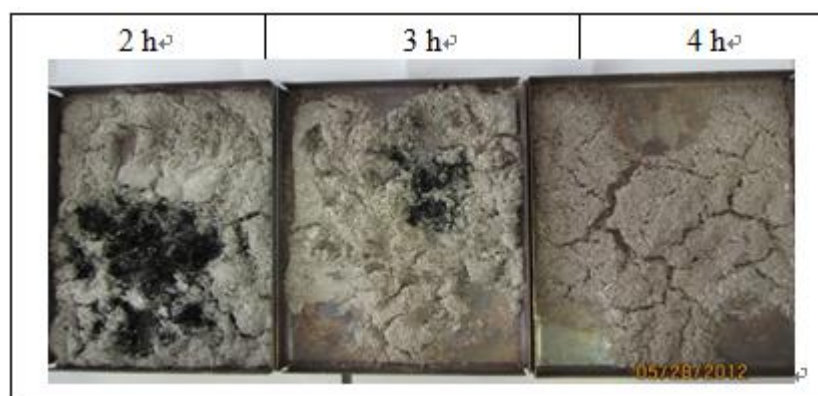


Figure 2. 4: Appearance of the ashes of *A. murale* 6 after different durations of combustion at 550 °C

3.4 Concentrations in the ashes of the 14 hyperaccumulators

Combustion was run at 550 °C for 3 h with the leaves, flowers, stems and seeds (if present) of all the hyperaccumulators and the concentrations of the major elements (Ca, Fe, K, Mg and Ni) are listed in Tables 2.8 & 2.9.

The Ni concentrations in ashes were very high, from 6 to 20 %, but depended on different plants. The highest Ni concentration was recorded in the ashes made from the leaves of *Leptoplax emarginata* (Table 2.9), which was about 203 mg (g_{ashes})⁻¹.

We calculated the mass ratio R (biomass: ashes) for all the organs of all the plants. We obtained 17.6 ± 3.6 for stems (n=13), 7.4 ± 1.1 for leaves (n=13), 7.9 ± 1.2 for flowers (n=8) and 12.3 ± 2.2 (n=3) for seeds. The concentration ratios R_i (concentration in the ashes: concentration in the biomass) for all the monitored elements i was in perfect accordance with R for Ni and Mg and in fairly good accordance for the others ones.

These factors were consistent with previous results concerning *Berkheya coddii* for which an enrichment factor of about 17 was recorded (Boominathan et al., 2004).

Then, the obtained ashes had a very high Ni content and could be considered as Ni bio-ore (Boominathan et al., 2004) in the next chapter. But in the same way, they were also highly concentrated in Mg, Ca, K and Fe as well as in minor elements, especially in Zn (Table 2.10 & 2.11 & 2.12). Such trace metal enrichments can be a drawback for further use. However, we designed different steps to remove these elements and to obtain the highest purity of nickel products.

Table 2. 8: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the ashes obtained from the different organs of the *Alyssum* hyperaccumulator species (except *A. murale* 6 for which the total biomass was mixed) (mg (g_{ashes})⁻¹)

	<i>A.markgrafii</i>			<i>A.bertolonii</i>			<i>A. murale 1</i>			<i>A. murale 2</i>			<i>A. murale 3</i>			<i>A. murale4</i>			<i>A. murale5</i>			<i>A. murale7</i>		
	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower
Ca	281 ±11	138 ±2.3	272 ±9.4	236 ±26	162 ±24	233 ±32	369 ±21	199 ±6.4	354 ±51	352 ±3.3	239 ±10	353 ±23	384 ±40	225 ±16	284 ±28	341 ±31	281 ±103	255 ±15	262 ±4.7	153 ±35	272 ±15	214 ±2.4	99.6 ±3.5	210 ±1.4
Fe	2.6 ±0.0	1.6 ±0.0	0.9 ±0.0	7.5 ±0.7	6.4 ±0.6	4.7 ±0.4	4.5 ±1.0	2.6 ±0.0	2.2 ±0.2	3.9 ±0.2	1.2 ±0.0	1.6 ±0.0	2.0 ±0.3	1.5 ±0.4	1.6 ±0.0	4.9 ±1.0	4.9 ±2.6	1.7 ±0.0	7.6 ±1.0	2.5 ±0.0	1.8 ±0.0	2.4 ±0.0	0.3 ±0.4	1.0 ±0.0
K	85.0 ±3.1	224 ±1.0	77.6 ±4.0	75.6 ±8.1	194 ±28	97.2 ±11	121 ±12	325 ±10	175 ±25	126 ±4.5	292 ±18	175 ±10	171 ±19	377 ±3.6	221 ±20	161 ±15	555 ±210	266 ±19	149 ±8.5	337 ±52	194 ±14	128 ±1.5	236 ±7.2	93.7 ±2.1
Mg	18.8 ±0.7	21.7 ±0.1	21.6 ±1.1	63.1 ±6.1	53.9 ±6.9	58.4 ±6.1	39.5 ±2.0	36.1 ±1.8	49.3 ±6.4	29.3 ±1.0	30.5 ±2.0	36.7 ±2.4	35.0 ±3.6	24.4 ±2.1	40.0 ±2.7	31.5 ±2.7	60.8 ±22	27.7 ±1.7	50.9 ±2.2	48.4 ±5.9	46.8 ±2.9	38.3 ±0.6	22.6 ±0.9	44.9 ±0.9
Ni	61.2 ±2.0	55.5 ±0.3	63.1 ±3.2	52.5 ±5.5	55.3 ±7.1	45.7 ±5.4	90.7 ±4.4	77.8 ±6.3	70.7 ±6.3	89.0 ±0.8	55.9 ±2.0	71.0 ±3.1	91.1 ±10	49.0 ±4.0	85.8 ±3.7	88.8 ±8.3	94.6 ±34	92.8 ±3.2	122 ±5.2	100 ±8.0	92.4 ±2.9	53.8 ±0.8	33.4 ±1.1	49.4 ±0.8

Table 2. 9: Concentrations of the major elements (Ca, Fe, K, Mg) and Nickel in the ashes of the leaves, stems, flowers and seeds of *Leptoplax* and *Bornmuellera* hyperaccumulator species (mg (g_{ashes})⁻¹)

	Leptoplax1		Leptoplax2			Bornmuellera1		Bornmuellera2			Bornmuellera3		
	leaf	stem	leaf	stem	seed	leaf	stem	leaf	stem	seed	leaf	stem	seed
Ca	222 ±167	61.3 ±6.5	63.7 ±0.6	130 ±6.7	131 ±7.6	236 ±121	105 ±2.8	335 ±5.4	223 ±15	159 ±16	240 ±11	132 ±10	0.0 ±0.0
Fe	1.5 ±0.1	6.1 ±0.3	1.4 ±0.0	1.2 ±0.0	1.4 ±0.1	2.2 ±0.1	1.3 ±0.1	2.8 ±0.1	13.9 ±2.3	14.6 ±0.1	2.6 ±1.2	14.3 ±0.5	56.6 ±0.6
K	182 ±7.1	416 ±28	17.8 ±0.2	35.0 ±22.1	229 ±12	243 ±6.8	485 ±6.3	179 ±4.3	311 ±15	171 ±1.7	162 ±4.8	277 ±15	60.0 ±0.6
Mg	32.0 ±1.6	43.5 ±2.4	54.8 ±0.5	48.2 ±1.9	61.0 ±1.5	37.7 ±1.0	28.0 ±0.4	36.1 ±0.0	37.6 ±3.1	42.8 ±0.4	57.7 ±5.2	53.2 ±10	117 ±1.2
Ni	203 ±5.4	126 ±4.2	189 ±1.9	103 ±3.8	161 ±3.7	174 ±4.7	92.0 ±1.2	101 ±2.8	93.7 ±6.7	107 ±1.1	165 ±3.7	89.0 ±1.6	114 ±1.1

Table 2. 10: Concentration of the minor elements in the whole hyperaccumulator species ($\mu\text{g (g}_{\text{dry plant}})^{-1}$)

	<i>A.markgrafii</i>	<i>A.bertolonii</i>	<i>A. murale</i> 1	<i>A. murale</i> 2	<i>A. murale</i> 3	<i>A. murale</i> 4	<i>A. murale</i> 5	<i>A. murale</i> 6	<i>A. murale</i> 7	<i>Leptoplax</i> 1	<i>Leptoplax</i> 2	<i>Bornmuellera</i> 1	<i>Bornmuellera</i> 2	<i>Bornmuellera</i> 3
Co	3.7 ±0.3	12.9 ±0.9	9.0 ±0.3	13.3 ±1.5	5.3±0.6	7.0 ±0.3	6.1±0.7	5.2 ±1.0	4.1 ±0.1	7.1 ±0.6	2.7 ±1.5	6.6 ±1.5	3.4 ±0.1	4.8 ±0.3
Cr	3.4 ±1.2	14.5 ±1.8	3.3 ±1.3	1.1 ±0.3	0.8±0.0	1.6 ±0.2	2.0±2.8	4.1 ±7.6	2.1 ±0.3	2.4 ±0.4	0.3 ±0.3	0.5 ±0.2	2.3 ±0.1	2.3 ±0.3
Cu	9.2 ±0.2	3.3 ±0.5	3.0 ±0.1	3.0 ±0.1	2.4±0.1	2.4 ±0.1	3.0±1.4	1.9 ±0.2	4.4 ±0.1	1.5 ±0.1	1.7 ±1.1	1.9 ±0.0	2.4 ±0.2	2.3 ±0.1
Mn	27.8 ±0.8	69.6 ±2.7	45.0 ±1.1	46.9 ±1.9	17.3±0.4	31.0 ±2.2	23.0±4.2	21.3 ±3.7	25.1 ±0.6	20.4 ±0.5	11.2 ±6.4	21.0 ±1.2	27.9 ±37	32.4 ±37
Mo	0.9 ±0.0	0.3 ±0.1	0.9 ±1.0	0.3 ±0.1	0.4±0.3	0.5 ±0.1	0.5±0.0	0.2 ±0.3	0.5 ±0.0	1.5 ±0.1	0.5 ±0.4	0.4 ±0.2	0.1 ±0.6	0.1 ±2.1
Pb	1.5 ±0.1	2.0 ±0.1	1.6 ±0.1	1.4 ±0.1	1.3±0.2	1.0 ±0.1	1.7±0.3	1.4 ±0.1	1.4 ±0.1	2.5 ±0.0	1.5 ±0.9	2.27 ±0.3	1.6 ±0.2	1.8 ±0.2
Se	0.9 ±0.2	0.4 ±0.2	0.6 ±0.3	1.0 ±0.1	0.9±0.2	0.3 ±0.1	1.3±0.1	1.19 ±0.1	0.9 ±0.2	0.8 ±0.2	1.0 ±0.5	1.1 ±0.0	1.3 ±0.1	1.5 ±0.1
Zn	224 ±8.7	95.4 ±3.9	83 ±3.5	57.6 ±3.9	78.3±2.2	72.6 ±2.5	78.7±7.1	52.6 ±9.8	85.5±2.6	145 ±12	87.7 ±48	123 ±4.1	81.6 ±0.1	72.0±0.2

Table 2. 11: Concentration of the minor elements in the ashes of the leaves, stems, flowers and seeds of *Alyssum* hyperaccumulators species ($\mu\text{g}(\text{gashes})^{-1}$)

	<i>A.markgrafii</i>			<i>A.bertolonii</i>			<i>A. murale 1</i>			<i>A. murale 2</i>			<i>A. murale 3</i>			<i>A. murale4</i>			<i>A. murale5</i>			<i>A. murale7</i>		
	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower	leaf	stem	flower
Co	45.6 ±0.3	13.1 ±0.1	19.1 ±0.2	122 ±12	59.5 ±5.2	50.4 ±5.4	137 ±33	42 ±0.3	58 ±3.4	419 ±8.3	72 ±0.4	114 ±6.6	148 ±13	18 ±4.8	70 ±5.0	227 ±52	57 ±28	86 ±1.0	194 ±4.8	38 ±1.9	43 ±1.0	55 ±0.9	5.8 ±7.4	21 ±0.3
Cr	46 ±9.0	171 ±17	38.0 ±12	113 ±6	131 ±6.1	75.6 ±4.7	29 ±4.6	35 ±3.8	69 ±13	21 ±1.6	80 ±0.7	12 ±0.2	214 ±31	15 ±3.8	14 ±0.6	37 ±2.7	44 ±23	24 ±16	42 ±6.8	32 ±0.8	14 ±5.0	30 ±0.5	304 ±0.4	428 ±43
Cu	64 ±0.7	120 ±2.1	46 ±0.3	20 ±2.4	43 ±4.3	28.7 ±4.4	21 ±3.8	54 ±1.1	38 ±10	39 ±0.7	46 ±0.8	59 ±2.7	24 ±2.0	24 ±6.2	46 ±3.0	25 ±6.0	54 ±29	29 ±1.0	19 ±0.1	39 ±6.7	44 ±0.4	42.5 ±0.7	19.3 ±27	38 ±0.8
Mn	254 ±2.5	137 ±2.1	297 ±4.1	560 ±51	431 ±39	549 ±58.4	514 ±126	370 ±0.3	443 ±25	970 ±27	475 ±2.4	806 ±4.0	290 ±41	207 ±64	329 ±9.0	577 ±128	486 ±251	626 ±6.0	493 ±26	317 ±14	368 ±4.7	270 ±3.2	49.0 ±68	322 ±3.5
Mo	1.8 ±0.0	7.0 ±0.7	4.8 ±0.1	2.3 ±0.1	5.6 ±0.3	5.5 ±0.6	2.4 ±0.5	5.6 ±0.1	11 ±3.8	4.1 ±0.0	8.4 ±1.0	7.2 ±0.2	16 ±2.4	3.2 ±0.8	7.4 ±0.3	3.0 ±0.6	7.1 ±3.6	7.7 ±0.2	3.1 ±0.2	8.0 ±0.8	7.6 ±0.5	3.8 ±0.2	8.5 ±11	11.0 ±0.1
Pb	12 ±0.2	11 ±0.1	11 ±0.2	12.8 ±1.3	13.6 ±1.3	10.1 ±1.2	18 ±4.7	17 ±0.2	13 ±0.8	26 ±0.9	14 ±0.1	19 ±0.7	17 ±2.3	10 ±2.6	20 ±1.0	21 ±4.2	18 ±8.8	19 ±0.5	30 ±0.4	22 ±1.2	21 ±0.5	12.1 ±0.2	3.4 ±4.0	9.7 ±0.0
Se	0.5 ±0.0	0.8 ±0.2	0.7 ±0.2	0.0 ±0.0	0.0 ±0.0	0.7 ±0.1	0.4 ±0.4	0.3 ±0.4	0.7 ±0.1	1.7 ±0.2	1.4 ±0.4	2.1 ±0.1	0.4 ±0.5	1.2 ±0.1	0.7 ±0.9	0.4 ±0.4	0.0 ±0.0	1.7 ±0.4	0.0 ±0.0	1.8 ±0.6	1.7 ±0.6	0.8 ±0.3	0.7 ±0.3	0.8 ±0.1
Zn	1204 ±12	3451 ±61	1139 ±22	496 ±49	959 ±87	552 ±59	843 ±196	1315 ±5.2	731 ±53	934 ±29	813 ±6.8	913 ±11	925 ±124	1231 ±378	1293 ±65	880 ±129	1329 ±683	1167 ±3.0	1314 ±30	1569 ±52	1213 ±17	611 ±5.4	448 ±632	764 ±15

Table 2. 12: Concentration of the minor elements in the ashes of the leaves, stems, flowers and seeds of *Leptoplax* and *Bornmuellera* hyperaccumulator species ($\mu\text{g (g}_{\text{ashes}})^{-1}$)

	<i>Leptoplax1</i>		<i>Leptoplax2</i>			<i>Bornmuellera1</i>		<i>Bornmuellera2</i>			<i>Bornmuellera3</i>		
	leaf	stem	leaf	stem	seed	leaf	stem	leaf	stem	seed	leaf	stem	seed
Co	183 ±3.9	35 ±1.4	153 ±1.5	27.6 ±0.1	58.7 ±4.5	98 ±0.8	23.5 ±1.1	68.8 ±3.1	41.5 ±5.5	110 ±1.1	152 ±0.6	59 ±1.2	227 ±2.3
Cr	15.4 ±2.1	122 ±12	21.9 ±0.2	156 ±19	10.0 ±1.4	13.8 ±0.2	16.3 ±2.4	18.8 ±1.3	95.8 ±29	139 ±1.4	16 ±3.7	73 ±1.3	407 ±4.1
Cu	18 ±1.5	25 ±0.1	20.3 ±0.2	27 ±0.7	27.6 ±1.5	21 ±1.7	43 ±5.3	13.1 ±1.0	54.3 ±5.5	36.5 ±0.4	13.3 ±0.1	42.4 ±1.9	225 ±2.3
Mn	375 ±9.3	258 ±11	265 ±2.7	191 ±0.7	229 ±14	338 ±4.8	234 ±15	393 ±0.8	667 ±86	598 ±6.0	621 ±9.8	753 ±17	153 ±15
Mo	16.2 ±0.2	24 ±1.3	4.4 ±0.0	14.8 ±1.3	13 ±0.1	3.9 ±0.1	5.8 ±0.8	2.9 ±0.2	5.1 ±0.9	4.0 ±0.0	1.2 ±0.7	1.8 ±0.1	0.0 ±0.0
Pb	39.2 ±1.4	26 ±1.0	37.9 ±0.4	20.4 ±0.1	29 ±1.3	32 ±0.5	19.8 ±1.4	20.2 ±0.3	31.9 ±3.2	31 ±0.3	32.7 ±0.2	27.6 ±0.7	54.4 ±0.5
Se	1.6 ±1.1	0.0 ±0.0	0.0 ±0.0	0.22 ±0.3	2.1 ±1.5	0.5 ±0.6	0.8 ±0.2	0.6 ±0.2	0.0 ±0.0	0.0 ±0.0	4.0 ±0.9	0.3 ±0.5	0.0 ±0.0
Zn	1970 ±76	2136 ±73	2032 ±20	1585 ±12	1703 ±96	1702 ±5.0	1779 ±98	1131 ±12	1859 ±201	1391 ±14	1575 ±4.8	1445 ±12	1864 ±19

3.5 XRD of the ashes of the hyperaccumulators

The ashes made at 550 °C after 3 h were characterized by XRD, with the help of the database of the International Centre for Diffraction Data. NiO was clearly identified as the predominant phases in all the ashes. MgO, CaCO₃, K₂CO₃, Ca₅(PO₄)₃OH, CaPO₃OH and K₂Mn₂(SO₄)₃ were also present clearly as in the Fig.2.5.

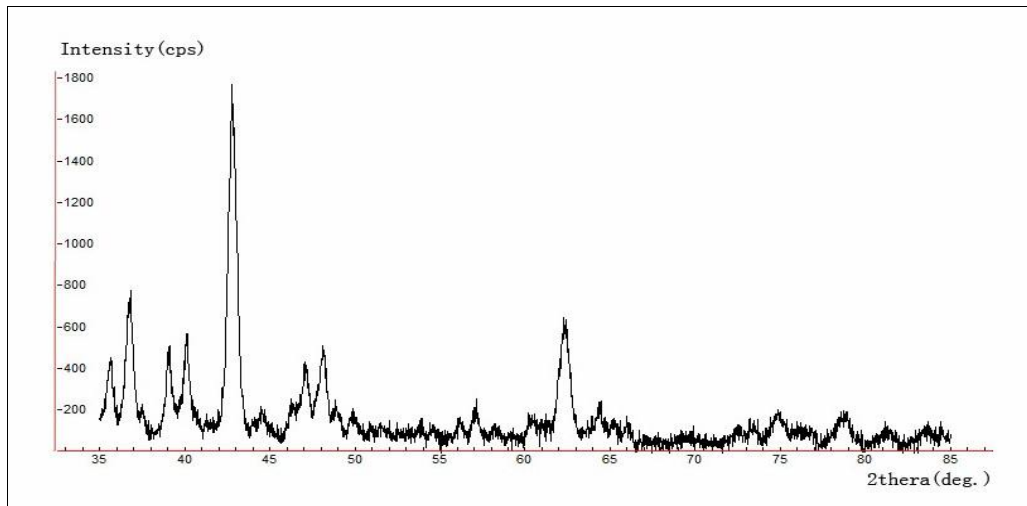


Figure 2. 5: XRD pattern of *A. murale* ashes

3.6 Particle size distribution, SEM and EDX analysis

The particle size distribution of the raw ashes does not obey log-normal distribution as a peak with shoulders (which is usually found for a population of spherical beads) (Fig.2.6.). This could be expected because of a non-spherical particle morphology (Fig.2.7). In these conditions, the mean particle size has little significance but gives an order of magnitude. We found a median particle diameter in volume ($d_{v,50}$) of 134.5 μm , (50 wt % of the particles had a lower size).

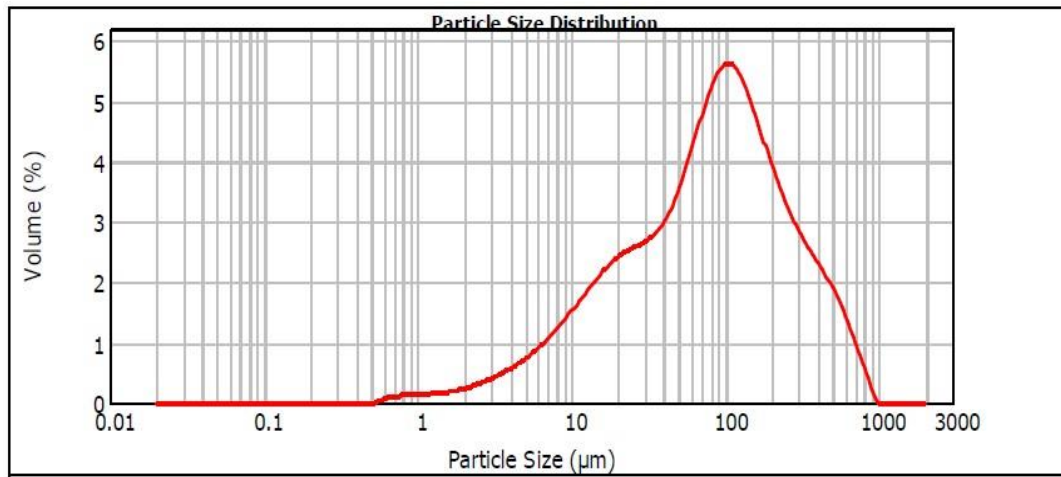


Figure 2. 6: Particle size distribution of the raw *A.murale* ashes

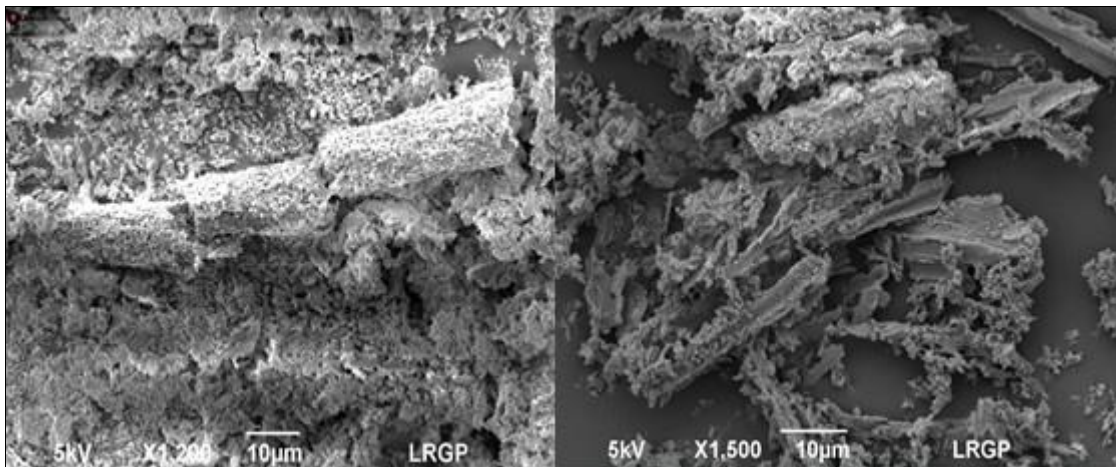


Figure 2. 7: SEM images of *A.murale* raw ashes

The SEM photographs of *A. murale* show that particles were non spherical and of irregular shapes (Fig.2.7.). A thin and long clot was observed on the photograph on the left (magnification of 1,200) and was more visible with a higher magnification (photograph on the right, magnification of 1,500). This clot was supposed to be originated from stem ash, because of its cylindrical shape. EDX spectra collected from this sample at three different locations are shown in Fig.2.8 (a, b, c). Results were in accordance with our assumption because we found a high concentration of K and a small one of Ca (Fig. 2.8 a & c). The opposite was observed in Fig. 2.8 b, and then point b was originated from ashes of leaves or flowers. To investigate the detail K and Ca content rate in the stems, leaves and flowers, we did another set of EDX analyses of stem ash, leaf ash and flower ash (Fig.2.9). As expected, in stem and flower ash, K was much higher than that in leaf ash. However, Ca was less concentrated in the leaf ash than in the stem ash.

