CHAPTER 3

Methodology

3.1 Study sites description:

This study was carried out in two locations. Ex situ germination trials were conducted in a small tree nursery at the Biology department of Science Faculty in Chiang Mai University, whilst field trials were conducted at the Siam Cement Groups Mae Than Mine near Mae Than Village, Sop Prab Sub-district, Mae Than District and Lampang Province.

3.2 Field Plots:

The lignite mine is situated at the end of Highway 1043, about 20 km NE of Sop Prap village (17°58ʾN, 99°26ʾ E, elevation about 400 meters). The lower hills around the mine have deciduous, seasonal, hardwood + bamboo (bb-df) vegetation, while the upper parts include disturbed deciduous, dipterocarp-oak, seasonal, hardwood (dof) forest (Maxwell, unpublished data) (Appendix-C). Both forest types have been extensively and severely damaged by locals for decades for timber, bamboo products, medicinal plants, edible mushrooms, and excessive hunting (Maxwell, unpublished data).The bedrock is shale and the soil ranges from moderately to extensively rocky with no or little organic top soil in the forests. Mine rubble has been dumped throughout the area, forming irregular hillocks of debris. Some introduced trees have been planted along some of the dirt roads there (Appendix-c). The overall vegetation of the SCG area is typical of lowland Northern Thailand. The climate of Northern Thailand, including the SCG site, has three distinct seasons. From November to mid-March it is cool and dry, then hot and dry until May and rainy from June until the end of October. In 2011, the total amount of rain at the SCG mine office was 1,326 mm.

3.3 Nursery:

The Biology Department nursery (18° 47' 25N latitude and longitude 98° 58' 54E, 310 m elevation) had an average annual temperature of 25◦C and annual rainfall of 1.098 mm.

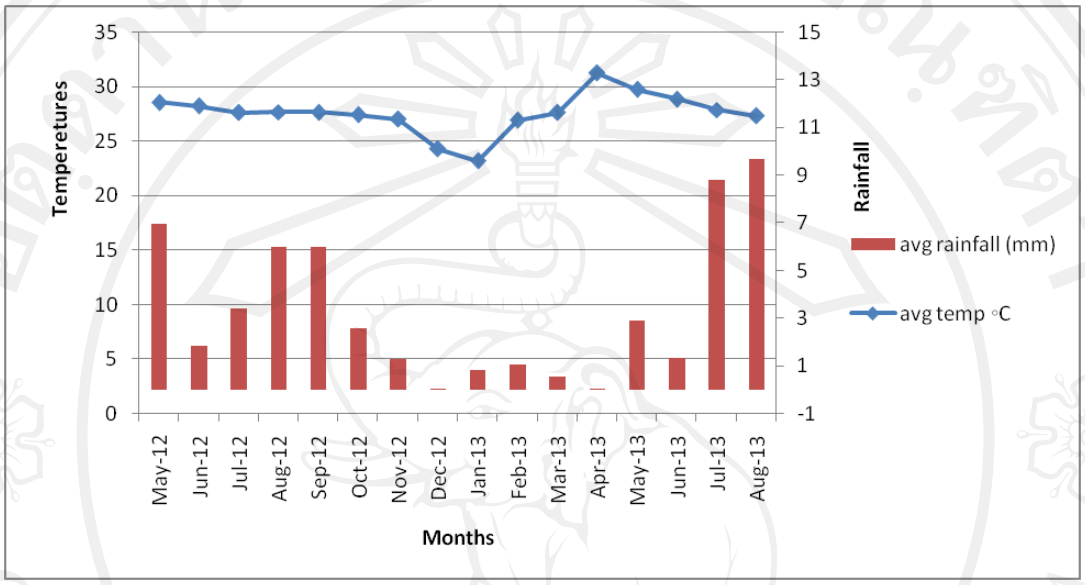

Figure 0-1: Average temperature (◦C) and rainfall (mm) in Chiang Mai (about 300m elevation, 9km distance between Chiang Mai Meteorological station and Chiang Mai University).