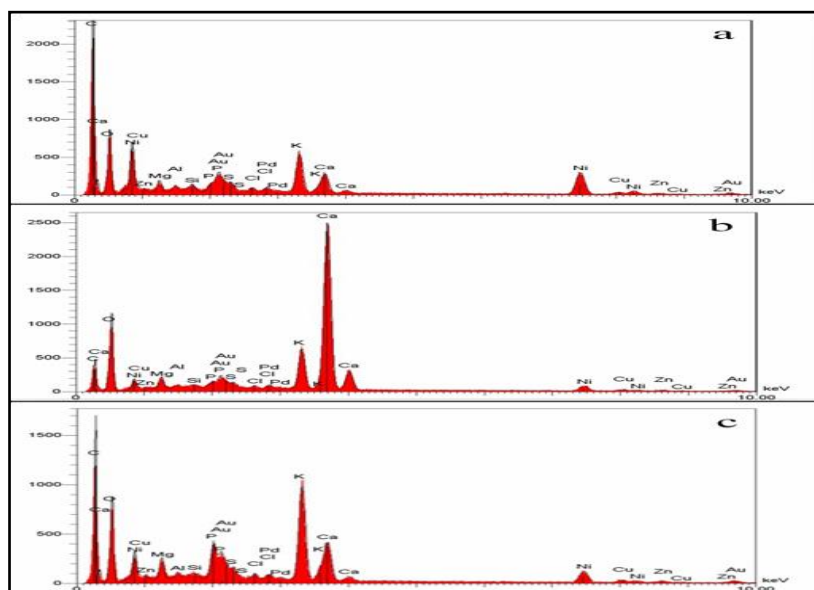


Figure 2. 8: (a,b,c) EDX of *A.murale* raw ashes at three different points

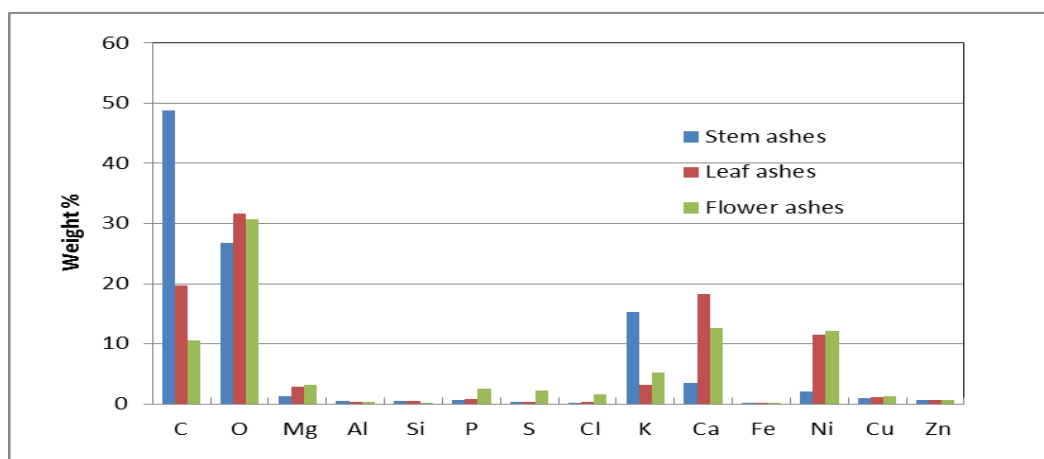


Figure 2. 9: Elemental weight percentage of the stem, leaf and flower ashes

4 Conclusions

The composition of the different organs of 15 Ni-hyperaccumulators was carefully recorded and discussed (Zhang et al., 2014). Results showed that Ni reached 0.8-1.6 % in *Alyssum* species, 1.4-2.2 % in *B. tymphaea* and 3.2-3.4 % in *L. emarginata*, and that the highest concentrations of Ni were always recorded in leaves (especially of *L. emarginata*) whatever the species. Other elements (e.g. K and Ca) gave information on plant needs for phytomining purpose, which may require appropriate fertilization to sustain long-term practice. *A. murale* was chosen for all the following experiments as this plant was easily cropped to obtain large biomass quantities.

Combustion assays proved that a temperature as low as possible (550 °C) was the most adapted to avoid metal loss and save energy. In our experimental system, the best combustion duration was 3 h for the small furnace but 2 h for the big furnace; but these figures should be adapted according to the furnace characteristics and amounts of processed biomass. Ashes contained up to 20 % Ni, which represents a relatively high content of potential interest for further Ni-recycling processes.

As the high grade bio-Ni-ore was easily prepared after the incineration process, the Ni-phytomining seems feasible provided that all the optimizations of the hydrometallurgical process to produce ANSH salt are achieved. This is the objective of the next chapter.

Chapter 3: Optimization of the process to produce ANSH (ammonium nickel sulfate hexahydrate) from ashes of *A. murale*

Science never solves a problem
without creating ten more.

George Bernard

1 Introduction

A hydrometallurgical process was designed to produce a high value Ni salt (ANSH) from the ashes of *A. murale* (Fig.3.1) (Barbaroux, 2010; Barbaroux et al., 2011; Barbaroux et al., 2012; Mercier et al., 2012).

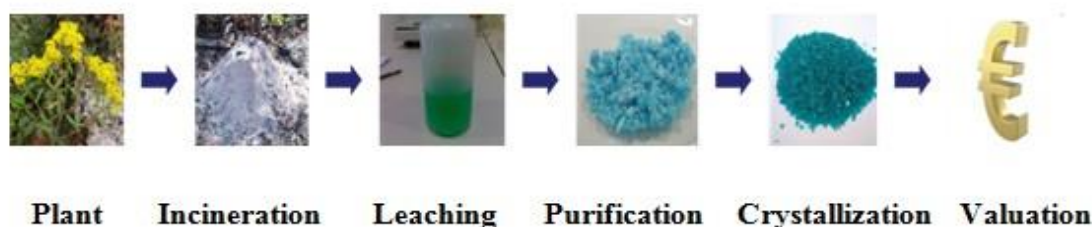


Figure 3. 1: The representative photos of the existed hydrometallurgical process

The objective of this chapter is to re-visit this process in order to:

- ❖ investigate each step in detail, to optimize its performance,
- ❖ save water, energy and reactants and minimize the formation of by-products.

The performance criteria of the process are the production yield and the salt purity. As a matter of fact, ANSH price increases drastically with purity.

Also this process should be considered as an eco-technology in the field of “green chemistry”. For this reason, as well as for economic reasons, mass and energy must be saved, residual solutions and wastes must be minimized and their re-use must be taken into account.

This chapter is organized in four parts corresponding to the main steps of the process: **ash washing** (Part I), **acid leaching** (Part II), **purification of Ni-rich leachate** (Part III) and **ANSH crystallization** (Part IV).

2 Part I–Ash washing stage

2.1 Introduction

In the previous study (Barbaroux et al., 2012), ash was washed with deionized water in a suspension containing 20 % solid, stirred for 15 min. Then the supernatant was separated and the ash dried in a stove at 100 °C for 2 h. Then, to maximize K elimination, a second step of washing was done. The objective of this part is to study carefully the ash washing step in order to maximize the removal of K while saving water and energy.

2.2 Materials and methods

Sulfuric acid (95.0-97.0 %), ethanol (99.8 %), and ammonium hydroxide (25.0 % NH₃) were provided by Sigma Aldrich, ammonium sulfate (99.5 %) and dimethylglyoxime (HDMG) (99.0 %) by Fluka, sodium hydroxide (99.0 % by Merck), calcium hydroxide (96.0 %) by BDH Prolabo and sodium fluoride NaF (analytical reagent grade) by Fisher Scientific were used in this chapter.

2.2.1 Composition of the raw ashes

The ICP-AES analysis of the raw ashes showed that the major elements were Ca, Mg, Fe, K and Ni (Chapter 2: 3.5). XRD analyses have shown that Mg and Ni were present as oxides (MgO, NiO), Ca was in the form of calcite CaCO₃, while K was mostly in the form of potassium carbonate K₂CO₃, and partly in the form of potassium and calcium phosphate KCaPO₄. If sulfuric acid was directly added to the raw ashes, there will be a great wasted volume of H₂SO₄, and the K, Ca and Mg in the raw ashes (K 113.9 g (kg ashes)⁻¹; Ca 129.1 g (kg ashes)⁻¹; Mg 33.2 g (kg ashes)⁻¹) would surely affect the purity of

the final Ni products. K_2CO_3 has a high solubility (111 g K_2CO_3 per 100 g water at 20 °C (Linke, 1965), then it was chosen to wash the raw ashes to remove K.

2.2.2 Influence of the washing duration

The influence of washing duration was studied in the same conditions as in the previous process (Barbaroux et al., 2012): 30 g of raw ash and 120 mL deionized water (ash mass fraction 20 %) were put into a 250 mL beaker and stirred with a three-blade turbine (Heidolph Bioblock Scientific). Supernatant samples (1 mL) were taken at the beginning and after 1, 5, 10, 15, and 20 min. They were diluted into a volumetric flask of 50 mL, separately, and analyzed by ICP-AES.

2.2.3 Influence of ash mass fraction

Three mass fractions were tested, 10, 15 and 20 %: 11.1 g, 17.7 g and 25.0 g of raw ash with 100 mL deionized water were put into a 250 mL beaker and stirred for 15 min. Supernatant samples (1 mL) were taken and analyzed as previously.

2.2.4 Influence of the stirring speed

The washing duration was fixed at 15 min and the ash mass fraction at 10 % solids. Washing was performed at 3 stirring speeds, 500, 1000 and 2000 rpm. In the same way, samples were taken and analyzed.

2.2.5 Influence the washing methods

In the previous study (Barbaroux et al., 2012), the ash was washed once and dried in a stove at 100 °C during 2 h; afterwards it was washed a second time. In this part, two methods were followed to optimize the washing stage.

The first method (M#1) consisted in washing the ash several times successively with deionized water without drying (Fig.3.2). The operating conditions were: ash mass fraction: 20 %, stirring speed: 500 rpm; duration: 15 min. Between 2 washing operations, the suspensions were vacuum filtered with filter papers (40 Ashless, Diameter 90 mm, Whatman). Beginning with the raw ash A_{10} , the first washing gave rise to the washed ash A_{11} to process effluent PE_1 and the washing #n gave rise to A_{1n} and PE_n (Fig.3.2). The method was tested until 8 washing steps.

The effluents and washed ashes were analyzed (ICP-AES) and in some cases ashes were analyzed by XRD.

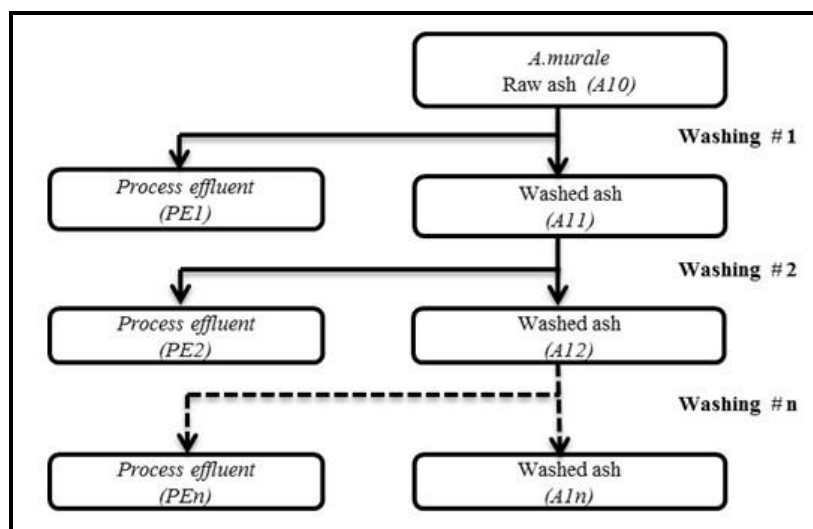


Figure 3. 2: Diagram of the washing process following method M#1

The second method (M#2), a so-called counter-current method, has been designed to minimize water consumption. It consisted in 3 successive steps (Fig.3.3): a 1st batch of raw ash A_{10} was washed as in M#1. The second batch of raw ash A_{20} was washed with PE_{12} to give A_{21} and PE_{21} and ash A_{21} was washed with deionized water to give A_{22} and PE_{22} . The third batch was a repetition of the second one: raw ash A_{30} was washed with PE_{22} to give A_{31} and PE_{31} then A_{31} was washed with deionized water to give A_{32} and PE_{32} . In the following experiences, the process will be continued as: the process effluent (PE_n , $n=3, 4\dots$) could be used to wash the raw *A. murale* ashes (A_{n0} , $n=4, 5\dots$) for the first washing and then using the fresh deionized water to wash the washed ashes (A_{n1} , $n=4, 5\dots$) for the second washing. Elemental composition of the solutions was measured by ICP-AES for each treatment.

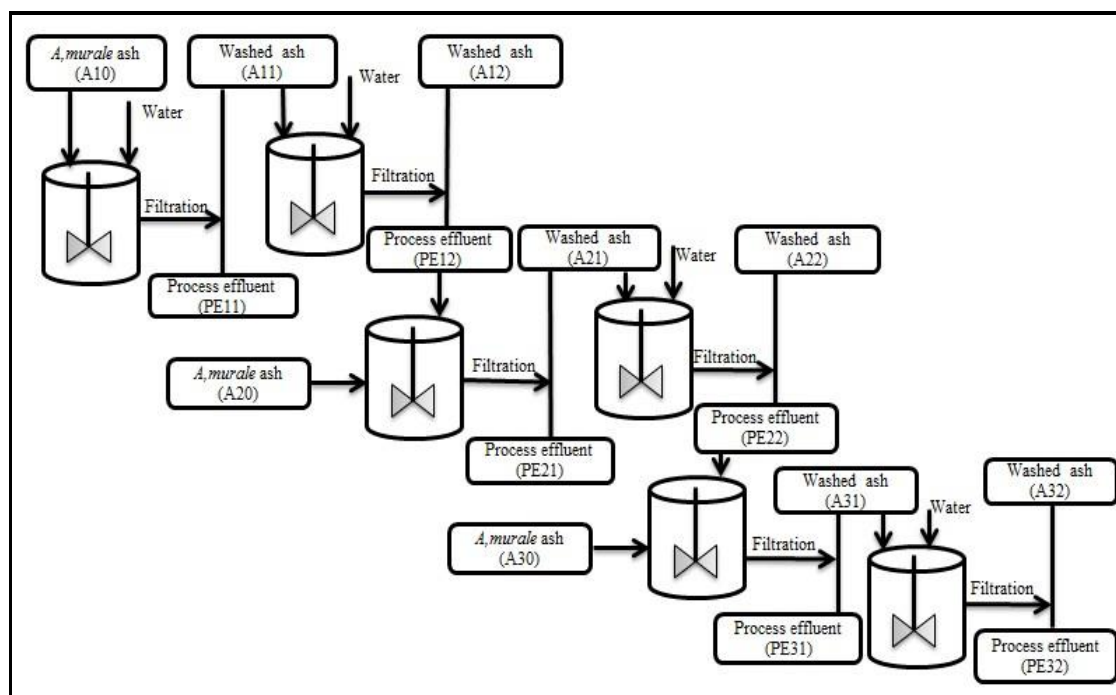


Figure 3. 3: Process diagram of simulating counter-current washing following the 2nd method

2.3 Results and discussion

2.3.1 Influence of the stirring duration

In the previous contribution (Barbaroux et al., 2012), the agitation duration was 15 min.

In Fig.3.4, K concentration in the supernatant was plotted against time.

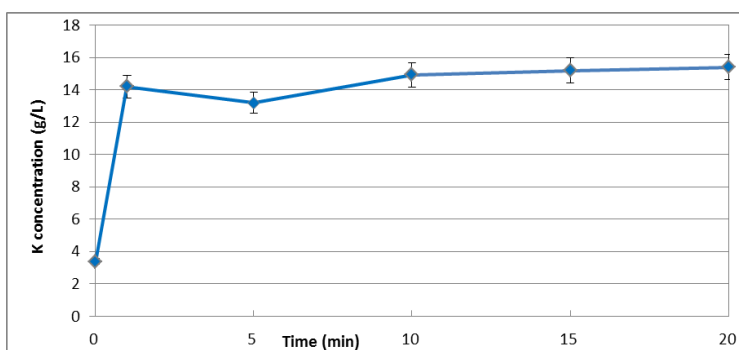


Figure 3. 4: Variation of K concentration in the supernatant with the agitation time

K concentration was immediately high and reached a plateau (about 15 g L^{-1}) after 10 min. This experiment was repeated seven times, the results were almost the same except when the ashes were not made in the same combustion batch, and then the minimum and maximum K concentrations in the supernatant were a little different (the deviation was also shown in the figure). At last, in order to make sure all the K_2CO_3 in the raw ash was well dissolved in the water, for further experiments, we kept a duration of 15 min.

2.3.2 Influence of the ash mass fraction

One could think that the best conditions would be reached with the highest solid mass fraction possible. In our experiments, the highest mass fraction was 20 %, because above

20 %, the suspension became too dense (like a mud); moreover, the ash was light and could not be immersed in a very small water volume, as shown in Fig.3.5.

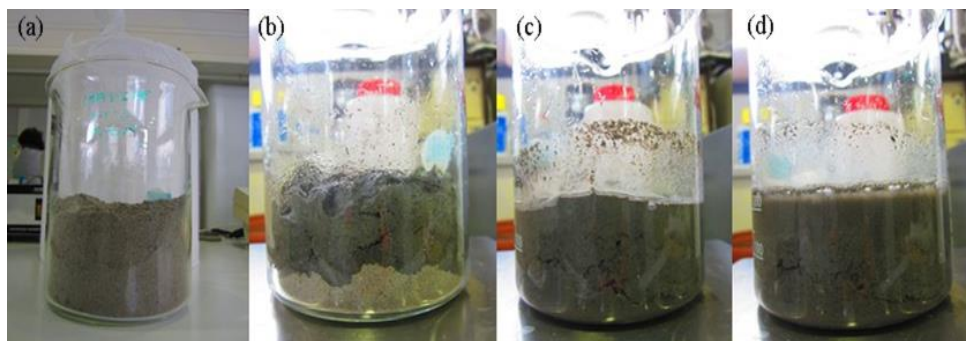


Figure 3. 5: Raw ashes and mixtures observed at different ash mass fractions:

a) Raw ashes; b) mass fraction of 50 %; c) mass fraction of 30 %; d) mass fraction of 20 %

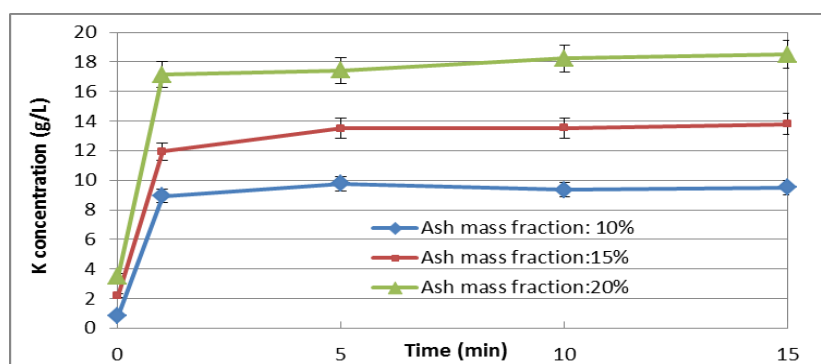


Figure 3. 6: K concentrations evolution at different ash mass fractions

In Fig. 3.6, we plotted the evolution of K concentration for the 3 selected mass fractions, 10, 15 and 20 %. In all cases, the concentrations reached a plateau that began at 5-10 min. The final concentrations were 9.5 ± 0.6 , 13.8 ± 0.8 and $18.4 \pm 1.1 \text{ g L}^{-1}$, at 10, 15 and 20 % ash which corresponded to nearly the same K removal rates of $75.0 \pm 5.0 \%$ after the first washing step. K removal was not limited by the solubility of K_2CO_3 , which is 111 g in 100 g water at 20 °C (Linke, 1965). The removal rate of 75 % was assigned to the presence of another phase containing K.

As a conclusion, it is better to use a solid mass fraction as high as possible in order to save water: for 1 kg ashes, the washing stage at 10 % needs 9 L deionized water, while it only needs 4 L at 20 %. Then a mass fraction of 20 % will be chosen for the other experiments.

2.3.3 Influence of the stirring speed

In Figure 3.7, we plotted the evolution of K concentration in the supernatant at 3 stirring speeds. The curves could be superimposed, meaning that K removal was not affected by the stirring speed. About the K concentration reached a plateau of about 10 g L^{-1} as here the mass fraction was of 10 %, and this result was correspond with that in Fig.3.6. Then, in order to save energy, the lowest speed, 500 rpm, was selected for further experiments.

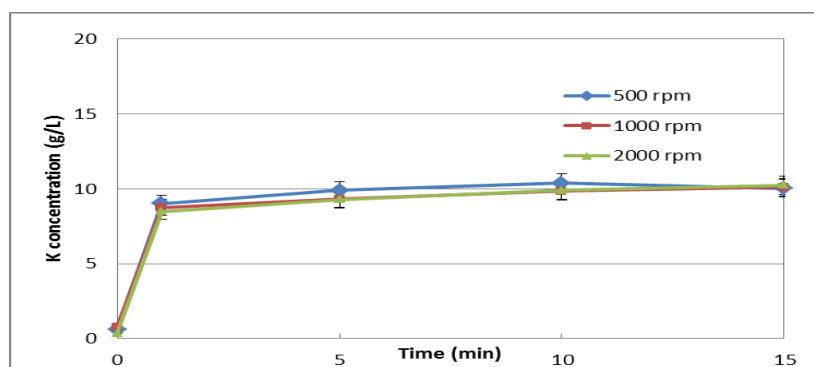


Figure 3. 7: K concentration evolution in the supernatant at 3 stirring speeds, 500, 1000 and 2000 rpm

2.3.4 Influence of the washing methods

a) Results obtained with method M#1

Method M#1 was tested with 3 successive washing steps ($n=3$) (Fig.3.2). The condition was of 60.0 g raw ash washed with 240 mL deionised water. The final concentrations of

K in the effluents were $16.2 \pm 1.1 \text{ g L}^{-1}$ in PE1, $1.7 \pm 0.1 \text{ g L}^{-1}$ in PE2 and lower than $0.5 \pm 0.0 \text{ g L}^{-1}$ in PE3 (Fig.3.8).

The mass balance and K removal yields are given in Table 3.1: $76.2 \pm 6.9 \%$ K was eliminated after the second washing. The K elimination in third step washing was about $2.8 \pm 0.3 \%$. A fraction of $15.5 \pm 1.5 \%$ of K remained in the washed ashes (A_{13}).

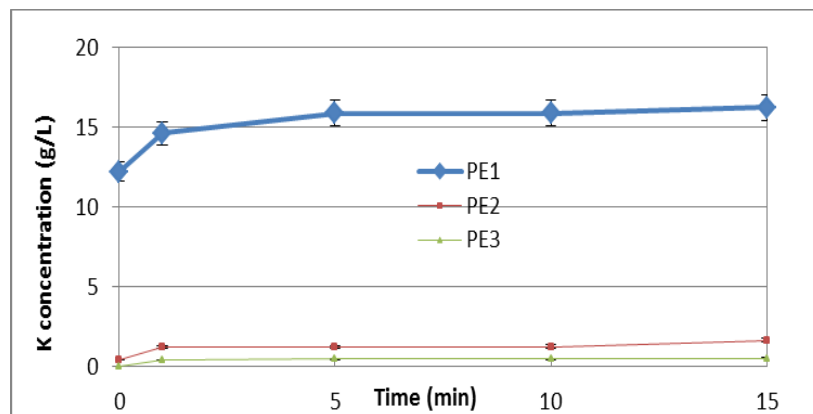


Figure 3. 8: K concentrations in the supernatants PE1, PE2 and PE3, following method M#1

Table 3. 1: K mass balance and removal yields in three steps washing

	Mass (mg)	Removal (%)
Raw ash (A_{10})	6003 ± 360.2	100.0
Effluent (PE_1)	4021 ± 201.0	67.2 ± 6.1
Effluent (PE_2)	537.2 ± 26.9	9.0 ± 0.8
Effluent (PE_3)	170.0 ± 8.5	2.8 ± 0.3
Washed ashes (A_{13})	924.5 ± 55.5	15.5 ± 1.5

Furthermore, XRD analysis of the raw (A_{10}) and washed ash (A_{11} and A_{12}) was carried out (Fig.3.9). The peaks marked 1, 2 and 3 in A_{10} have disappeared in A_{12} . According to the database of the International Centre for Diffraction Data, these peaks corresponded to K_2CO_3 . This proved that the washing process was really effective to dissolve K_2CO_3 in the raw ash. The comparison between the ashes washed once (A_{11}), twice (A_{12}) and three

times (A₁₃) showed no difference (Fig.3.10). This result was expected since after the first washing, the low amount of K₂CO₃ remaining in the ash was hardly visible by XRD.

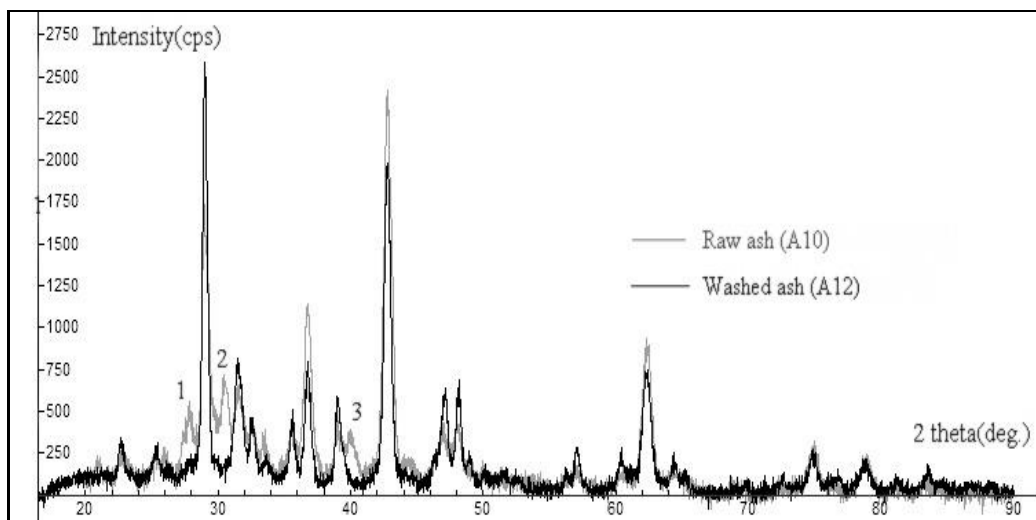


Figure 3. 9: XRD pattern of the raw and washed ashes (A10 & A12)

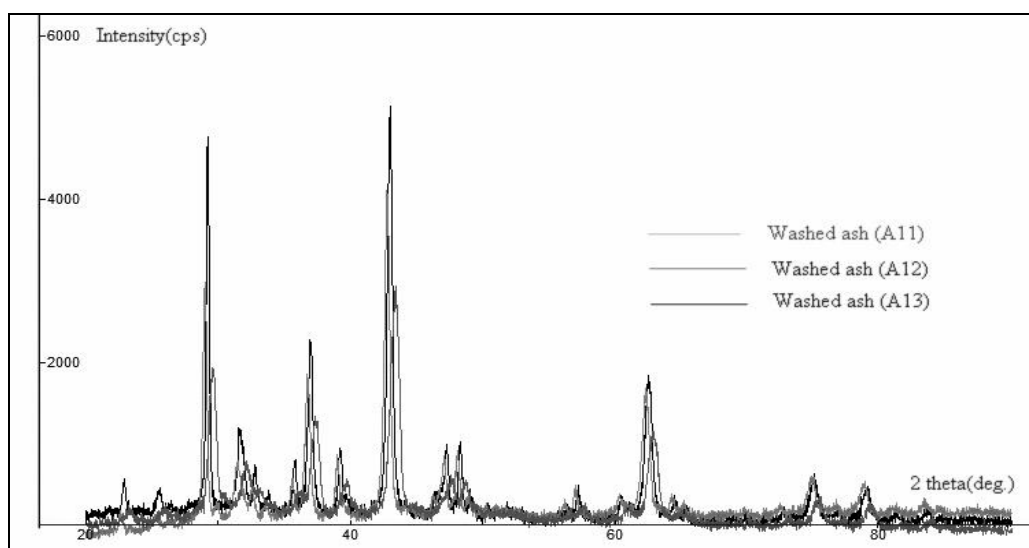


Figure 3. 10: XRD pattern of the washed ashes (A11 & A12 & A13)

Method M#1 was then tested with 2 washing steps (n=2). The mass balance is presented in Table 3.2. After, experiments were repeated seven times. 39.7 g dried ashes (A₁₂) were obtained from 50.0 g raw ashes (A₁₀), the mass reduction was 19.1 %, a little lower than

the reduction of 23.6 % (Barbaroux et al., 2012). K concentration in the ash was reduced from 101.1 g kg⁻¹ in A₁₀ to 23.7 g kg⁻¹ in A₁₂, and K concentration in the effluent (PE₁) was 15.9 ± 0.8 g L⁻¹ and 3.3 ± 0.2 g L⁻¹ in PE₂. The results in Table 3.2 showed that 63.2 ± 5.7 % K was removed in the first washing, and 12.9 ± 1.2 % in the second step, and total 76.1 ± 6.9 %, which is consistent with the previous results.

Table 3. 2: K mass balance and recovery yields with method M#1, two steps

	Mass (mg)	Removal (%)
Raw ash (A ₁₀)	5059 ± 303.6	100.0
Effluent (PE ₁)	3186 ± 159.3	63.2 ± 5.7
Effluent (PE ₂)	651.8 ± 32.6	12.9 ± 1.2
Washed ashes (A ₁₂)	914.0 ± 54.8	18.1 ± 1.8

To finish, method M#1 was tested with 8 steps (n=8), actually, washing was continued until pH was constant. After 8 steps, 38.5 g dried ash (A₁₈) were obtained from 50.0 g raw ashes (A₁₀). The pH of the effluents decreased from 12.5 to 10.9 and the K content was reduced from 19.2 ± 1.0 g L⁻¹ to 0.2 g L⁻¹ (Fig.3.11).

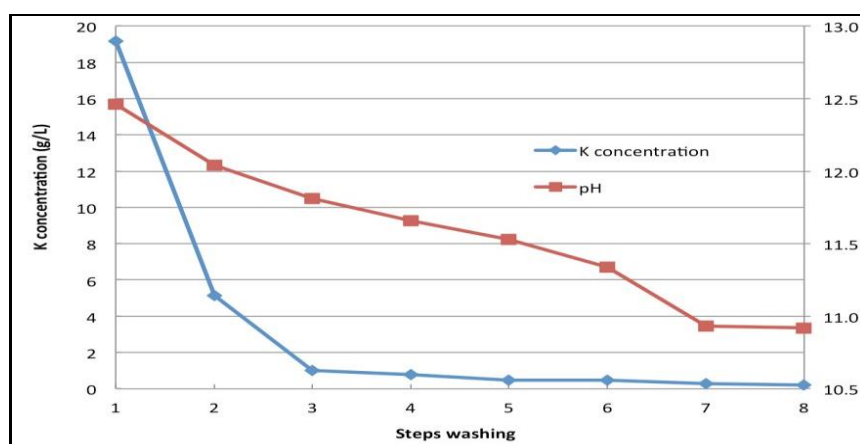


Figure 3. 11: K concentrations and pH change with the washing steps

Table 3. 3: K mass balance and removal yields with method M#1, eight steps

	Mass (mg)	Removal (%)
Raw ash (A ₁₀)	5059 ± 304.0	100.0
Effluent (PE ₁)	3069 ± 153.0	60.9 ± 5.5
Effluent (PE ₂)	829.2 ± 41.5	16.4 ± 1.5
Effluent (PE ₃)	194.6 ± 9.7	3.9 ± 0.3
Effluent (PE ₄)	147.9 ± 7.4	2.9 ± 0.3
Effluent (PE ₅)	92.8 ± 4.6	1.8 ± 0.2
Effluent (PE ₆)	91.6 ± 3.8	1.8 ± 0.2
Effluent (PE ₇)	54.6 ± 2.7	1.1 ± 0.1
Effluent (PE ₈)	56.9 ± 3.2	1.1 ± 0.1
Washed ashes (A ₁₈)	867.4 ± 52.0	17.2 ± 1.7

At the end, K removal ratio was 90.0 ± 8.1 %. As shown in Table 3.3, the first steps were the most important and we considered that 2 steps (removal ratio of 77.3 ± 7.0 %) were enough for the washing process.

To conclude this part, method M#1 performed with 2 steps enabled us to reach an efficiency as high as previously (Barbaroux et al. 2012) but without intermediate drying. Then energy, time and water were saved.

b) Results obtained with method M#2

Compared with M#1 that required at least 600 mL of deionized water to wash 75 g ashes (2 washing steps, 25 g per time, ash mass fraction 20 %), method M#2, inspired from counter-current washing (Fig. 3.12), only needed 400 mL deionized water. Then one-third was saved. The last effluent PE₃₂ can also be used to wash new raw ashes A₄₀. Then nearly half volume of deionized water was saved. Moreover, for 25 g raw ash washed in

100 mL water, K concentration was higher in the effluent PE₃₁ (23.9 g L⁻¹, Fig. 3.12) than in the washing solution of M#1 (the maximum was of 18.4 g L⁻¹, Fig. 3.6).

In view of this, we speculated that in the pilot process, with the simulating counter-current washing method, the K concentration in the first process effluent (PE_{n1}, n=2, 3...) will be more and more concentrated. The mass balance is given in Table 3.4.

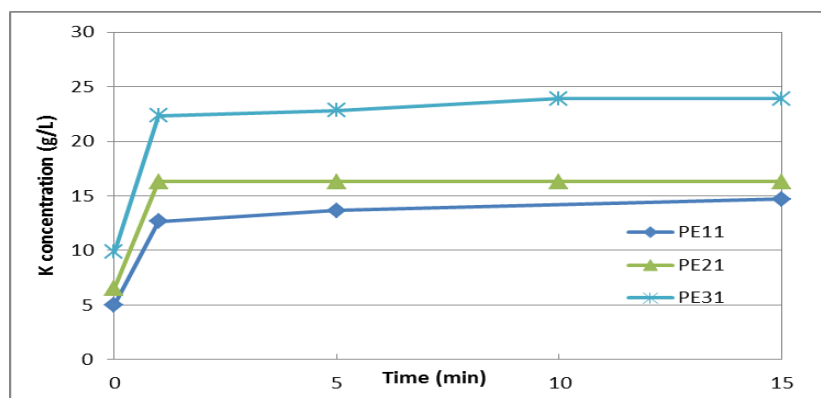


Figure 3. 12: K concentrations in each first effluent of simulating counter-current washing M#2

Table 3. 4: K mass balance and removal yields with method M#2

	Mass (mg)	Removal (%)
Raw ashes (A ₁₀)	2495 ± 150.0	100.0
Effluent (PE ₁₁)	1556 ± 78.0	62.6 ± 5.6
Effluent (PE ₁₂)	482.9 ± 24.1	19.4 ± 1.8
Washed ashes (A ₁₂)	346.7 ± 20.8	13.9 ± 1.4
Total (A ₂₀ +E ₁₂)	2770 ± 166.0	
Effluent (PE ₂₁)	1632 ± 82.0	59.1 ± 5.3
Effluent (PE ₂₂)	436.5 ± 21.8	15.8 ± 1.4
Washed ashes (A ₂₂)	320.4 ± 19.2	11.6 ± 1.1
Total ashes (A ₃₀ +E ₂₂)	2462 ± 148.0	
Effluent (PE ₃₁)	1639 ± 82.0	66.8 ± 6.0
Effluent (PE ₃₂)	434 ± 21.7	17.7 ± 1.6
Washed ashes (A ₃₂)	317.5 ± 19.1	12.9 ± 1.3

After the 1st washing with 100 mL deionized water, 15.3 g dried ashes (A₁₂) were obtained from 25.0 g ashes (A₁₀). After filtration, 93 mL PE₁₂ were obtained to wash 23.0 g of new raw ashes (A₂₀) at 20 % mass fraction. K removal rate from the raw ash was $82.0 \pm 7.4 \%$, $74.9 \pm 6.8 \%$, and $84.5 \pm 7.6 \%$ (Table 3.4), which was nearly the same or even a little higher than that with M#1 (table 3.1-3.3).

2.3.5 Dissolution equilibrium calculations in the washing step

Using method M#1 (n=2), we noticed that K concentration increased with time and reached a plateau both in PE1 and PE2 (Fig.3.8) while Ca and Mg concentrations in PE₁ and PE₂ changed with different trends (Fig.3.13 & 15). We suspected that precipitation and dissolution of CaCO₃ and MgCO₃ were likely to occur. To check these assumptions, we calculated the ion products in solution that we compared to the solubility products (K_{sp}) (Weast, 1985-1986).

The ion product for CaCO₃ is defined as:

$$Q_{\text{c CaCO}_3} = \gamma_{\text{Ca}} \gamma_{\text{CO}_3} [\text{Ca}^{2+}][\text{CO}_3^{2-}] \quad (\text{Eq. 3.1})$$

Where $Q_{\text{c CaCO}_3}$ is the ion product (mol L⁻¹)², γ_{Ca} and γ_{CO_3} are the activity coefficients of Ca²⁺ and CO₃²⁻, [Ca²⁺] and [CO₃²⁻] are the concentrations of Ca²⁺ and CO₃²⁻ (mol L⁻¹).

The activity coefficients were calculated from:

$$\log \gamma_{\pm} = -A|Z + Z -| \left[\frac{\sqrt{I}}{1+a\sqrt{I}} + bI \right] \quad (\text{Eq. 3.2})$$

$$I = 0.5 \sum c_i z_i^2 \quad (\text{Eq. 3.3})$$

With c_i: molar concentrations of ion I (mol L⁻¹), z_i: ionic charge of ion I; A=0.51, a=1.0, b=-0.2

For Ca, in PE1, the concentration decreased with time from 45.5 to 28.0 mg L⁻¹ and reached a plateau. However, in PE2, the concentration increased a little and then kept nearly at the same level (Fig.3.13).

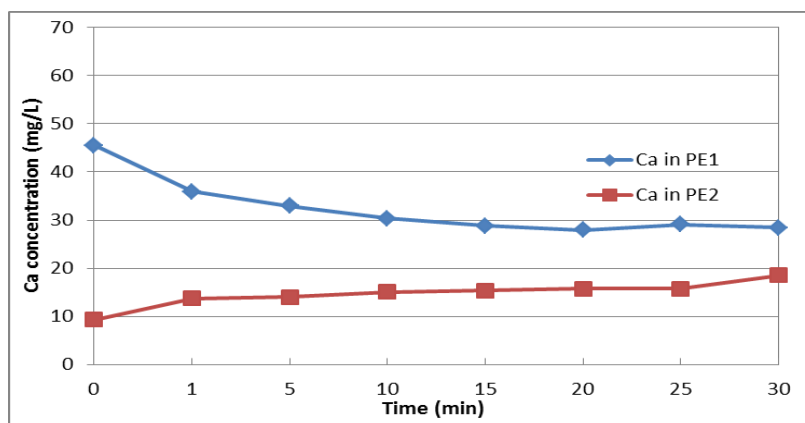


Figure 3. 13: Evolution of Ca concentrations in PE1 and PE2

This can be explained that Ca in the ashes was dissolved in the water as Ca²⁺ at the beginning. With the agitation, pH after 15 min was of 11.3.

Calculations showed with Eq. 3.1 to 3.3 showed that: $I=1.38 \text{ mol L}^{-1}$,

And $Q_{\text{CaCO}_3} = 9.7 \cdot 10^{-5} (\text{mol L}^{-1})^2$, that was much higher than the solubility product $K_{\text{spCaCO}_3} = 4.95 \cdot 10^{-9} (\text{mol L}^{-1})^2$ (Weast, 1985-1986)

Then, precipitation of CaCO₃ occurred and Ca²⁺ concentration decreased in PE1.

This was confirmed by visual observation: some small white spots in the washed ashes (A₁₂) were recorded (Fig. 3.14). This was also proved by adding H₂SO₄ into the ashes, much CO₂ was generated.



Figure 3. 14: Appearance of the ashes after filtration (the white spots are CaCO_3)

In PE2, $Q_{\text{Ca(OH)}_2} = 9.1 \cdot 10^{-11} \text{ (mol L}^{-1}\text{)}^3$ lower than $K_{\text{spCa(OH)}_2} = 7.88 \cdot 10^{-6} \text{ (mol L}^{-1}\text{)}^3$

Then, no precipitation of Ca(OH)_2 was expected. Therefore, Ca^{2+} reappeared in the effluent solution and Ca^{2+} concentration increased a little.

Considering Mg (Fig.3.15), in PE1, the transfer of CO_3^{2-} from the ash to the aqueous phase with the dissolution of K_2CO_3 implied CaCO_3 precipitation. But given that $K_{\text{spCaCO}_3} = 4.95 \cdot 10^{-9} \text{ (mol L}^{-1}\text{)}^2 \ll K_{\text{spMgCO}_3} = 1.15 \cdot 10^{-5} \text{ (mol L}^{-1}\text{)}^2$, no precipitation of MgCO_3 occurred since there was not enough CO_3^{2-} to react with Mg^{2+} .

$$Q_{\text{MgCO}_3} = 4.55 \cdot 10^{-6} \text{ (mol L}^{-1}\text{)}^2 < K_{\text{spMgCO}_3}$$

Then Mg^{2+} concentration reached a plateau.

In PE2, as new deionized water was used to wash A_{11} , some more K_2CO_3 dissolved and then there was more CO_3^{2-} in PE2.

Then $Q_{\text{MgCO}_3} = 6.7 \cdot 10^{-5} \text{ (mol L}^{-1}\text{)}^2 > K_{\text{spMgCO}_3}$, then MgCO_3 was generated, Mg^{2+} concentration decreased.

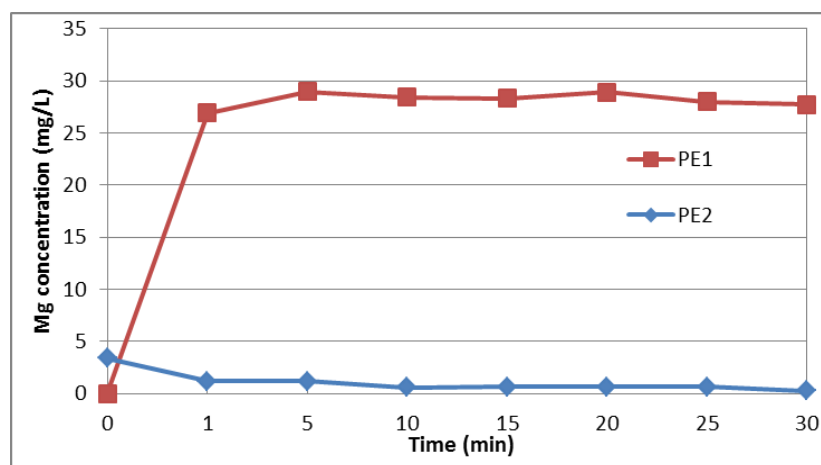


Figure 3. 15: Evolution of Mg concentrations in PE1 and PE2

2.4 Conclusions

The detailed study of the washing step enabled us to determine the best conditions. At the bench scale, we consider that the best method is M#1: two washing steps, 20 % solid, 15 min with a stirring speed of 500 rpm. It enabled us to reach a K removal ratio of *ca.* 80.0 ± 5.0 %. Compared to (Barbaroux et al., 2012), 50 % of water was saved when using method M#2 as well as energy since there is no drying at 100 °C between the two steps washing.

In the lab, we continued to use M#1 with two steps washing was chosen. While at the pilot scale, method M#2 will be implemented to shorten the process time, save energy and economize the producing cost. At a higher scale, the effluent containing K could be reused as fertilizing agent.

In all cases, Ni remains in the ashes.

3 Part II-Acid leaching

3.1 Introduction

After the washing step, the ash (A_{12}) contained Ni, Ca, Mg and Fe as major elements. The next step is Ni transfer from the ash into the liquid phase, that was can be achieved by leaching with H_2SO_4 (Barbaroux et al., 2012). The objective of this part is to re-visit this step and search the best conditions in order to save matter and energy while solubilizing Ni, and if possible to separate Ni from Mg at this stage.

The first idea was to save energy and try to solubilize Ni at room temperature. The second idea was to solubilize Ni selectively, leaching Mg first and Ni after. Eventually, leaching at high temperatures, 70 and 95 °C, was also studied.

3.2 Materials and methods

3.2.1 Experiments to test acid leaching at room temperature

a) Tests with washed ash

12.5 g of washed ash (A_{12}) were put into 50 mL deionized water; H_2SO_4 (0.5 M in a 1st trial then 2M) was introduced drop by drop from a burette to reach a pH between 7 and 8. The solution was stirred by magnetic stirring (Agitateur magnetique 35011, Bioblock Scientific) and pH was measured by a pH meter (PHM210, standard pH meter).

Then, 3 experiments were carried out, starting from 12.5 g of A_{12} in 50 mL deionized water:

1. 1 mL H_2SO_4 (2.0 M) was dropped into the beaker per 10 min for 8 times: pH went up slowly so another 2 mL H_2SO_4 was added drop by drop for 40 h to reach a pH of 8.0.
2. 5 mL H_2SO_4 (2.0 M) were introduced once at the beginning. After 4 h, the pH was 8.9,

- but it continued to increase slowly (0.01 per 10 min). Samples were withdrawn (solutions and solids). Another 5 mL H_2SO_4 (2.0 M) was dropped into the solution, and pH decreased to 5.4. However, the pH increased quickly at the first hour, then went up slowly. After 40 h, this test was stopped and pH was 8.6.
3. 10 mL H_2SO_4 (2.0 M) were dropped into the beaker once at the beginning. pH dropped from 11.5 to 4.7 immediately, then it increased quickly at the beginning and more slowly after. This test was stopped also after 40 h and the pH was 8.9.

b) Tests of selective leaching

The previous experiments showed that the metal concentrations in L_1 were different at different pHs. A series of studies was done to select the best pH. Automatic Titrator (Titrator DL50 Graphix) was used to control the pH of 5, 4, 3, 2, and 1, separately. Every test was done in the same situation (5.0 g of washed ashes (A_{12}) with 20 mL water, stirring speed of 100 rpm, and duration for 8 h). The only difference was that 0.5 M H_2SO_4 was used to control the pH at 5, 4 and 3, but 1.0 M H_2SO_4 was used to control the pH of 2 and 1 in order to reduce the reaction time and the acid volume. After 8 h, 500 mL deionized water was used to wash the leached ashes, and then the element concentrations in L_1 and washing effluents were analyzed by ICP-AES. The leached ashes after washing were also analyzed by XRD to be compared with the ash before acid leaching.

3.2.2 Acid leaching at high temperature

Leaching was tested at 70 ° and 95 °C, at different ash mass fraction, concentration of acid, and duration.

Firstly, we searched a suitable ash mass fraction. 5 g of A₁₂ were put into 20 mL H₂SO₄ (2 M) (ash mass fraction of 20 %) in the beaker using heating plate to control the temperature at 70 °C (Thermometer HD8601). After 30 min, there was no more liquid in the beaker, another 20 mL acid were added to continue the experiment for 2 h. This time, the solid ratio was suitable for agitation.

Next, batches were conducted by mixing 5 g of A₁₂ with 45 mL H₂SO₄ (1 M, 2 M, 3 M) (ash mass fraction of 10 %), using heating plate to control the temperature at 70 °C and 95 °C. To compensate water evaporation, deionized water was added every 20 min to keep the total volume constant. Samples (1 mL) were withdrawn after 1, 2, 3 and 4 h and filtered.

3.3 Results and discussion

3.3.1 Controlling the pH

The composition of the washed ash A₁₂ obtained by ICP-AES analysis was as follows: Ni (188.5 mg·g⁻¹), Ca (177.3 mg g⁻¹), Mg (50.5 mg·g⁻¹), Fe (23.7 mg·g⁻¹). We supposed that Ni was in the form of NiO and Ni(OH)₂ and Mg in the form of Mg(OH)₂ and MgCO₃. Then we calculated the best pH zone to remove Mg. We used the following values at 25 °C: $K_{spMg(OH)_2}=5.66 \cdot 10^{-12} \text{ (mol L}^{-1}\text{)}^3$, $K_{spMgCO_3}=1.15 \cdot 10^{-5} \text{ (mol L}^{-1}\text{)}^2$ and $K_{spNi(OH)_2}=5.54 \cdot 10^{-16} \text{ (mol L}^{-1}\text{)}^3$ (Weast, 1985-1986).

The pHs corresponding to the dissolution at equilibrium of Mg(OH)₂ and Ni(OH)₂ were calculated from:

$$pH = -\lg Kw - \frac{1}{2} \lg Mg^{2+} - \frac{1}{2} \lg K_{spMg(OH)_2} \quad (\text{Eq. 3.4})$$

$$pH = -\lg Kw - \frac{1}{2} \lg Mg^{2+} - \frac{1}{2} \lg K_{spMgCO_3} \quad (\text{Eq. 3.5})$$

$$pH = -\lg K_w - \frac{1}{2} \lg Ni^{2+} - \frac{1}{2} \lg K_{spNi(OH)_2} \quad (\text{Eq. 3.6})$$

Then, for $(Mg^{2+}) = 0.05 \text{ mol L}^{-1}$, we obtained $pH=9.0$ (Eq.3.4). Then, if $pH \geq 9.0$, Mg would be in the form of $Mg(OH)_2$. Or else, the reaction would move to the right, and Mg would be in the form of Mg^{2+} . For $MgCO_3$, pH would be 12.2 (Eq.3.5). So if we control the $pH < 9.0$, Mg would be in the form of Mg^{2+} .

The pH corresponding to the dissolution of $Ni(OH)_2$ would be 6.9 for $(Ni^{2+}) = 0.1 \text{ mol L}^{-1}$ (Eq. 3.6). Then at pH higher than 6.9, Ni will be in the form of $Ni(OH)_2$. Hence, the pH zone between 7 and 8 was chosen to keep Mg as Mg^{2+} and Ni as $Ni(OH)_2$. Then, Mg and Ni can be separated after filtration.

3.3.2 Recovery rates of Mg and Ni from A_{12}

In Table 3.5, we present the recovery rates of Mg and Ni attained at room temperature corresponding to the different acid additions.

Table 3. 5: Recovery rates of Mg and Ni from washed ashes (A_{12}) and raw ashes (A_{10})

Ashes	conditions		Recovery %	
	H_2SO_4 (mL)	Duration (h)	Mg	Ni
A_{12}	10	4	6.0	0.0
A_{12}	10	40	8.0	0.0
A_{12}	5	4	4.0	0.0
A_{12}	10	4	6.0	0.0
A_{12}	30	4	28.0	3.0
A_{12}	10 (1 mL min^{-1})	40	15.0	0.0
A_{12}	10 (10 mL once)	40	8.0	0.0

Three conclusions were drawn from these balances:

1. Adding the same volume of sulfuric acid (10 mL), after 4 and 40 h, the recovery of Mg

remained low (4 to 6 %).

2. For the same duration (4 h), with increasing the volume of sulfuric acid added (from 5 to 30 mL), Mg recovery rate increased up to 38 % but Ni was lost (3 %).
3. Using the different methods of adding the acid, the results were different. If the acid was introduced slowly, more Mg could be solubilized at this step, as much as 15.0 %. If all the needed acid dropped at once time, the recovery of Mg was lower than adding slowly.

We conclude that it was not possible to remove Mg without dissolving Ni by simple acid addition at room temperature.

3.3.3 Selective leaching

With the help of Automatic Titrator, the volume of H₂SO₄ for controlling pH was known (Table 3.6). Mg and Ni concentrations and recovery rates at different pHs are shown in Fig.3.16.

Table 3. 6: Materials of selective leaching

pH	H ₂ SO ₄ (mL)		Water of washing the leached ashes(SW ₁)
	0.5 M	1.0 M	mL
5	41.5		500
4	46.7		500
3	48.4		500
2		27.6	500
1		37.6	500

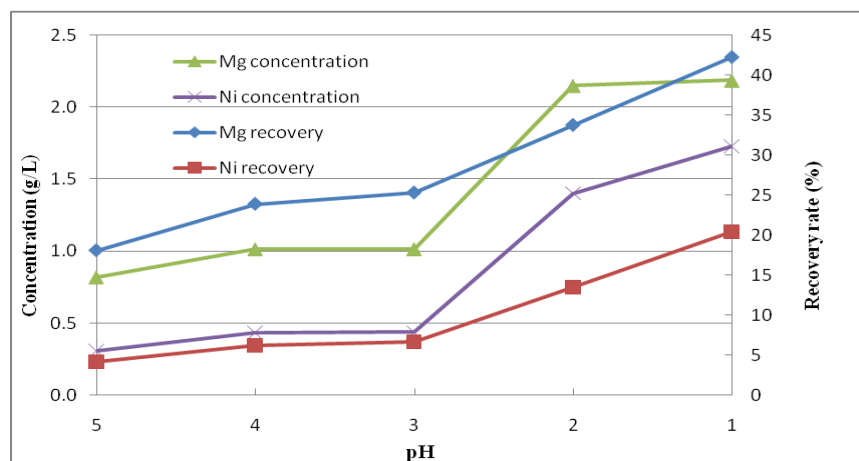


Figure 3. 16: Mg and Ni concentrations and recovery rates at different pH

Results showed that at pH=1, Ni concentration was the highest among those five points (1.7 g L^{-1}). With pH decreasing from 5 to 3, Ni concentration increased a little but a sharp increase was recorded between 2 and 1. At the same time, Mg changed in the same trend. Decreasing pH, Mg concentration went from 0.8 g L^{-1} to 2.2 g L^{-1} .

Mg and Ni recovery rates at this selective leaching step showed that the maximum recovery of Ni was 20.0 %. Mg followed the same trend with a recovery rate increasing to 42.0 %.

Ni and Mg concentrations in the effluents (500 mL washing water) at different pH were in the same trends. This proved these results were interrelated, after using the same volume deionized water washing SW_1 , Ni and Mg concentrations were still highest in the effluent at pH=1 than at other higher pH.

Then Ni leaching could not be conducted at room temperature.

3.3.4 Optimal parameters for acid leaching

According to (Barbaroux et al., 2012), the parameters of acid leaching were 95 °C, 1.9 M H₂SO₄, 240 min, solid ratio = 150 g L⁻¹, the obtained leachate contained 10.2 g L⁻¹ of Ni, corresponding to 96.0 % of Ni solubilization.

The results that we obtained at 70 and 95 °C at different acid concentrations are presented in Table 3.7. Very high Ni solubilization rates could be attained even at 70 °C. But the required acid concentration was high (3 M). Then the gain obtained would be lost at the neutralization step. For this reason, we selected a temperature of 95 °C and 2 M acid: after 2 h, 99.4 ± 7.9 % Ni was transferred from A₁₂ into L₁.

Table 3. 7: Ni extraction yield from *A. murale* washed ashes

H ₂ SO ₄ (M)	Temperature (°C)	Extraction (Ni %)			
		Time (h)			
		1	2	3	4
1	70	56.8 ± 4.0	61.8 ± 4.3	69.2 ± 4.8	78.8 ± 5.5
2	70	67.1 ± 5.4	78.8 ± 6.3	80.1 ± 6.4	94.0 ± 7.5
3	70	74.8 ± 6.0	88.0 ± 7.0	98.7 ± 7.9	100.2 ± 7.5
2	95	95.9 ± 7.7	99.4 ± 7.9	100.6 ± 7.9	101.9 ± 8.1

The comparison between XRD analysis (Fig.3.17) of the washed ash (A₁₂) and the ashes after acid leaching (SW₁) have shown the disappearance of peaks (I, II, III, IV, V and VI) corresponding to CaCO₃ and MgCO₃ (database of the International Centre for Diffraction Data). In SW₁, the peaks (1, 2, 3-6) corresponding only to CaSO₄, and no peaks matched with MgSO₄ and NiSO₄, which was another evidence of Ni transfer from the washed ashes (A₁₂) into the leachate (L₁).

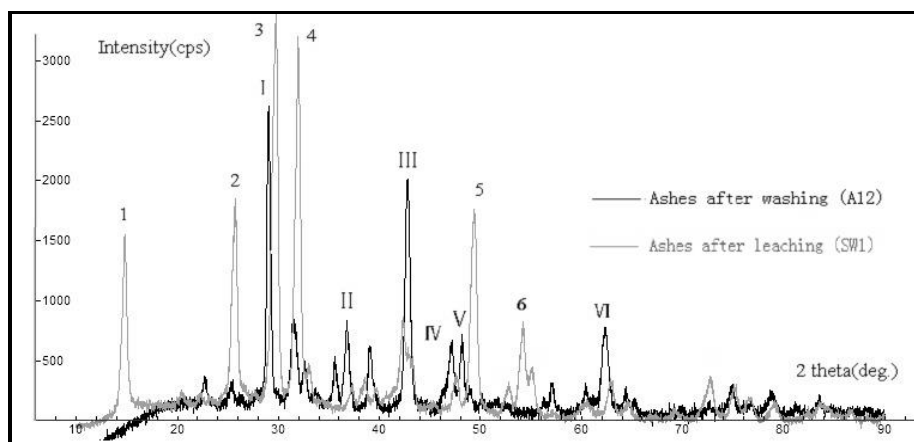


Figure 3. 17: XRD pattern of the ashes (A_{12} & SW_1)

Finally, the following conditions were chosen as the best ones for acid leaching: **95 °C as in (Barbaroux et al., 2012), 2 h instead of 4 h, solid concentration = 111 g L⁻¹ (ash mass fraction of 10 %).**

3.4 Conclusion

In this part, we have investigated a wide range of conditions for acid leaching. Previous calculations had shown that selective leaching of Ca, Mg and Ni should be possible at room temperature. But actually our results have shown that only a small fraction of Ni (less than 20 %) could be leached in these conditions. From a scientific point of view, further calculations and modelling could be done to refine the research, explain the results and predict the results in other conditions.

From an applied point of view, in order to optimize the ANSH production process, we have confirmed that a high temperature was required to solubilize Ni from the washed ash. Increasing temperature up to 70 °C would be enough, but to avoid the use of a concentrated acid, 95 °C is better. The optimal conditions were then 10 % solid ratio, 95 °C, sulfuric acid of 2 M and 2 h reaction.

4 Part III – Leachate purification before crystallization

4.1 Introduction

The objective is to improve this step in order to purify the solution (L_1) as much as possible before crystallization. The solution has a very low pH (0.0-0.2) and contains Ni (10.2 g L^{-1}), Mg (2.6 g L^{-1}) and Fe (0.4 g L^{-1}).

Then neutralization was performed to increase pH and remove Fe by precipitating $\text{Fe}(\text{OH})_3$. It would be done by addition of NaOH 5 M (Barbaroux et al., 2012). In this work we replaced NaOH by $\text{Ca}(\text{OH})_2$ to avoid the formation of Na_2SO_4 . Then the solution volume is decreased by evaporation and Mg is removed by precipitating MgF_2 .

4.2 Materials and methods

4.2.1 Neutralization

The pH of L_1 was about 0.0-0.2. At first we repeated once neutralization by NaOH 5 M. Then we used a suspension of $\text{Ca}(\text{OH})_2$ (10 or 20 wt %) which was added drop by drop into 400 mL L_1 under magnetic agitation. The reaction was conducted for 1 h, until pH reached 4 to 5 (Mettler Toledo, DG 111-SC). The suspension was filtered by a vacuum pump. The resulting leachate was called L_2 .

4.2.2 Adding NaF to eliminate Mg

In order to remove Mg from L_2 , according to the solubility of MgF_2 (0.013 per 100 g water at 25 °C and insoluble at 90 °C) and NiF_2 (2.56 per 100 g water at 25 °C)(Lide, 2003), a mass of NaF corresponding to an excess of 10 % compared to the stoichiometric quantity (3.5 g of NaF for 30 g of raw ashes) was added into L_2 under magnetic stirring at

room temperature (20 ± 5 °C) for 30 min in order to dissolve all NaF. The suspension was filtered and the filtrate called L₃.

4.2.3 Evaporation

L₃ was heated to 100 °C on a hot plate to evaporate water and reduce the volume by a factor of 3- 4. After evaporation, a small amount of insoluble residue was observed, and the final solution obtained after filtration was called L₄.

4.3 Results and discussion

4.3.1 Neutralization

The use of NaOH 5 M generated a significant amount of Na₂SO₄ at the first crystallization step since the solubility of Na₂SO₄ is 4.8 g per 100 g of water at 0 °C, while the solubility of Ni(NH₄)₂(SO₄)₂·6H₂O is 1.4 g per 100 g of water at 0 °C. Calculations have shown that if NaOH (5 M) was added to L₁ (obtained from 30 g of raw ashes A₁₀), 152 mL of NaOH (5 M) was consumed. Then the production of Ni(NH₄)₂(SO₄)₂·6H₂O salt was 24.3 g but with 34.6 g Na₂SO₄. These calculations have been confirmed by the experimental tests.

The photographs, XRD and IR analysis of Na₂SO₄ which generated in the crystallization process when NaOH was used as neutral agent are given in Fig.3.20 (a, b & c). High peaks of Na₂SO₄ (database of the International Centre for Diffraction Data) were recorded at 19, 28, 32 and 49° in Fig.3.21 (b). And according to the IR spectral data (Socrates, 2004), the significant band between 1080-1130 cm⁻¹ indicated the presence of

SO_4^{2-} ion in the crystal structure (Fig.3.21 (c)). Both analysis demonstrated that a rather high amount of Na_2SO_4 crystals were generated.

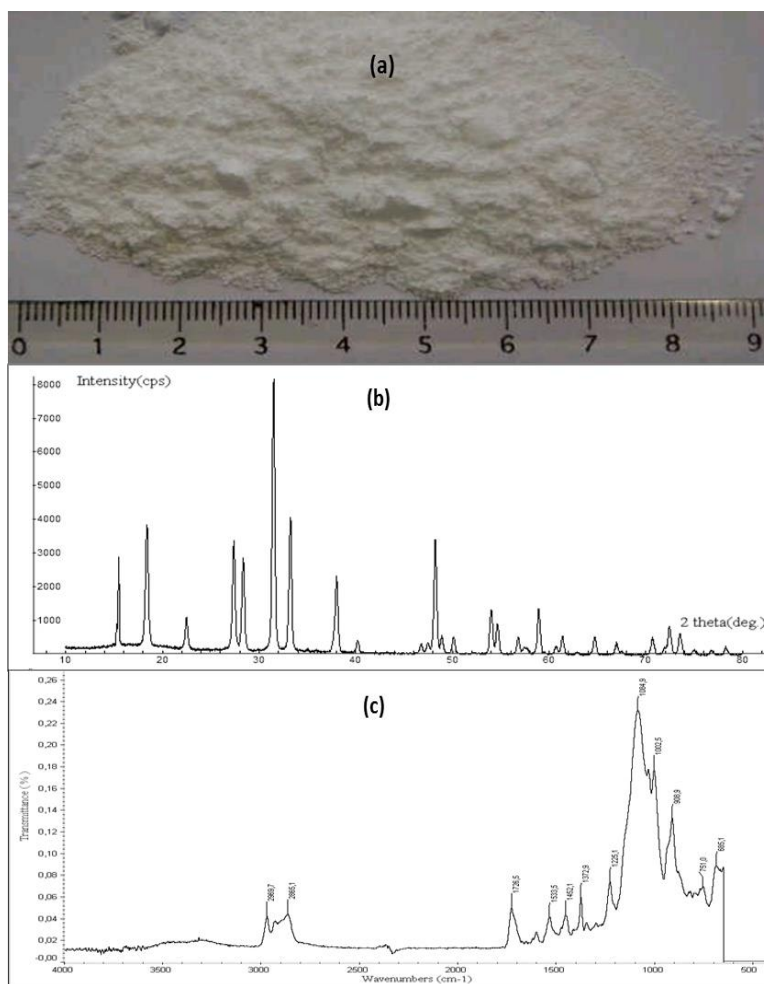


**Figure 3. 18: a) Photograph of Na_2SO_4 generated in crystallization process when using NaOH as neutral agent;
b) XRD patterns of the Na_2SO_4 ; and c) IR spectrum of the Na_2SO_4**

Replacing NaOH by $\text{Ca}(\text{OH})_2$ made CaSO_4 precipitate since the solubility of CaSO_4 is 0.205 g per 100 g of water at 25 °C (Lide, 2003). After filtration, we obtained a leachate (L_2) without CaSO_4 and Na_2SO_4 . The solid waste (SW_2) was mostly composed of CaSO_4 . The XRD pattern of SW_2 showed high peaks of CaSO_4 (peaks (1, 2- 5 at 15, 30, 33, 49 and 55°) (Fig. 3.19).

Since $\text{Fe}(\text{OH})_3$ solubility is $2.097 \cdot 10^{-9}$ g per 100 g of water at 15 °C (IUPAC-NIST Solubility Database, Version 1.0), it was precipitated and confirmed by peaks (I, II-V) (Fig.3.19).

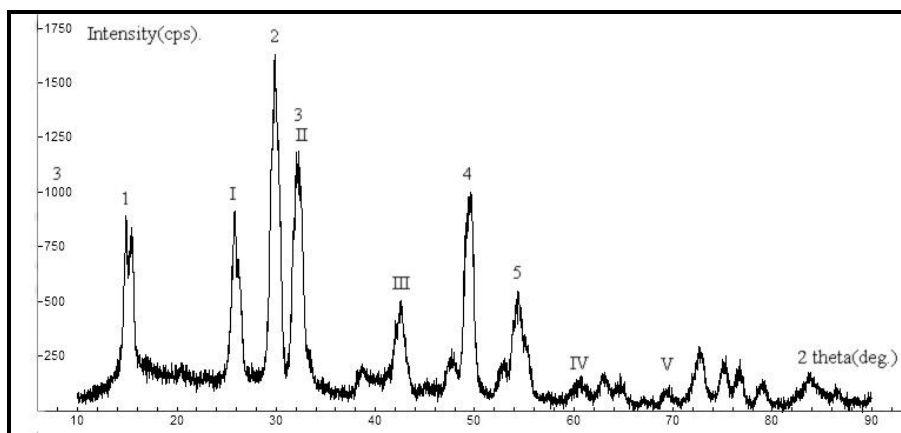


Figure 3. 19: XRD pattern of the solid waste (SW₂)

We tested different mass fractions of $\text{Ca}(\text{OH})_2$ suspension. At 20 % (27 g $\text{Ca}(\text{OH})_2$ in 110 mL water), the mixture became too dense (like a mud) and nearly 20 % of Ni was trapped with the precipitate of CaSO_4 and lost. Then a suspension of 10 % (27 g $\text{Ca}(\text{OH})_2$ in 245 mL water) was used to avoid these problems, the increase volume (the same mass of $\text{Ca}(\text{OH})_2$, but more than twofold volume of water) in L₂ can be easily compensated by evaporation. The Ni loss was confirmed by the green color of SW₂ obtained with a 20 % suspension while it remained white with a 10 % suspension (Fig.3.20).

Table 3. 8: Mass balance of the whole process

	Mass (mg)					Recovery (%)				
	K	Ca	Mg	Ni	Fe	K	Ca	Mg	Ni	Fe
Raw ash (30 g)	3208	3753	889.0	3803	145.3	100.0	100.0	100.0	100.0	100.0
Process effluent 1 (PE ₁)	2552±135.0	6.0±0.2	5.8±2.3	5.1±0.5	0.5±0.3	79.5±4.2	0.2±0.0	2.3±0.3	0.5±0.0	0.3±0.2
Process effluent 1 (PE ₂)	325.4±64.9	9.3±1.8	2.1±1.4	0.0 ±0.0	0.0±0.0	10.1±2.0	1.8±0.0	1.4±0.2	0.0±0.0	0.0±0.0
Acid leachate (L ₁)	565.7±62.3	232.4± 46.7	940.7±5.5	3718±18	140.0±1.7	17.6±1.9	6.2±1.2	105.8±0.6	97.8±0.5	96.4±1.2
Solid waste (SW ₁)	25.0±10.0	3299±131	21.0±9.1	443.3±30.7	26.4±1.4	0.8±0.3	87.9±3.5	2.4±1.0	11.7±0.8	18.2±1.0
Treated leachate (L ₂)	515.3±47.2	344.6±66.7	979.4±32.9	3692 ± 27.6	3.3±0.6	16.1±1.5	9.2±1.8	110.2±3.7	97.1±0.7	2.3±0.4
Solid waste (SW ₂)	44.8±14.5	6024.2±335.5	36.4± 14.0	171.9±45.4	138.8±7.8	1.4±0.5	160.5±8.9	4.1±1.6	4.5±1.2	95.5±5.3
Treated leachate (L ₃)	569.8±59.2	56.5±12.3	200.2±36.3	3555 ±71.6	3.5±1.5	17.8±1.8	1.5±0.3	22.5±4.1	93.5±1.9	2.4±1.0
Solid waste (SW ₃)	29.5±5.0	317.5±155.5	753.7±53.6	40.5±1.0	9.4±2.3	0.9±0.2	8.5±4.1	84.8±6.0	1.1±0.0	6.5±1.6
After evaporation (L ₄)	514.6±40.9	47.4±10.3	133.1±44.3	3554 ±127.1	0.4±0.2	16.0±1.3	1.3±0.3	15.0±5.0	93.5±3.3	0.3±0.2
Crystals	229.6±20.4	1.7±1.4	32.5 ± 11.6	2438 ± 51.4	0.0±0.0	7.2±0.6	0.0±0.0	3.7±1.3	64.1±1.4	0.0±0.0
Effluents	316.4±89.9	44.9±9.6	94.9 ± 32.9	1021 ± 33.9	0.3±0.2	9.9±2.8	1.2±0.3	10.7±3.7	26.8±0.9	0.2±0.1

In these conditions, the leachate was neutralized to pH 4.5 and 95.5 ± 5.3 % of Fe was removed (Table 3.8). Thence, Fe will not be an impurity to affect the purity of the final Ni salt (ANSH).

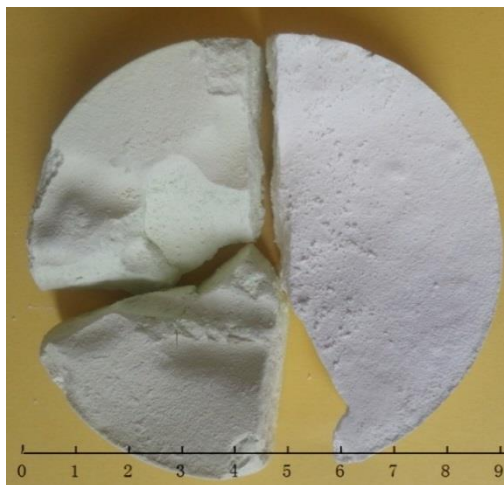


Figure 3. 20: Photograph of different colors of SW₂

4.3.2 NaF addition to remove Mg followed by evaporation

Magnesium was eliminated from the treated leachate 2 (L₂) after precipitation of MgF₂ after addition of NaF. After filtration, 84.8 ± 6.0 % of Mg was removed (Table 3.8).

Then the treated leachate 3 (L₃) of 660 mL was evaporated to concentrate Ni in the purified leachate (L₄) of 210 mL. The volume of L₄ was calculated from the results of Ni concentration with the volume of L₃ and the solubility of NiSO₄ at 25 °C. For instance, with the Ni concentration and volume of L₃, the Ni molar was obtained. So a controlled volume must be calculated to make sure all the NiSO₄ still dissolved in L₄ and without crystal of NiSO₄ appeared in L₄. Such as if there is 90 g of NiSO₄ in L₃ (E.g. 600 mL), we cannot evaporate L₃ to a volume of 200 mL as the solubility of NiSO₄ at 25 °C is 40.4 g per 100 mL water(Weast, 1985-1986), because only 80.8 g of NiSO₄ can be dissolved in 200 mL solution, the other 10 g of NiSO₄ will appear in the form of crystals. However, as

our objective is to add $(\text{NH}_4)_2\text{SO}_4$ into L_4 to react with all NiSO_4 in the solution, so in this case, a volume of 225 mL could be accepted for the final volume of L_4 .

What is important, after this evaporation step, $93.5 \pm 3.3 \%$ of nickel was still in the purified leachate (L_4) (Table 3.8).

4.4 Conclusion

This purification step was very important because the purity of the final Ni salt product (ANSH) depends on the purity of this treated leachate (L_4). Firstly, considering the production cost, all analysis confirmed that a suspension of 10 % $\text{Ca}(\text{OH})_2$ was a better neutralizing agent than NaOH (5 M). After the neutralization step, $95.5 \pm 5.3 \%$ of Fe was removed from L_1 .

Secondly, adding NaF to remove Mg was also effective (removal rate: $84.8 \pm 6.0 \%$). Evaporation enabled us to obtain a leachate L_4 pure and concentrated enough for crystallization. The MgF_2 waste could be used in different applications provided that its purity is high enough. This point has to be studied.

5 Part IV- Crystallization

5.1 Introduction

Crystallization is a conventional industrial operation in chemical manufacturing processes (Tanner, 1976). With the development of techniques, we can produce different sizes of optically perfect single crystals of various compounds. For example, the current state of on-line optical particle description in reaction crystallization and cooling crystallization techniques were investigated (Abbas et al., 2002). The crystallization and agglomeration kinetics process of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in a mixed suspension mixed product removal (MSMPER) crystallizer was studied (Randolph and Larson, 1988a; Randolph and Larson, 1988b; Tavaré et al., 1985). $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ crystallization from solution using magnetic weigh method was proved possible (Xinxin et al., 2002). A new method to grow large single $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ crystals was described (Genbo et al., 2002).

Crystallization was the most critical step in this process. Two steps of crystallizations were used before to obtain ANSH crystals with a purity of 88.8 % (Barbaroux, 2010; Barbaroux et al., 2012). However, we found that after the first step of crystallization, NaOH, $(\text{NH}_4)_2\text{SO}_4$ and NaF were added again into the Ni solution, and a second evaporation had to be done. These steps increased process complexity and production cost.

In this part, the whole objective is to simplify the process and get ANSH crystals with high purity from the treated leachate (L_4) produced from ashes of *A. murale*.

5.2 Materials and methods

5.2.1 Different methods of crystallization

To crystallize ANSH, a mass of ammonium sulfate corresponding to an excess of 20 % from the stoichiometric quantity (9.4 g) was added to L₄ (Eq.3.7). The suspension was heated at 60 °C to dissolve the solids and kept at room temperature.



Two methods have been tested: the former consisted in keeping the solution in the fridge at 4 °C for one night as previously done (Barbaroux et al., 2012). The latter consisted in performing the crystallization in a cooled reactor with magnetic stirring. The reactor was cooled thanks to a cooling fluid (*ASCAGEL MEG*: antigel base monoethylene glycol) flowing in the jacket at 0 °C, the temperature being maintained at 0 °C by a cooling bath (Bioblock Scientific 18207) for 4 h. The suspension was filtered by a vacuum pump (Fisher scientific).

At the end of the crystallization, the crystals were washed with 5-10 mL of deionized water at 0 °C. The process effluent (PE₃) was kept for reusing (adding concentrated H₂SO₄ into it for acid leaching). To increase crystal purity, a second crystallization was performed at 0 °C. Firstly, 17 g of ANSH crystals dissolved in 100 mL of deionized water at about 60 °C (the solubility of ANSH salt is 17.5 g per 100 g water at 60 °C (IUPAC *Solubility Data Series*). Then crystallization was performed in the same condition as the first one. At last, the suspension was filtered and the filtrate (PE₄), which contained a little Ni, was reused as PE₃.

5.2.2 Characterization of the ANSH crystals

Five methods (ICP-AES, XRD, IR and gravimetric) were used to characterize our crystals (S.J.Baviskar et al., 2011).

- ❖ For ICP-AES analysis, we prepared solutions by dissolving about 0.1 g of ANSH crystals obtained from the optimized process into 200 mL of deionized water (the solubility of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ is 1.9 g per 100 g water at 0 °C, so we are sure that all the ANSH salt was dissolved in the water (Lide, 2003)). We also prepared solutions with two commercial salts (Sigma Aldrich): salt#1 (purity 99.99 %) and salt #2 (purity at least 98 %) at the same condition.
- ❖ XRD analysis of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ crystals was carried on Rigaku – Miniflex II diffractometer at LRGP, with a scan range from 10 to 80 ° at a scanning speed of 1.0 ° min⁻¹. Cu K α radiation was used with 30 kV and 15 mA.
- ❖ IR spectra of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ crystals was measured by AVATAR 370 FT-IR (Thermo Nicolet) spectrometer. The sample spectrum is usually compared with a background spectrum, so a new background was determined for each sample of our products.
- ❖ The gravimetric analysis was done in triplicate, and Eq.3.8 showed the reacting molecules (with the chemical structure underneath (Fig.3.21)). About 0.5-0.6 g of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ crystals were precisely weighed three times, put in a 250 mL beaker, dissolved in 50 mL deionized water at 60 °C. The reagent (50 mL of ethanol containing 10 g L⁻¹ of HDMG) was added, and then concentrated ammonia (25 % NH₃) was added drop by drop to reach pH 9. The red complex of $\text{Ni}(\text{HDMG})_2$ appeared. The reaction was run for 20-30 min under magnetic stirring and the suspension was filtered. The solid $\text{Ni}(\text{HDMG})_2$ was washed with deionized water and dried at 105 °C in the oven until constant weight.

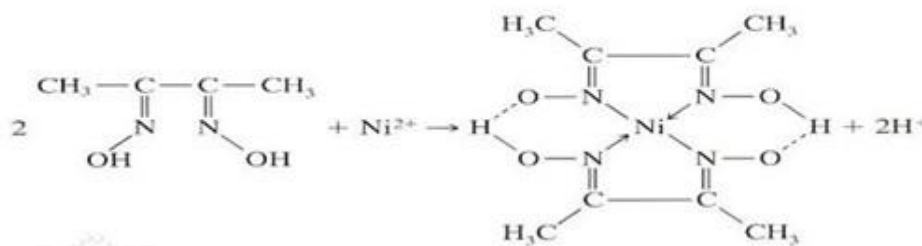
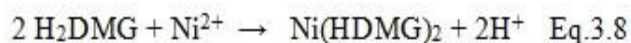


Figure 3. 21: Chemical structure of HDMG and Ni(HDMG)₂

5.3 Results and discussion

5.3.1 Crystals

ANSH crystallization was performed with and without stirring, and, as expected, stirring led to crystals of smaller particle size (Fig. 3.22). Stirring made also purity increase as detailed after.



Figure 3. 22: ANSH crystals obtained a) with stirring and b) without stirring

The characterization of salts #1 and 2 were as follows:

- ❖ For salt #1, a purity of $99.6 \pm 0.3 \%$ was obtained by ICP-AES and $99.7 \pm 0.1 \%$ by gravimetric analysis.
- ❖ For salt #2, we found $98.8 \pm 1.0 \%$ and $98.6 \pm 0.1 \%$.

Then both techniques gave the expected results (slightly underestimated for salt#1).

The elemental concentrations in the powder crystals (Fig.3.22 (a)) measured by ICP-AES after each crystallization step is given in Table 3.9. The purity was 92.4 ± 0.9 % after the first step and reached 100.6 ± 0.7 % after the second one. The results obtained by gravimetric analysis were of the same order: 89.2 ± 0.3 % after the first step and 99.1 ± 0.2 % after the second one. These data were lower than the ICP-AES results as when we did the ICP-AES analysis, even compared with the control standard solution, there was about 1.0 - 5.0 % error (deviation). However, this purity was higher than 88.8 % in the previous study (Barbaroux et al., 2012).

The purity of the big crystals (Fig.3.22 (b)) was 80.1 ± 1.1 % and 93.7 ± 0.3 % after the first and second crystallization (Table 3.10), lower than the one of powder crystals. Because without stirring, all the other possible salts were co-crystallized at the same time to affect the purity. Then we stopped the tests for these crystals and focused on the powder.

XRD patterns (Fig. 3.23(a)) show that the peaks of the crystals made under stirring corresponded to most peaks of salt #1. Moreover, peaks marked 1 (16.46°), 2 (21.50°), 3 (23.60°), 4 (29.50°), 5 (31.99°), 6 (36.97°), 7 (40.86°) and 8 (41.90°) were in excellent agreement with the peaks of $\text{Ni}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ from AMS data for ANSH (Treushnikov et al., 1978). The peaks marked *, large for salt #1 but not so large in our crystals neither appeared strongly in AMS data for ANSH (Treushnikov et al., 1978). So the peaks of 1-8 demonstrated well our crystals are ANSH salts.

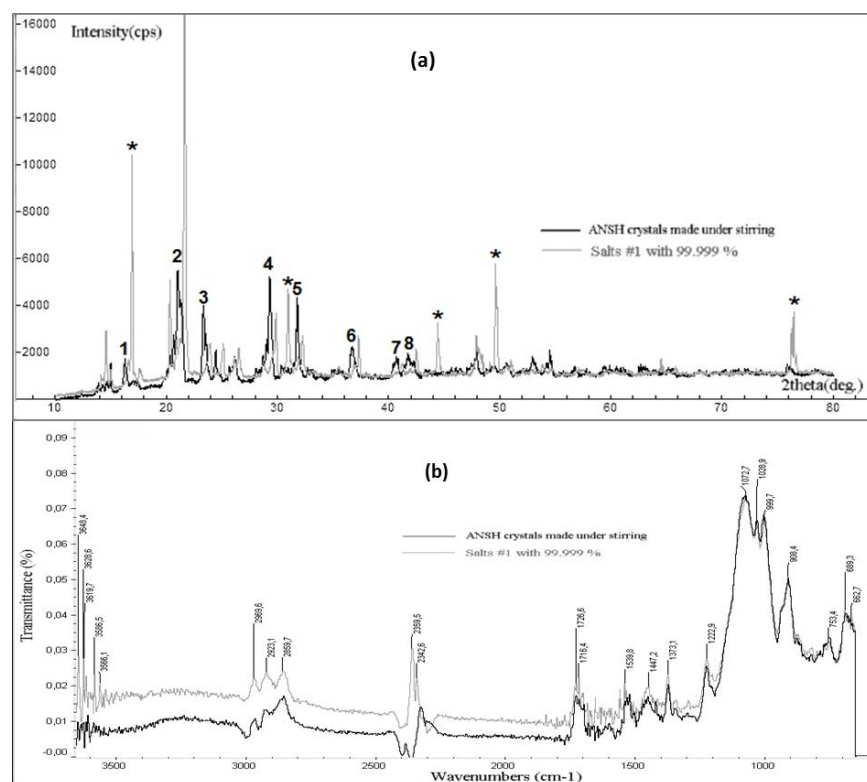


Figure 3. 23: a) X-ray diffraction patterns of ANSH crystals made under stirring and the commercial salt of high purity (salt#1 of 99.999 % purity); and b) Infra-red spectrum

Infra-red spectrum of the crystals showed a strong band between 1080-1130 cm⁻¹ indicating the presence of SO₄²⁻ ion in the crystal structure as predicted (Socrates, 2004) (Fig. 3.23(b)). The band of N-H should be located between 3300-3500 cm⁻¹ for -NH₂ and -NH, but there was no absorption peaks for -NH₃. So there were no peaks between 3300 and 3500 cm⁻¹ to show -NH₃ in the crystals as in Fig.3.23 (b). And there was no significant difference between our crystal and salt#1.

Table 3. 9: Elemental concentrations in the powder crystals after the first (1st) and second (2nd) crystallization

Elements		As	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Zn
Concentration (mg g ⁻¹)	1 st	0.00	0.18	0.00	0.03	0.00	0.00	0.03	17.65	2.85	0.01	0.52	137.36	0.79	0.02	0.88
		±0.00	±0.14	±0.00	±0.01	±0.00	±0.01	±0.03	±3.41	±2.01	±0.00	±0.33	±1.28	±0.04	±0.00	±0.04
	2 nd	0.00	0.03	0.00	0.03	0.00	0.00	0.01	18.80	0.87	0.00	0.01	149.43	0.77	0.03	0.95
		±0.00	±0.03	±0.00	±0.00	±0.00	±0.01	±0.01	±2.99	±0.64	±0.00	±0.01	±1.06	±0.45	±0.00	±0.02

Table 3. 10: Elemental concentrations in the big crystals after the first (1st) and second (2nd) crystallization

Elements		As	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Zn
Concentration (mg g ⁻¹)	1 st	0.00	0.06	0.00	0.03	0.00	0.02	0.24	17.02	7.92	0.05	6.30	119.00	1.07	0.03	0.88
		±0.00	±0.02	±0.00	±0.00	±0.00	±0.01	±0.01	±0.61	±0.01	±0.00	±0.57	±1.59	±0.10	±0.00	±0.01
	2 nd	0.00	0.06	0.00	0.02	0.00	0.00	0.02	23.82	0.50	0.00	0.09	139.31	0.87	0.03	0.79
		±0.00	±0.09	±0.00	±0.00	±0.00	±0.01	±0.00	±0.79	±0.01	±0.00	±0.05	±0.50	±0.01	±0.00	±0.01

5.4 Conclusion

ANSH crystals were produced by 2 crystallization steps without and with stirring, the best process being under stirring. Purity was assessed by several analytical methods and the salt was compared to commercial salts of high purity.

Crystals made under stirring reached a **purity of 99.1 ± 0.2 % (Ni: 149.13 ± 1.06 g Kg⁻¹)** which was significantly higher than previously, since a purity of 88.8 % (Ni: 132 ± 3.13 g Kg⁻¹) was obtained by Barbaroux et al.(Barbaroux et al., 2012). This higher purity can be explained by the changes that were done in the process, such as using Ca(OH)₂ as neutralizing agent instead of NaOH, adding NaF to remove Mg before the crystallization, adding once (NH₄)₂SO₄ and running once evaporation instead of twice, and doing two continuous crystallization steps than once.

6 Conclusion

Compared with the previous process (Barbaroux et al., 2012), the new process flow sheet and the photograph are presented in Fig.3.24 & 3.25.

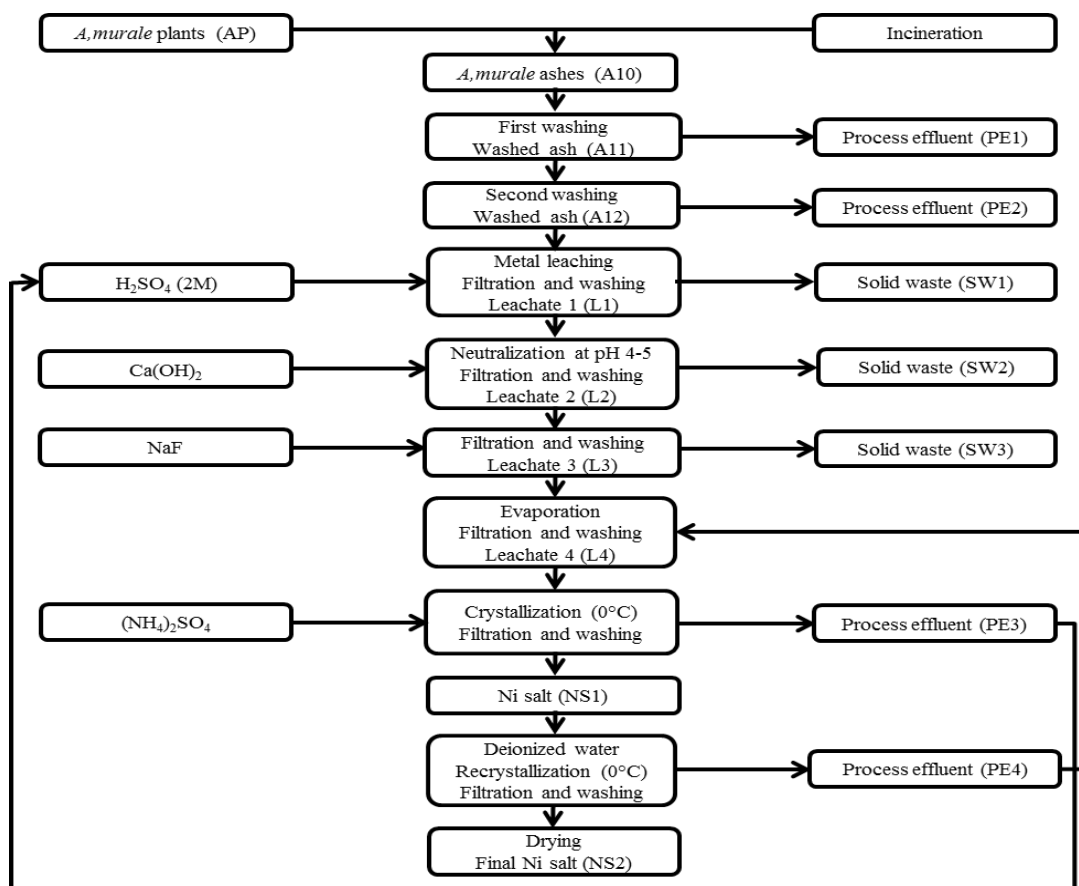


Figure 3.24: The new flow sheet of the process to produce ANSH salt from ashes of *A. murale*



Figure 3. 25: Photograph of the whole process

The main differences compared to the previous one (Barbaroux et al., 2012) are as follows. Firstly, the leaching duration was divided by two, which saves energy, since leaching is conducted at 95 °C. Secondly, for the neutralization, 10 % suspension of Ca(OH)_2 was used instead of NaOH (5 M), which reduced the economic cost, and also avoided the production of a great quantity of Na_2SO_4 in the process. In addition, almost all H_2SO_4 in excess was precipitated in the form of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, and 95.5 ± 5.3 % of Fe was eliminated from the raw ashes. Thirdly, magnesium was removed before ANSH crystallizations, and the removal rate was 84.8 ± 6.0 %. One step of evaporation did reduce the volume of the pure leachate (L_4), and concentrated Ni before crystallization. And also, one step of adding ammonium sulfate was enough and lowered the production cost. To finish, ANSH crystals were produced and characterized by IR, XRD analysis, ICP-AES and gravimetric analysis. The purity reached 99.1 ± 0.2 %, which was higher than previously (88.8 %).

Table 3. 11: Amounts of plant, reactants and water needed to prepare 1 kg of ANSH

	Mass (kg)	Volume (L)	Condition
Plant	18.4		
Ash	1.2		550 °C, 2 h
Washing (water)		4.8	Washing twice, 15 min once, 20 °C
Washed ash	1.0		
Leaching (H_2SO_4 , 2M)		8.6	95 °C, 2 h
Neutralization: (Ca(OH)_2) in water (20%)	1.2	4.8	20-25 °C, 1 h
NaF in water	0.2	0.5	20-25 °C, 30 min
Evaporation			100 °C, 2-4 h
$(\text{NH}_4)_2\text{SO}_4$ in water	0.5	0.5	60 °C, 5 min
ANSH crystals	1.0		0 °C, 4 h

The quantities of reactants needed to produce 1 kg of ANSH crystals are given in Table 3.11. The process requires a high mass of hyperaccumulator plant: 18.4 kg to produce 1 kg of salt.

The reactant quantities could be a little different at the pilot scale. An economic study and a LCA will be done to have accurate data.

The mass balance (Table 3.8) was obtained from the general results. The test has been done in triplicate with the whole process from the raw ashes to the final crystals. We found that at least 89.6 ± 6.2 % of K was removed from the raw ash, and this ratio can be improved by using more water to wash the raw ashes. Using Ca(OH)_2 increases the pH and eliminate 95.5 ± 5.3 % of Fe from the raw ashes. The step of adding NaF can precipitate 84.8 ± 6.0 % of Mg from the leachate L₂. 64.1 ± 1.4 % yield of Ni in the crystals from the raw ashes was not high, but all the other nickel (26.8 ± 0.9 %) in the effluents can be recycled and recovered, so the total Ni yield can attain more than 90.9 ± 2.3 %. Moreover, the method to improve the yield is ongoing.

About process effluents and waste solids generated in this process, PE₁ and PE₂ are K₂CO₃ solution, which could be reused as fertilizing agents. If needed, this solution can also be evaporated into solid to be transported more easily. PE₃ and PE₄ could be reused directly in this process at the acid leaching step by adding concentrated H₂SO₄ into it. SW₁ consists mainly of CaSO₄ and small part of SiO₂, and SW₂ mainly contains of CaSO₄·2H₂O and minor portion of Fe(OH)₃, these solids could be used directly as cement and chemical filler materials. Or after being dried, these solids can be used desiccant. About SW₃, a very small amount of this waste solid is generated in this process, but which mainly comprises MgF₂, we will carefully consider the handling and application.

Chapter 4: New ideas of producing nickel products from *A. murale* biomass

Learn from yesterday, live for today, hope
for tomorrow. The important thing is
not to stop questioning.

Albert Einstein

1 Introduction

In the previous chapter, we have seen the whole process to produce ANSH from the ash of *A. murale*. The objective of the present part is to explore other methods to prepare different Ni salts and to extract Ni from the plant biomass. To begin, we will focus on the preparation of nickel sulfide (NiS) from the ash of *A. murale*, NiS can be considered as a final product or as an intermediate for the synthesis of ANSH or nickel sulfate (NiSO₄). Then we will investigate the possibility of working directly from the plant biomass, replacing the burning step by an extraction step with hot water.

Besides increasing the knowledge on the production of Ni products from hyperaccumulator biomass, the result will enable us to evaluate the interest of these approaches for phytomining.

2 Part I-Producing nickel products from *A. murale* ash

2.1 Introduction

The ANSH production process presented in Chapter 3 requires several purification steps to separate Ni from the other major elements (K, Ca, Mg and Fe). To avoid these steps, which are time and cost consuming, a possibility would be to find a chemical compound that precipitates selectively with Ni. Then, the goal of the following experiments is to simplify the whole process to get pure Ni salts.

Two conditions should be met to generate a nickel salt by this method: 1) this salt must be easily obtained by precipitation at room temperature; and 2) its solubility must be significantly different from the ones of the salts that could be formed with the other cations (Ca²⁺, Mg²⁺, K⁺, Fe²⁺ and Na⁺). Table 4.1 gives the solubility data for some common salts (Lide, 2005).

Table 4. 1: Solubility (expressed in g / 100g H₂O) of some common inorganic salts at 25 °C

	Ni ²⁺	Ca ²⁺	Mg ²⁺	Fe ³⁺	K ⁺	Na ⁺
SO ₄ ²⁻	40.4	9.205	35.7	444*	12.0	28.1
Cl ⁻	67.5	81.3	56.0	91.2	35.5	36.0
CO ₃ ²⁻	0.0043*	0.00066*	0.18*	-	111	30.7
NO ₃ ⁻	99.2	144	71.2	82.5*	38.3	91.2
S ²⁻	Insoluble	Slightly soluble	Reacts with water	-	Soluble	20.6

* at 20 °C

For SO₄²⁻, Cl⁻ and NO₃⁻, these data show high solubility of nickel salts with the corresponding calcium, magnesium, iron, potassium and sodium compounds. At first, NiCO₃ seems corresponding to the two criteria. However, CaCO₃ is definitely less soluble than NiCO₃ but there are relatively less Ca²⁺ ions in solution. However, this compound may be difficult to prepare at high purity because i) it requires CO₂ addition at high pressure or ii) it is poorly crystallized with a very variable composition in presence of an alkali-carbonate (CaCO₃ + Ni(OH)₂) (Amiel and Besson, 1963).

Hydrometallurgical processing of laterite ores classically uses H₂S to selectively precipitate nickel-cobalt sulfide. Despite the lack of quantitative solubility data, table 4.1 shows that NiS seems to be a good chemical intermediate to simplify ANSH preparation. This reaction typically occurs at 80-120 °C and 1-10 bar (Crundwell et al., 2011). To avoid these drastic conditions and the use of this toxic gas at very low concentration, H₂S is substituted by sodium sulfide hydrate (Na₂S·9H₂O).

Table 4. 2: Examples of prices of common nickel salts

Nickel products	Chemical formula	Purity (%)	Price	Price(€ kg ⁻¹)	Source
Ammonium nickel sulfate hexahydrate	Ni(NH ₄) ₂ (SO ₄) ₂ •6H ₂ O	99,999	121 €/25g	4 840	http://www.sigmaaldrich.com/catalog/product/aldrich/574988?lang=fr&region=FR
		≥98	38,4 €/100g	3 840	http://www.sigmaaldrich.com/catalog/product/aldrich/a1827?lang=fr&region=FR
Nickel(II) sulfate hexahydrate	NiSO ₄ •6H ₂ O	99	22,2 €/100g	2 200	http://www.sigmaaldrich.com/catalog/product/sial/227676?lang=fr&region=FR
Nickel(II) sulfate	NiSO ₄	99,99	57,9 €/10g	57 900	http://www.sigmaaldrich.com/catalog/product/aldrich/656895?lang=fr&region=FR
Nickel(II) sulfide	NiS	99,90	105 €/10g	10 500	https://fr.vwr.com/app/catalog/Product?article_number=35661.09
Nickel(II) hydroxide	Ni(OH) ₂		67,7 €/250g	271	http://www.sigmaaldrich.com/catalog/product/aldrich/283622?lang=fr&region=FR
Nickel(II) sulfamate tetrahydrate	Ni(NH ₂ SO ₃) ₂ •4H ₂ O	98	146 €/500g	292	http://www.sigmaaldrich.com/catalog/product/aldrich/262277?lang=fr&region=FR
Nickel(II) citrate hydrate	Ni ₃ (C ₆ H ₅ O ₇) ₂ •xH ₂ O	≥98	345 €/500g	690	https://fr.vwr.com/app/catalog/Product?article_number=39634.36

Therefore, a selective reaction is possible with $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ to give NiS but without K_2S , MgS and CaS . NiS can be viewed as the final product or be transformed, such as it could be oxidized by hydrogen peroxide to obtain a pure NiSO_4 solution, which could be sold as a solution or be crystallized. Moreover, NiSO_4 solution can also be used to produce ANSH salt of very high purity or to produce nickel hydroxide $\text{Ni}(\text{OH})_2$. Nickel hydroxide can be considered as the final product or transformed as well. Its reaction with sulfamic acid ($\text{H}_2\text{NSO}_3\text{H}$), would lead to nickel sulfamate ($\text{Ni}(\text{NH}_2\text{SO}_3)_2$). Sulfamate nickel plating is used much in nickel electroplating and has a big market in the world. Its reaction with citric acid ($\text{C}_6\text{H}_8\text{O}_7$) would lead to nickel citrate ($\text{Ni}_3(\text{C}_6\text{H}_5\text{O}_7)_2$).

Examples of prices are given in Table 4.2; however they should be considered as examples since prices depend on different parameters (market, distributors, quantity, purity etc.).

2.2 Materials and methods

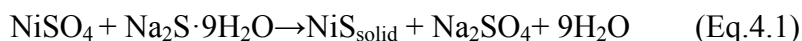
2.2.1 Preparing NiSO_4 at pH between 4 and 5

A. murale harvested in July, 2012 from Albania was used in this chapter. Batches of 100 g dry plants (2 mm) were burnt in a big furnace (Barnstead Thermolyne 1400 Furnace) at 550 °C for 2 h. The following step was acid leaching with an ash mass fraction of 10 % (30 g raw ash (A_{10}) with 270 mL 2 M H_2SO_4), heating on a hot plate at 95 °C for 2 h. The suspension was filtered by vacuum pump (Labbox) to obtain leachate 1 L_1 . A suspension of 10 % $\text{Ca}(\text{OH})_2$ was added to raise pH of L_1 between 4 and 5. Then, the mixture solution was filtrated at the previous filtration condition, the filtrate was called leachate 2 (L_2).

2.2.2 Producing NiS, ANSH salt and other nickel products

A mass of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ corresponding to an excess of 10 % from the stoichiometric quantity (other metal ions did not react with $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$, so an excess of 10 % was enough to consume

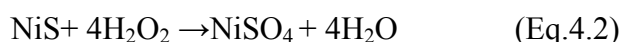
all the Ni^{2+} in the leachate) was added drop by drop into L_2 to precipitate nickel sulfide at room temperature (20 °C):



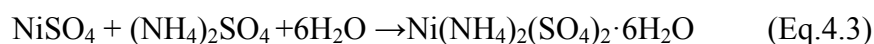
We consider this equation since the leachate contains Ni^{2+} and SO_4^{2-} ; Na_2SO_4 is soluble in these conditions since its solubility is 28.1 g per 100 g water at 25 °C.

The suspension was filtered by a vacuum pump (Labbox), all the solid was NiS.

Afterwards, a mass of H_2O_2 (30 %) corresponding to an excess of 100 % from the stoichiometric quantity was added into a beaker with the solids of NiS. We used an excess of 100% to make sure to dissolve all the NiS. But even in these conditions, a small quantity of residual solid was observed. The suspension was heated at 95 °C to dissolve all the NiS as in Eq.4.2.



Then, the production of ANSH salt was carried out with a mass of ammonium sulfate corresponding to an excess of 20 % from the stoichiometric quantity (Eq.4.3) added to the NiSO_4 solution. The suspension was heated at 60 °C to dissolve the solid, and kept at room temperature. Crystallization was conducted in a cooled reactor with magnetic stirring. The reactor was cooled thanks to a cooling fluid (*ASCAGEL MEG*: antigel base monoethylene glycol) flowing in the jacket at 0 °C, the temperature being maintained at 0 °C by a cooling bath. The suspension was filtered by a vacuum pump and the crystals were washed with 5-10 mL of deionized water at 0 °C. The process effluent (PE_3) was kept for reusing (adding concentrated H_2SO_4 into it for acid leaching).



About 0.1 g of ANSH crystals produced from NiS was dissolved in 200 mL water to generate ANSH solution samples, and then the elemental concentrations in these samples were

analyzed by ICP-AES. NiS solids and ANSH salt were characterized by XRD and IR using the method as in the previous chapters.

2.3 Results and discussion

2.3.1 Production of ANSH with NiS as an intermediate product

In the previous chapter, acid leaching was carried out with the washed ash (A_{12}) –K was removed from the raw ash (A_{10}) by washing-. However, in this part, as $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ only precipitated Ni, Cu and Zn, not K, Ca, Mg, and Fe; acid leaching can be done directly with the raw ash (A_{10}), without the ash washing step.

The main steps of this designed process are presented in Figure 4.1. At first, the raw ash was attacked with H_2SO_4 , releasing CO_2 to give L_1 (Fig.4.1 (a)). Then pH was adjusted between 4 and 5, this step is quite significant as if the pH was below 4, H_2S would be generated in the next step (H^+ reacts with S^{2-} from $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$). H_2S is very toxic and corrosive, so pH must absolutely be controlled to avoid its formation. The addition of sodium sulfide led to the precipitation of NiSO_4 solution (Fig.4.1 b & c). NiS was separated by filtration (Fig.4.1 (d)). The solution after oxidation is shown in Fig.4.1 (e) and the final crystals of ANSH in Fig. 4.1 (f).

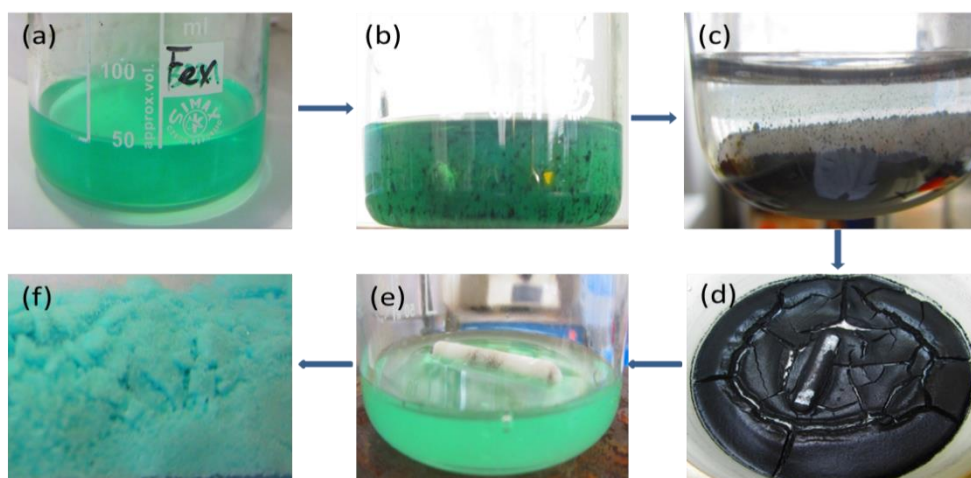


Figure 4. 1: Main steps of ANSH production with NiS as an intermediate product

The XRD diffractogram of NiS is given in Fig.4.2. It was interpreted with the data of AMCSD (American Mineralogist Crystal Structure Database) for NiS. Actually, the information of 2-theta and intensity given of the X-ray diffractogram of NiS (Fig.4.2) almost matched with AMCSD data for NiS: the peaks marked from 1 to 6 were in good agreement with the strong peaks of NiS from AMS data, but the peaks noted with * were not found in the AMCSD data.

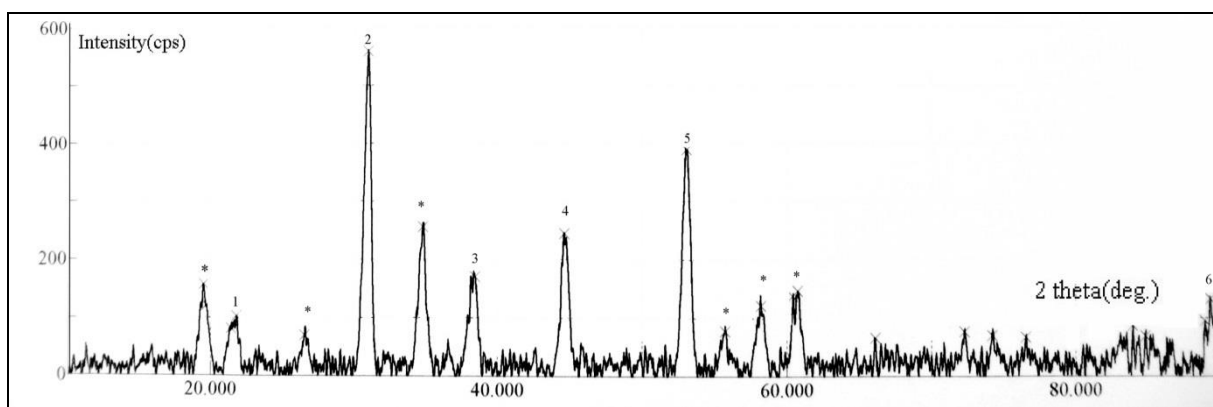


Figure 4. 2: XRD pattern of NiS

Except the XRD analysis, the solid was analyzed by ICP AES after mineralization. 0.1-0.2 g of NiS were added into 8.5 mL HNO₃ and 1.5 mL H₂O₂ for micro-digestion, and then samples drawn from the solution after micro-digestion were analyzed. Ni concentration in the NiS was between 228.3-371.4 mg g⁻¹ at different times. This difference may result from the step of washing the NiS and there was still impurity with the NiS (Na and Ca). However, until now we have not found another method to determine the purity of NiS and neither for are not composition of NiS, so we suppose the composition is a mixture of Ni_xS_y.

The results of ICP-AES for ANSH salt are given in Table 4.3. Compared with the purities previously obtained (88.0 % in (Barbaroux et al., 2012), 99.1± 0.2 % in chapter 3), with this method, the purity was 99.4 ± 0.8 %. Except nickel, the other elements concentrations were all below 5 mg g⁻¹ (ppm), which demonstrated the high purity of ANSH salt.

It was not possible to do an accurate mass balance, because despite the process was done in triplicate; the same difficulties were met to dissolve NiS. Then we are aware that Ni is lost at this step.

Table 4.3: Elemental concentrations in ANSH crystals produced from the process of using $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$

Elements	As	Ca	Cd	Co	Cr	Cu	Fe	K
Concentration (mg g ⁻¹)	0.00 ±0.00	0.07 ±0.01	0.00 ±0.00	0.03 ±0.00	0.00 ±0.00	0.06 ±0.01	0.00 ±0.00	2.44 ±1.96
Elements	Mg	Mn	Na	Ni	P	Pb	Zn	
Concentration (mg g ⁻¹)	1.62 ±0.02	0.00 ±0.00	0.02 ±0.01	147.65 ±0.12	0.18 ±0.07	0.02 ±0.00	0.93 ±0.00	

However, we present an estimation of the amount of reactants needed (Table 4.4). Starting from the same quantity of plants as previously (18.4 kg), we obtained 0.58 kg of salt (against 1 kg in chapter 3).

Table 4.4: Amounts of reactants needed to prepare ANSH from *A. murale* ash with NiS as an intermediate

	Mass (kg)	Volume (L)	Condition
Plant	18.4		
Ash	1.2		550 °C; 2 h
Leaching (H_2SO_4 2M)		10.8	95 °C; 2 h
$\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ in water	0.6	1	20-25 °C; 30 min
NiS	0.2		
H_2O_2 (30 %)	0.5	0.5	
Evaporation			100 °C; 1-2 h
$(\text{NH}_4)_2\text{SO}_4$ in water	0.3	0.2	60 °C; 5 min
Crystallization (ANSH salt)	0.6		0 °C; 4 h

2.4 Conclusion

We showed that it was possible to use $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ to produce NiS and ANSH salt, and the process of ANSH preparation was simplified. Compared with the optimized process in chapter 3, this process (Fig.4.3) did not need the step of washing the raw ashes, neither the purification. The most important was that only after one step of crystallization, the purity of the ANSH crystals produced from NiS was $99.4 \pm 0.8 \%$, which met our main objective of producing a high purity ANSH salt.

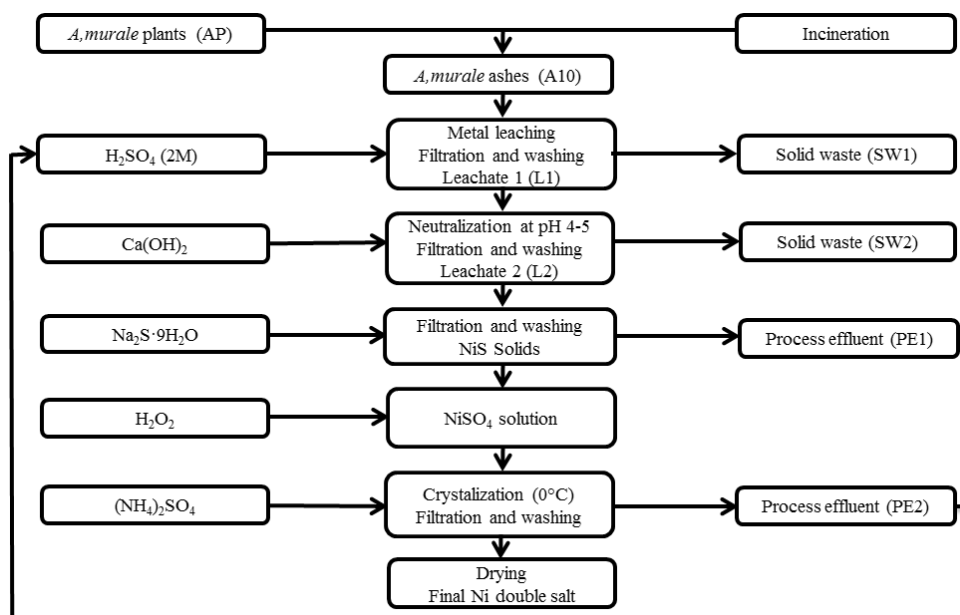


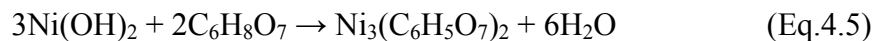
Figure 4.3: Diagram of producing ANSH salt from *A. murale* ash with NiS as intermediate product

But the drawback was the difficulty we have met to dissolve NiS and the subsequent Ni loss. This point has to be more investigated.

At last, this study gives us some perspective to produce other nickel products. Such as adding NaOH to L_1 (leachate of NiSO_4 solution) under magnetic stirring, the suspension filtered by a vacuum pump to get Ni(OH)_2 . Then, a mass of $\text{H}_2\text{NSO}_3\text{H}$ corresponding to an excess of 20 % from the stoichiometric quantity (Eq.4.4) add into Ni(OH)_2 to obtain nickel sulfamate $(\text{Ni(NH}_2\text{SO}_3)_2)$.



Or a mass of citric acid corresponding to an excess of 20 % from the stoichiometric quantity (Eq.4.5) added into $\text{Ni}(\text{OH})_2$ to produce nickel citrate ($\text{Ni}_3(\text{C}_6\text{H}_5\text{O}_7)_2$).



3 Part II--Producing nickel products directly from *A. murale* plants

3.1. Introduction

Nickel was found concentrated in the leaf and stem tissues of *A. murale* and majorly bound to malic acid along with other organic ligands of lower molecular weight (McNear et al., 2010). In the leaves, malate acid was found as the predominant chelating organic acid to accumulate metal (Montargès-Pelletier et al., 2008), and this was also proved by a study of reporting Ni and malic acid molar ratio was 1.0 and the ligand between Ni and malate played a role of supporting Ni detoxification/transport and storage in the plant of *S. tryonii* (Bhatia et al., 2005).

The driving idea here is to preserve these organic compounds in the plants. Herein, the first idea was to investigate if nickel citrate, nickel malate, and other nickel compounds could be extracted by boiling the Ni-hyperaccumulating plants (*A. murale*) in deionized water at 100 °C. Previous studies have reported that such an extraction should be possible. Ni was recovered from *Berkheya coddii* (*B. coddii*) biomass from a three-step hydrothermal process (le Clercq et al., 2001). And also heavy metals (Zn, Pb and Cu) were obtained from the hyperaccumulating plants (*Sedum alfredii* Hance) using hydrothermal process (Yang et al., 2009a; Yang et al., 2010; Yang et al., 2009b).

3.2. Materials and methods

3.2.1 Extracting nickel from A. murale plants in deionized water at 100 °C

For the very first trial, 15 g of dry plants with leaves, stems and flowers together (not crushed) were put into a flask (1 L) with 900 mL deionized water, then the temperature increased to 100 °C (Fisher brand agitateur-magnétique chauffant 10515) to boil the solution with condenser tube. Supernatant samples (1mL) were taken at the beginning of boiling and after 5, 10, 15, 20, 25, 30, 45, and 60 min. At last, vacuum filtration was carried out to separate the boiled plants from the solution. The boiled wet plants were brought to an oven for 12 h and digested by micro-wave to be analyzed by ICP-AES.

Then, 40 g of dry crushed plants (leaves, stems and flowers mixed together, batches of 40 kg ground with an industrial grinder to particle size of 2 mm) were put with 960 mL deionized water into a flask (1 L) to do the same experiment. Samples were taken and analyzed using the same method as above. The organic acid concentration was analyzed by High Performance Liquid Chromatography (HPLC).

Firstly, 10 g of crushed plants (2 mm) in 240 mL deionized water (a mass fraction of 4 %) and 30 g of crushed plants in 270 mL deionized water (a mass fraction of 10 %) were tested to compare the influence of different mass fraction.

Next, for the particle size, 30 g crushed plants with 270 mL water (a mass fraction of 10 %) but with very finely crushed plants (particle size < 1 mm) was carried out to compare the results of particle size of 2 mm. The boiling time was 15 min and supernatant samples (1 mL) were taken after the filtration.

3.2.2 Ni extraction by heating with ultrasonic wave or autoclave

To begin, 10 g of plants with 240 mL deionized water (a mass fraction of 4 %) were put into a flask in glass (1 L). The flask was heated by ultrasonic wave (Fisher Bioblock scientific,

25 KHz, power 100 %) at 80 °C (the maximum temperature of the ultrasonic wave) for 30 min and then heated to 100 °C for 15 min by a hot plate.

Then, we prepared 3 flasks with mass fractions of 4 % (10 g of plants - 240 mL water), 10 % (26.7 g of plants - 240 mL water) and 15 % (42.4 g of plants - 240 mL water). But as shown in Fig. 4.4, there was not enough water in the 3rd case.

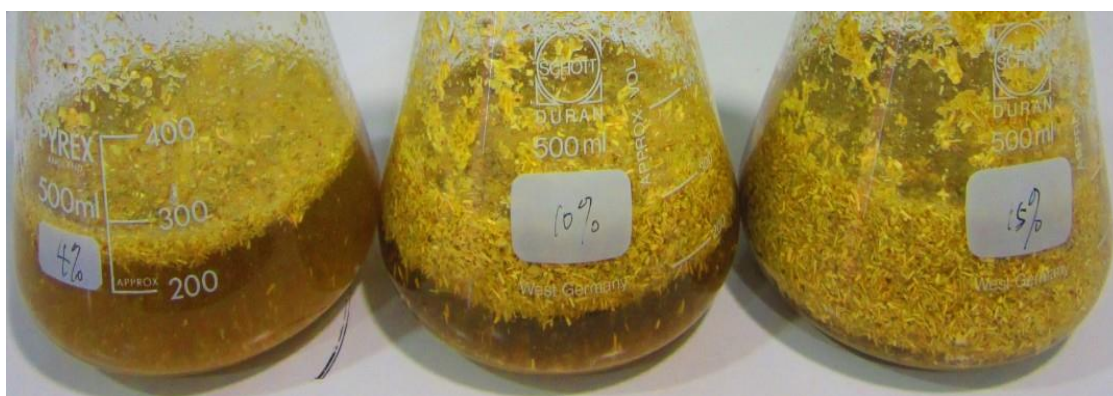


Figure 4.4: Comparison of the different solid/liquid ratios (4, 10 and 15%)

Then, only the flasks of mass fractions of 4 and 10 % were put into the autoclave (Adolf wolf, Robert-Bosch-Str.13). Pressure was set at 3.0 bar (relative pressure) and temperature at 143 °C for 1 h. Then, pressure and temperature were gradually decreased to 0.0 bar and below 60 °C. Samples were taken out from the autoclave and filtered by a vacuum pump. The filtrate was greenish brown. The boiled wet plants were washed with 1000 mL water. For the mass fraction of 10 %, 5 washing steps were run (1000 mL deionized water per step) to wash the wet plants until the filtrate was colorless. The boiled wet plants were brought in an oven to be dried and then digested by micro-wave to analyze ICP-AES.

3.2.3 Producing nickel sulfide, nickel sulfate

As more than 80 % of Ni was transferred from *A. murale* plants into water, NiS, NiSO₄ and ANSH were produced as follows.

Firstly, 2.0 g of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ was added slowly into the boiled solution (110 mL) to precipitate Ni (Eq.4.1). NiS appeared immediately and the solution turned to black. Filtration by a vacuum pump (Labbox) and centrifugation (Allegra X-22R Centrifuge) (6000 rpm, 25 °C for 3 h) were used to separate NiS from the suspension.

As organic matter was present in the boiled solution, the solution was very dense and NiS was difficult to separate from the suspension. In order to solve this problem, 10 g of crushed plants (particle size 2 mm) were put in 90 mL H_2SO_4 (0.05 M) at 100 °C for 15 min carried out for once. Then 30 g of crushed plants (particle size 2 mm) were put in 270 mL H_2SO_4 (0.05 M) at 100 °C for 15 min three times. Samples were withdrawn and analyzed by ICP-AES.

As NiS was well produced, NiSO_4 and ANSH salt were produced using the same process as in section 2.2.2.

3.3. Results and discussion

3.3.1 Extraction of the major elements

The evolution of the concentrations of the major elements (Ca, K, Mg and Ni) in the boiled solution heating with hot plate is plotted (Fig. 4.5); Fe concentration was below 0.9 mg L^{-1} , so Fe concentration is not shown in Fig.4.4. Results showed that for the entire and crushed plants, the element concentrations kept nearly constant from the beginning to 1 h. Therefore, in the following tests, 15 min was chosen as a boiling duration.

For the entire plants, the major metal concentrations in the solution kept lower than 200 mg L^{-1} (Fig. 4.5(a)), much lower than in the concentrations obtained with the crushed plants (all the major elements concentrations were above 380 mg L^{-1} except Ca with ca 100 mg L^{-1}).

After 15 min, For Ni, the concentration in solution obtained with the crushed plants (Fig.4.5(b)) was 552.5 mg L^{-1} , nearly 6 times higher than with the entire plants (95.7 mg L^{-1}).

Concentrations of K, Ca and Mg were respectively 512.5, 370.0 and 104.5 mg L⁻¹ compared with 145.4, 47.2 and 19.6 mg L⁻¹ in the solution got with entire plants, that is to say about fourfold, eightfold, fivefold higher in the boiled solution obtained with crushed plants.

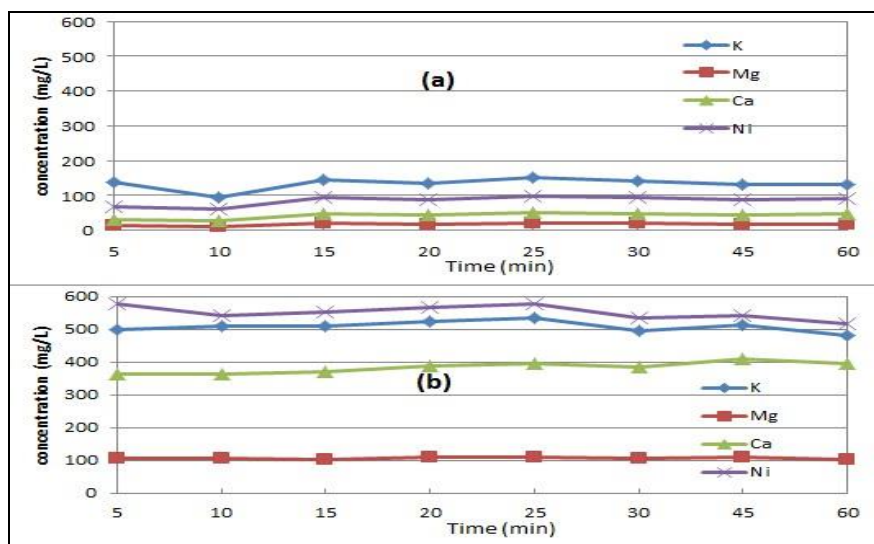


Figure 4.5: Evolution of K, Mg, Ca and Ni concentration in the solution obtained by boiling

a) the entire plants and b) the crushed plants (2 mm)

This result was expected since solid liquid transfer is easier when using smaller particles. Diffusion characteristic time is inversely proportional to the square of particle size (for spherical particles). But we tested both situations to make sure that the grinding step, which is energy consuming, was mandatory. Actually it was.

Then we investigated the influence of particle size by crushing the plants to particle size ≤ 1 mm. Results are given in Table 4.5.

Obviously, elemental concentrations in the boiled solution with smaller particle (≤ 1 mm) were little higher than with particle of 2 mm. As the test was carried out in the same condition (the same mass of plants and with the same volume de water), so Ni extraction in the boiled solution with particle (≤ 1 mm) (69.0 %) was also little higher than with particle of 2 mm (61.7 %).

This was in accordance with the effects of granularity studies (Yang, 2010; Yang et al., 2010).

For example, Yang and his team investigated plant granularity from 4 mesh (4.75 mm) to 18

mesh (1.00 mm) boiled in a stainless iron autoclave with distilled water. At the same condition (the same stirring speed, temperature, and reaction duration), the heavy metal removal efficiency was nearly the same: 99.2 % of the heavy metals (Zn, Pb and Cu) were transferred and dissolved in the boiled solution. Considering the time and energy consumption for grinding, they chose 18 mesh as the optimum granularity. In our experiments, considering also the finer granularity needs more energy and time for grinding, particle size of 2 mm was chosen as the optimal granularity.

Table 4.5: Major element concentrations and extraction in the boiled solution for two different particle sizes

Elements	Mass (mg)	Concentration (mg L ⁻¹)		Mass (mg)		Extraction (%)	
	Plants	Boiled solution		Boiled solution		(mass in solution)/(mass in plants)	
		(2 mm)	(≤1 mm)	(2 mm)	(≤1 mm)	(2 mm)	(≤1 mm)
Ca	233.3	421.5	500.5	83.9	99.6	36.0	42.7
Fe	9.2	0.3	10.2	0.1	2.0	0.6	22.1
Mg	84.4	178.9	207.9	35.6	41.4	42.2	49.0
K	256.3	930.0	1069.5	185.1	212.8	72.2	83.0
Ni	264.0	818.5	915.5	162.9	182.2	61.7	69.0

3.3.2 Organic acids in the boiled water

Fig.4.6 displayed the peaks of the organic acids in the boiled solution from the crushed plants.

The corresponding data are given in Table 4.6.

Here we noted that the most concentrated organic ligand was malate (2.609 g L⁻¹), followed by acetate and citrate (1.944 and 1.816 g L⁻¹). **These results demonstrated that the organic compounds present in the plant tissues were transferred into the solution.** Then with the help from the formula and pKa at 25 °C of these acids (Table 4.7), we tried to find the composition of these organic acids with Ni, Zn, Mg and K, however, even the correlations between the organic ligands and the metals are not clearly understood at the moment. In the

same way, it is difficult to calculate mass balances since the concentrations of these ligands in the plant tissues have not been measured.

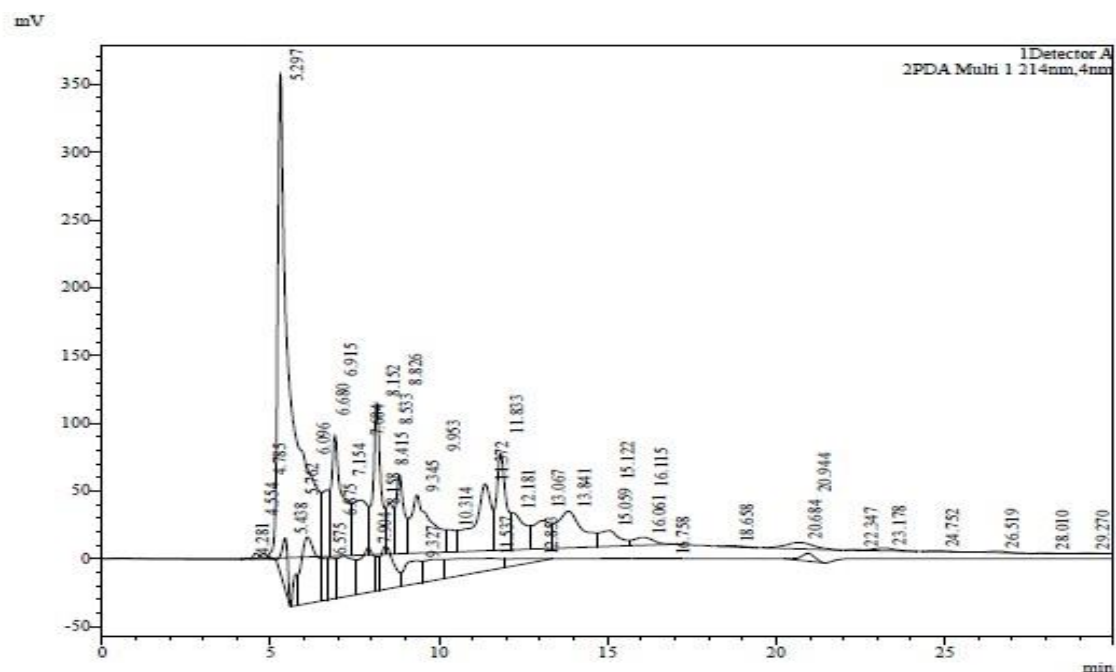


Figure 4.6: Chromatogram of organic acids in the boiled solution with crushed plants (2 mm)

Table 4.6: Concentration of organic acids in the boiled solution from the crushed plants

Acids	Ret.Time	Area	Height	Concentration
Name	min	mV*min	mV	g L ⁻¹
Acetate	13.067	735244	20708	1.944
Pyruvate	9.345	1785863	42955	0.689
Lactate	10.314	278440	16438	0.545
Fumarate	11.833	1421683	71360	0.032
Citrate	6.915	2077789	89008	1.816
Malate	8.152	1730086	111768	2.609

Table 4.7: Formula, mass molar and pKa at 25 °C of acids in the boiled solution

Name	Formula	Mass molar (g/mol)	pKa (25 °C)
Acetate	CH ₃ COOH	60.05	4.74
Lactate	CH ₃ CH(OH)COOH	90.08	3.9
Pyruvate	CH ₃ COCOOH	88.06	2.5
Fumaric	HOOCCHCHCOOH	116.07	pKa1=3.03 pKa2=4.44
Malate	HOOCCH ₂ CH(OH)COOH	134.09	pKa1=3.40 pKa2=5.20
Citrate	HOC(CH ₂ COOH) ₂ COOH	192.14	pKa1=3.13 pKa2=4.76 pKa3=6.4

3.3.3 Comparison of different heating methods

Microwave heating has been investigated to improve nickel leaching from nickel laterite ores (Al-Harashseh and Kingman, 2004; Che et al., 2010; Huang and Rowson, 2002; Zhai et al., 2009; Zhai et al., 2010). No suitable microwave heating device was available in the lab. But we have had the opportunity to try ultrasonic wave and autoclave and the metal extractions are listed in Table 4.8.

Table 4.8: Metal extractions of boiling plants (2 mm) using hot plate & ultrasonic wave & autoclave heating

Elements	Extraction % (mass in solution)/(mass in plants)		
	Hot plate	Ultrasonic wave	Autoclave
Ca	65.0	61.4	88.3
Fe	54.5	48.5	85.7
K	69.4	79.2	99.9
Mg	86.5	83.3	92.6
Ni	83.9	85.3	90.8

a) Heating by ultrasonic wave

As the maximum set temperature of our ultrasonic apparatus is 80 °C, so the mixed solution of plants and water could not be brought to the boiling temperature. Then, the flask was put on a hot plate to heat at 100 °C for 15 min in order to make sure more Ni could be transferred into the solution. Despite heating by ultrasonic wave for 30 min, no significant difference of Ca, Fe, K, Mg and Ni extraction rates were recorded between the results obtained by ultrasonic heating and hot plate. As listed in table 4.4, 83.9 % of Ni, and Mg, Ca and K were found 86.5 %, 65.0 % and 69.4 % transferred into the boiled solution from the crushed plants (2 mm) by heating on a hot plate, while 85.3 %, 83.3 %, 61.4 %, and 79.2 % of the corresponding elements were found in the boiled solution by heating in the ultrasonic wave equipment and on a hot plate together. Hence we found that there was no interest to use ultrasonic heating which is more expensive than hot plate.

b) Autoclave heating

Compared heating by a hot plate (100°C), the autoclave heating was done at relative higher pressure and temperature (3.0 bar; at 143 °C). Table 4.4 shows a little higher metal extraction by using autoclave. 90.8 % nickel was transformed into the boiled solution, 88.3 %, 99.9 %, and 92.6 % for Ca, K and Mg, and also a remarkable improvement extraction of Fe, from 54.5 % to 85.7 %. However, considering our goal was to get a high Ni concentration solution but not Fe and other metals, the Ni extraction rose not too much while the economic cost increased much for using autoclave, so in the following experiments, hot plate heating will be used.

3.3.4 Trial to prepare NiS from the aqueous solution

Extraction with hot water enabled us to transfer above 80 % of Ni from the plants to the solution. Then NiS was prepared by addition of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (Fig.4.7). Actually, we compared NiS precipitation in the boiled solution and in a NiSO_4 solution prepared with pure $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ at the same concentration of Ni (3.0 g L^{-1}). In Fig.4.7 (a), the boiled solution is on the left side while and the pure nickel sulfate solution is on the right. The boiled solution is brown because of the organic extract. Fig.4.7 (b) & (c) respectively illustrate the particles of NiS generated from the pure and from the boiled solution. Fig.4.7 (d) shows NiS obtained after filtration of the suspension coming from the pure Ni solution. Fig.4.7 (e) displays that it was not possible to filtrate NiS obtained from the boiled solution since the black solids entered into the filtrate. Then the suspension was centrifuged (Fig.4.7 (f)) to separate NiS from the solution. At the moment, we have not explained the reason why the NiS particles were so small. And since then, this method is not currently applicable since centrifugation is too complex and expensive to allow upscaling.

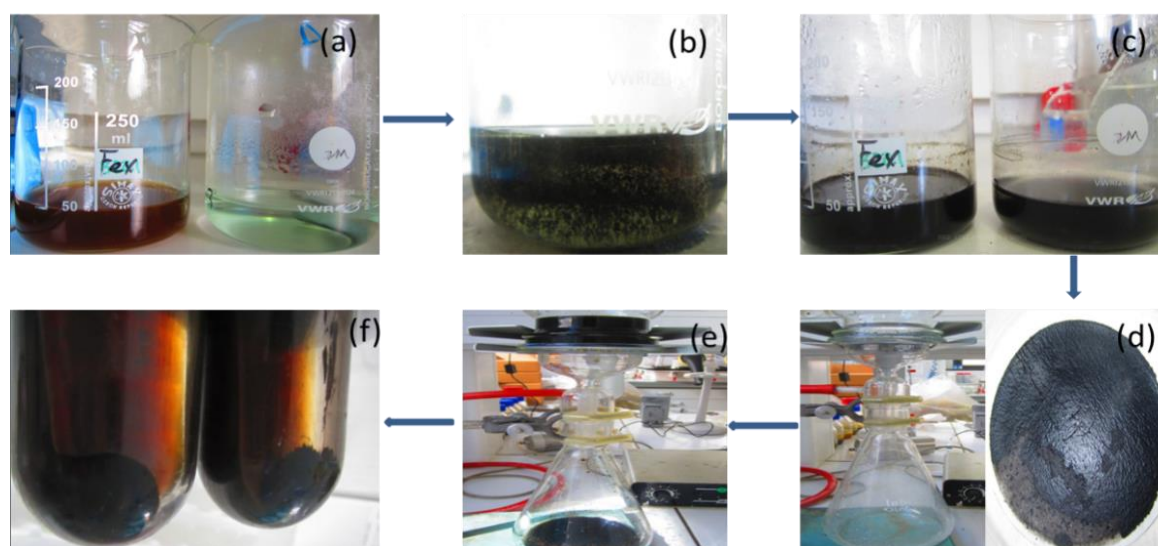


Figure 4. 7: Photos of adding $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ into the boiled solution

3.3.5 Ni extraction from the boiled solution at pH 1

As Ni was still bound to organic matter in the boiled water (section-3.3.2), we used 0.05 M H₂SO₄ (pH=1) for plant boiling *A. murale* plants in order to break the ligands between Ni and organic matters. This method had been used by Barbaroux (Barbaroux, 2010; Barbaroux et al. 2011) as a first step to try selective precipitation and further Ni metal recycling.

Nickel concentration in this boiled solution (30 g ground plants in 270 mL 0.05 M H₂SO₄ (pH=1), a mass fraction of 10 %) was about 1.8-2.6 g L⁻¹. Also, triplicate tests demonstrated that Ni extraction reached 96.5 ± 2.3 % (Table 4.9), which means that nearly all of nickel transformed into the boiled solution from the plants. The same conclusions had been obtained by Barbaroux et al. (2011). This extraction rate was higher than the one obtained by boiling the plants in water by hot plate (83.9 %) and even autoclave (90.8 %).

After adding Na₂S·9H₂O drop by drop into this boiled solution, 1.6 ± 0.2 g of NiS was obtained from 30 g ground plants. 55.7 % of Ni was found in NiS and the rest (35.8 %) was still remained in the filtrate. However, the formula and purity of NiS were still not confirmed as previously.

Table 4. 9: Mass balance of producing NiS from boiling plants (2 mm) in 0.05 M H₂SO₄

Elements	Mass (mg)		Extraction %	Mass (mg)		Yields (%)
	Plants	Boiled solution	Boiled solution	Ni in NiS	Ni in Filtrate	
Ca	242.5±10.3	227.2 ± 6.5	93.7 ± 2.7	/	38.5 ± 1.9	/
Fe	6.0 ± 1.2	6.7±0.5	113.2 ± 8.2	1.8 ± 0.1	2.6 ± 0.1	30.0
K	319.8 ±3.0	279.2±7.2	87.3 ± 2.3	2.0 ± 0.1	125.9 ± 6.3	0.6
Mg	53.3 ± 1.5	50.7±0.9	95.1 ± 1.7	0.13 ± 0.0	17.8 ± 0.9	0.2
Ni	289.9 ±12.5	279.8 ± 6.6	96.5 ± 2.3	161.4 ± 8.1	103.8 ± 5.2	55.7

At last, it is sure that there is still much work to do for investigates this method. Such as the analysis of Ni composition with organic acids in the boiled water, the kinetics of reaction

between $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ and boiled solution, the particle size & purity and formula of NiS should be investigated.

3.4 Conclusion

Exploratory work was carried out to search the best condition to extract Ni from the plant biomass without burning. We showed that Ni could be extracted by boiling water (whatever the heating method), using ground biomass preferentially with particle size of about 2 mm. In these conditions, we checked that the organic ligands present in the plant tissues were preserved. But there is a need of understanding the composition of the solution. Also, boiling ground biomass in sulfuric acid allowed Ni transfer to solution at a very high rate, as previously recorded (Barbaroux et al., 2011).

Then Ni can be precipitated into NiS and a new process can be proposed (Fig. 4.8). But further work is needed to improve and optimize the process.

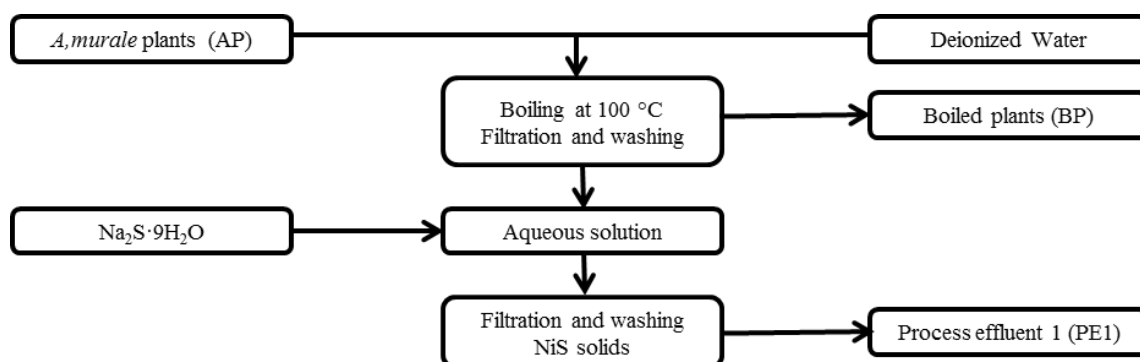


Figure 4. 8: Process of producing NiS from the *A. murale* plants

4 Conclusion

In this chapter, we have investigated new methods to prepare nickel salts from *A. murale*. The first driving idea was to use NiS as an intermediate product, since its solubility in water is very low. Then Ni could be efficiently and selectively separated from the other major elements. Other products, including ANSH, can be prepared from NiS that can be oxidized in NiSO₄. However, difficulties were met to assess the purity of NiS and to dissolve it to prepare the other products.

The second driving idea was to avoid the biomass burning step by extracting Ni directly from the biomass. We proved that it was possible with boiling water. This method is of paramount interest despite the difficulties met to prepare NiS. It offers new perspectives to separate Ni and its ligands from the solution and prepare new products.

Conclusion & perspectives

A man must be big enough to admit his mistakes,
smart enough to profit from them, and
strong enough to correct them.

John C. Maxwell

The objectives of this work devoted to Ni phytomining were:

- ❖ To select the best hyperaccumulators grown in the Balkans for Ni recovery,
- ❖ To optimize the ANSH production process (Mercier et al., 2012; Barbaroux et al., 2012) in view of upscaling, to produce a salt as pure as possible while saving water, reactant and energy,
- ❖ And to investigate other methods to prepare Ni products from plant biomass.

The characterization of 15 Ni hyperaccumulator plant taxa grown in the Balkans exhibited significant differences among them. The highest concentrations of Ni were recorded in leaves and mainly in *L. emarginata* (3.4 %). Other elements (e.g. K and Ca) gave information on plant needs for phytomining purpose, which may require appropriate fertilization to sustain long-term practice. However, further experiments were done with *A. murale* we could obtain high quantities of this plant, successfully cultivated at the plot scale in Albania. Also, with regard to the process itself (e.g. lesser waste production), plants containing more Ni and less Ca than *A. murale* could be of high interest (i.e. *L. emarginata*) in the future provided that we can successfully cultivate it.

Combustion assays proved that a temperature as low as possible (550 °C) should be chosen to avoid metal loss and lessen energy costs. The best combustion duration was set at 2 h but it has to be adapted following furnace characteristics. Ashes contained up to 20 % Ni, which is a very more than 10 times higher than usual laterite or saprolite ores that are currently exploited. Such ashes are considered as a bio-ore of high interest.

The detailed study of the ANSH production process enabled us to propose the following significant improvements:

- ❖ Recycling pathways were proposed at the ash washing step in order to save water.

- ❖ Acid leaching duration was reduced by a factor of two.
- ❖ Sodium hydroxide was replaced by calcium hydroxide, which decreases the cost, while avoiding the precipitation of Na_2SO_4 . In addition, almost all H_2SO_4 in excess was precipitated in the form of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, which induced almost total removal of iron.
- ❖ Magnesium was removed before ANSH crystallization, instead of after, which was more effective (removal of $84.8 \pm 6.0 \%$). The leachate volume was reduced by evaporation, which concentrated Ni; then only one step of ammonium sulfate addition was required for crystallization.
- ❖ After the second crystallization step, ANSH crystals were characterized by combined techniques and their purity reached $99.1 \pm 0.2 \%$, which was higher than previously.

The mass balance performed on the whole process proved that *ca* 91 % of the Ni present in the ash was recovered.

Finally, other alternative methods have been investigated to widen the possibilities of Ni recycling from *A. murale*; they are based on two driving ideas. The first one consisted in preparing other Ni products from the ash than ANSH. We showed that nickel sulfide and nickel sulfate could be obtained by precipitation with sodium sulfide and subsequent oxidation by hydrogen peroxide.

The second idea aimed at extracting Ni from the plant without burning the biomass. We proved that Ni could be extracted at a high rate just by boiling water. Then we explored the possibility of preparing Ni products from the extracting solution.

This research provides new evidence of the feasibility of Ni-phytomining. It gives rises to many perspectives, concerning:

❖ **The ANSH production process**

This process is ready to be upscaled. A pilot set-up has been designed and implemented in the lab and experiences are ongoing. The acquired data will enable us to refine the economic data and to evaluate the environmental impacts by Life Cycle Assessment.

❖ **Other processes leading to other Ni products**

Two routes have been followed to extract Ni from the biomass, either by burning the plants and working with ash or directly from plant biomass. Other routes are possible as well, e.g. by supercritical extraction. Also, other products will be prepared, mineral salts or organo-mineral products, e.g. made with carboxylate.

❖ **The whole agromining chain**

Recently, it was proposed to replace the word “phytomining” by “agromining”, which would be more relevant since agronomy is the first part of the chain. An integrative vision of the chain should be proposed to optimize the obtention of pure high-value products and more generally of services. As a matter of fact, the agromining chain provides various services beside the obtention of Ni products, which are soil depollution, decrease in soil toxicity and increase in soil fertility, landscape improvement and so on. Then the whole chain must be analyzed by life cycle assessment and other evaluation methods.

❖ **Extension to other plants and elements**

We have seen that other plants grown in the Balkans could be good candidates for Ni agromining. As a matter of fact, in many other parts of the world (e.g. in tropical regions), plants or trees containing far higher amounts of Ni have been recently identified. The current issue, which is of paramount importance, is to find conditions to grow these plants without competing with edible plant production or destroying the tropical forest. Mined Ni laterites can offer a great perspective for both land restoration with native hyperaccumulators and economic recovery of Ni. Then processes must be adapted or designed for these other plants.

More generally, agromining of other elements could be envisaged provided that plant cultivation is possible. Rare-earth element accumulating plants have just been identified and give new interesting perspectives.

Résumé étendu en français

To have another language is to
possess a second soul.

Emperor Charlemagne

Les plantes hyperaccumulatrices de métaux ont pleinement été découvertes dans les années 1970 (Jaffre et al., 1976). Ces plantes sont capable d'extraire les métaux des sols et de les accumuler à des concentrations très élevées dans leurs parties aériennes. Le procédé qui utilise ce trait est appelé phytoextraction. Tout d'abord, ces plantes peuvent être considérées comme des indicateurs de la présence de métaux dans les sols, mais très tôt, l'idée d'utiliser ces plantes pour dépolluer des sols a fait son apparition (Chaney, 1983; Huang et al., 1996; Kumar et al., 1995; Macek et al., 2007; Robinson et al., 1999; Salt et al., 1998; Sarma, 2011; Schroeder et al., 2010). Dans un second temps, il a été envisagé de récupérer les métaux présents dans la biomasse récoltée et cette technique a été baptisée « phytomine » (Bani et al., 2007; Brooks et al., 1998; Brooks et al., 2001; Chaney et al., 1998; Chaney et al., 2007; Robinson et al., 1997a; Robinson et al., 1997b). Parmi les métaux et les métalloïdes qui peuvent faire l'objet d'hyperaccumulation par les plantes (e.g. As, Au, Co, Cu, Cd, Ni, Pb, Zn), le nickel (Ni) est d'un grand intérêt car plus de 400 espèces d'hyperaccumulateurs de nickel ont été répertoriées dans le monde et ce métal à une valeur économique élevée (voir London Metal Exchange).

Au début du développement de la phytomine de Ni, le Ni était récupéré de la biomasse d'*Alyssum murale* par pyrométallurgie: après l'incinération de la biomasse, du Ni était produit par un procédé de fonderie à partir des cendres, considérées comme un minerai de Ni de haute qualité (Brooks et al., 2001; Chaney et al., 1998; Li et al., 2003a; Li et al., 2003b). En 2007, le Ni a atteint des prix de US\$ 40 kg⁻¹ voire plus, ce qui permettait de récupérer une valeur totale de 16 000 US\$ par une récolte annuelle sur un hectare (Chaney et al., 2007). Plus tard, l'hydrométallurgie a été appliquée aux biomasses d'hyperaccumulateurs. Il a été montré que le Ni pouvait être extrait des biomasses d'*A. murale* par lixiviation acide, sans incinération

(Barbaroux et al., 2009; Barbaroux et al., 2011). Le lixiviat acide a été traité pour obtenir du Ni pur après des étapes d'extraction et d'électrodéposition (Barbaroux et al., 2011).

D'autres contributions avaient pour but d'obtenir des produits de Ni à partir de cendres d'hyperaccumulateurs. Le Ni pur a été obtenu des cendres d'hyperaccumulateurs de Ni (*Hybanthus enneaspermus* (Violacée)) par procédé électrochimique (Tennakone et al., 2007). Des catalyseurs de Lewis ont aussi été produits à partir des cendres de *Psychotria douarrei* et de *Geissois pruinosa* ayant été cultivées en Nouvelle Calédonie (Losfeld et al., 2012a; Losfeld et al., 2012b). Enfin, un procédé a été mis au point et breveté par notre équipe pour produire un sel pur de nickel, ammonium nickel sulfate hexahydrate (ANSH), à partir des cendres de parties aériennes d'*A. murale* produit en Albanie (Barbaroux, 2010; Barbaroux et al., 2012; Mercier et al., 2012). Cette recherche a été menée dans un contexte interdisciplinaire et international entre le Laboratoire Sols et Environnement (Université de Lorraine, INRA, France), le Laboratoire Réactions et Génie des Procédés (Université de Lorraine, CNRS, France), l'Institut National de la Recherche Eau Terre Environnement (Québec, Canada) et l'Université Agricole de Tirana (Albanie).

En ce moment, il y a un intérêt croissant au niveau mondial pour le développement de la phytomine. Un des enjeux de ce développement est l'approvisionnement régulier de grandes quantités de biomasse possédant des concentrations de métal élevées. Ce point a été l'objet de nombreuses recherches au Laboratoire Sols & Environnement et à l'Université Agricole de Tirana. La construction d'une démarche agronomique a permis de mettre au point un itinéraire cultural pour *A. murale* et de produire plus de 100 kg Ni ha⁻¹ (Bani, 2009; Bani et al., 2014; Bani et al., 2009; Bani et al., 2007). La phytomine peut donc être considérée comme la combinaison de procédés agronomiques et métallurgiques. Pour mettre l'accent sur cette

nature spécifique de la phytomine, le terme « Agromine » a été mis en avant par les équipes de Nancy et repris par l'ensemble des spécialistes au niveau mondial en 2014.

Pour l'instant et bien qu'un nombre croissant d'équipes de recherche s'investissent dans l'agromine, il reste cependant de larges défauts de connaissance, tant sur le plan scientifique que celui des applications. Les domaines scientifiques concernés sont les suivants :

5. **Plantes hyperaccumulatrices** : La recherche doit porter sur les mécanismes de l'hyperaccumulation, sur l'écophysiologie de la plante et sur la génétique pour identifier les gènes impliqués dans l'hyperaccumulation et la sélection des meilleurs individus;
6. **Sols et agronomie des cultures d'hyperaccumulateurs** : les procédés agronomiques, tels que ceux mis au point pour *A. murale* en Albanie, doivent être étendus à différentes plantes, et les sols et climats correspondants partout dans le monde (en particulier pour les zones ultramafiques tropicales). Il y a un besoin de comprendre les paramètres gouvernant la disponibilité du Ni dans les sols et sa dynamique. De plus, la culture d'hyperaccumulateurs peut être couplée à d'autres enjeux, e.g. restauration d'écosystèmes (mines de Ni), Valorisation de zones délaissées (friches industrielles, sols construits) etc...
7. **Procédés métallurgiques** : les procédés existants doivent être améliorés et testés à l'échelle pilote ; ils doivent être étendus à d'autres plantes ou d'autres combinaisons plante/métal pour produire d'autres composés ;
8. **Evaluation de la filière agromine** : depuis les points de vue économiques et environnementaux et comparaison avec les techniques conventionnelles de production de Ni.

Parmi ces enjeux de recherche, nous avons porté notre attention sur les questions scientifiques suivantes :

4. Sélection des meilleures espèces hyperaccumulatrices natives des Balkans : les hyperaccumulateurs ont des caractéristiques d'homéostasie du Ni différentes. Parmi les hyperaccumulateurs de Ni des Balkans, quelles sont les espèces aux propriétés les plus intéressantes pour la récupération de Ni ?
5. Optimisation du procédé de production d'ANSH dans l'optique d'un passage à l'échelle pilote : Ce procédé a été conçu à l'échelle expérimentale de laboratoire et a permis de démontrer la possibilité de produire du ANSH. Cependant, est-il possible s'améliorer son efficacité et de l'optimiser en termes d'économie d'eau, de réactifs, d'énergie ? Mais aussi d'augmenter la pureté des sels produits ?
6. Rechercher d'autres méthodes pour produire des composés nickélifères à partir de la biomasse des plantes hyperaccumulatrices : Est-il possible de fabriquer d'autres produits ? Avec d'autres méthodes d'extraction ?

Pour répondre à ces questions, ce manuscrit est organisé en quatre chapitres rédigés en Anglais. Le premier chapitre présente l'état de l'art bibliographique sur la phytomine et l'agromine de Ni. Cela comprend le procédé agronomique de phytomine, les plantes hyperaccumulatrices de Ni et les procédés de récupération du Ni à partir des biomasses produites.

Le deuxième chapitre traite de la sélection d'hyperaccumulateurs des Balkans. Une série de plantes appartenant à trois genres différents a été échantillonnée dans un éventail large de conditions édaphiques et d'habitats en Albanie et en Grèce continentale. Nous avons analysé les teneurs en éléments majeurs (Ni inclus) des organes des plantes pour identifier les meilleurs individus. Ensuite, nous avons étudié les conditions optimales d'incinération de la

biomasse de la plus commune des espèces, *A. murale*, permettant de limiter les pertes en Ni. Nous avons précisé la composition de chacune des cendres obtenues et établi des bilans de masse entre la biomasse et les cendres.

Le troisième chapitre porte sur l'amélioration du procédé de production d'ANSH. Nous avons étudié chaque étape du procédé pour apporter une meilleure compréhension des mécanismes en jeu. Ce travail nous a permis d'améliorer le procédé en modifiant certaines des étapes et globalement en réduisant les consommations de matière et d'énergie nécessaire à la production d'une masse donnée de sel. La pureté du sel a aussi été considérablement améliorée.

Dans le quatrième chapitre, nous proposons d'obtenir d'autres produits nickélifères à partir de la biomasse d'*A. murale*, tels que le sulfure de Ni et le sulfate de Ni. Aussi avons-nous étudié la faisabilité de l'extraction de Ni directement à partir de la biomasse par de l'eau. Cette méthode ouvre de nouvelles possibilités pour produire d'autres composés à base de Ni.

Les principales conclusions de ce travail sont détaillées ci-dessous.

La caractérisation de 15 taxons de plantes hyperaccumulatrices de Ni natives des Balkans a montré des différences significatives au niveau de la phytoextraction. Les concentrations de Ni les plus élevées ont été enregistrées dans les feuilles et principalement pour l'espèce *L. emarginata* (3.4 %). Les autres éléments (e.g. K et Ca) ont permis de montrer les besoins des plantes dans le cadre de la phytomine, ce qui montre que la culture de ces plantes requiert une fertilisation appropriée au long terme pour des éléments fortement exportés comme le Ca et le K. Cependant, des expériences complémentaires menées avec *A. murale* nous ont permis d'obtenir de grandes quantités de biomasse de cette plante à l'échelle de la parcelle en Albanie. De plus, concernant le procédé à proprement parlé (e.g. plus faible production de

déchets), les plantes contenant plus de Ni et moins de Ca qu'*A. murale* (i.e. *L. emarginata*) pourraient faire l'objet d'une recherche plus intense à l'avenir, à condition de pouvoir les domestiquer.

Les tests de combustion ont prouvé que la température la plus basse possible (550 °C) devait être choisie pour éviter les pertes en métal et diminuer le coût énergétique de l'incinération des biomasses. Les meilleures conditions de combustion étaient de 2 h à 550 °C, mais elles doivent probablement être adaptées aux caractéristiques du four. Les cendres obtenues contenaient jusqu'à 20 % de Ni, ce qui est une concentration environ 10 fois supérieure à celle des minerais latéritiques exploités par la filière conventionnelle. Ces cendres peuvent donc être considérées comme des bio-minerais à forte valeur économique.

L'étude détaillée du procédé de production d'ANSH nous a permis de proposer les améliorations suivantes :

- ❖ Des voies de recyclages ont été proposées à l'étape de lavage des cendres dans le but d'économiser les quantités d'eau utilisée ;
- ❖ La durée de lavage acide a été réduite d'un facteur deux ;
- ❖ La soude a été remplacée par l'hydroxyde de calcium, ce qui diminue le coût tout en évitant la précipitation de Na_2SO_4 ; de plus, presque tout le H_2SO_4 en excès a été précipité sous forme de $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, ce qui a permis l'élimination quasi totale du fer ;
- ❖ Le magnésium a été éliminé avant la cristallisation d'ANSH au lieu d'après comme initialement dans le procédé, ce qui était plus efficace (élimination de 84.8 ± 6.0 %) ; le volume a été réduit par évaporation, ce qui a concentré le Ni ; alors, seulement une étape d'ajout de sulfate d'ammonium a été nécessaire pour la cristallisation.

- ❖ Après la seconde étape de cristallisation, les cristaux d'ANSH ont été caractérisés par des techniques croisées et leur pureté attestée à $99.1 \pm 0.2 \%$, une valeur supérieure à celles obtenues précédemment.

Le bilan de masse réalisé sur le procédé dans son intégralité a prouvé qu'environ 91 % du Ni des cendres était récupéré.

Enfin, des méthodes alternatives ont été testées pour élargir les possibilités de récupération de Ni à partir des biomasses d'*A. murale* ; elles étaient fondées sur deux idées directrices. La première était de préparer des produits nickélifères autres que l'ANSH à partir des cendres. Nous avons montré que le sulfure et le sulfate de Ni pouvaient tous les deux être produits par précipitation avec du sulfure de sodium et l'oxydation consécutive à l'ajout de peroxyde d'hydrogène pour obtenir le sulfate de Ni.

La seconde idée avait pour but d'extraire le Ni de la plante sans incinérer la biomasse. Nous avons montré que le Ni pouvait être extrait à un rendement élevé par l'eau bouillante. Nous avons ensuite exploré la possibilité de préparer des composés nickélifères à partir des solutions d'extraction.

Cette recherche apporte de nouveaux arguments démontrant la faisabilité de la phytomine de Ni. Elle soulève de nombreuses perspectives concernant :

❖ **Le procédé de production d'ANSH**

Le procédé est maintenant prêt à passer au stade pilote. Un dispositif pilote a été conçu et mise en place au LRGP ; les expériences sont en cours. Les données acquises nous permettront d'affiner les données économiques et d'évaluer l'analyse globale du cycle de vie de la filière.

❖ **D'autres procédés menant à des composés nickélifères différents**

Deux routes ont été suivies pour extraire Ni de la biomasse, soit par incinération et récupération à partir des cendres, soit directement à partir de la biomasse des plantes. D'autres routes sont possibles également, e.g. par extraction supercritique. Aussi, d'autres produits pourront-ils être préparés, sels minéraux ou composés organo-minéraux, e.g. carboxylates de Ni.

❖ **La filière globale d'agromine**

Récemment le terme « agromine » a été proposé plutôt que « phytomine », c'est un terme qui intègre mieux la notion de filière. Une vision intégratrice de la filière devrait être proposée pour optimiser l'obtention de produits purs à haute valeur et plus généralement de services. Les services fournis par l'agromine, en plus de la récupération de métal, sont la dépollution et la diminution de la toxicité des sols, l'amélioration de la fertilité des sols, la renaturation paysagère, etc. La filière entière doit donc être considérée par analyse de cycle de vie ou d'autres méthodes d'évaluation coûts-bénéfices.

❖ **L'extension à d'autres plantes and éléments**

Nous avons vu que d'autres plantes natives dans les Balkans pourraient être de bons candidats à l'agromine de Ni. En effet, dans de nombreuses parties du monde (e.g. régions tropicales), les plantes ou les arbres contenant des concentrations de Ni bien supérieures ont été identifiées dont certaines très récemment. L'enjeu actuel, qui est d'importance cruciale, est de définir les conditions de cultures adéquates pour ces plantes sans entrer en compétition avec la production de denrées alimentaires ou de conduire à la déforestation. Les latérites nickélifères après mine peuvent offrir une grande perspective pour la restauration des milieux avec des espèces hyperaccumulatrices natives et une récupération économiquement rentable de Ni à partir des substrats saprolitiques mis à nu et des stériles silicatés. Les procédés devront alors être adaptés ou conçus pour ces autres plantes. De manière générale, l'agromine d'éléments

autres que le Ni pourrait être envisagée si la culture de plantes cibles est possible. Des plantes hyperaccumulatrices de Co, de Cu, de Sc, de terres rares ont été identifiées très récemment et offrent de nouvelles perspectives.

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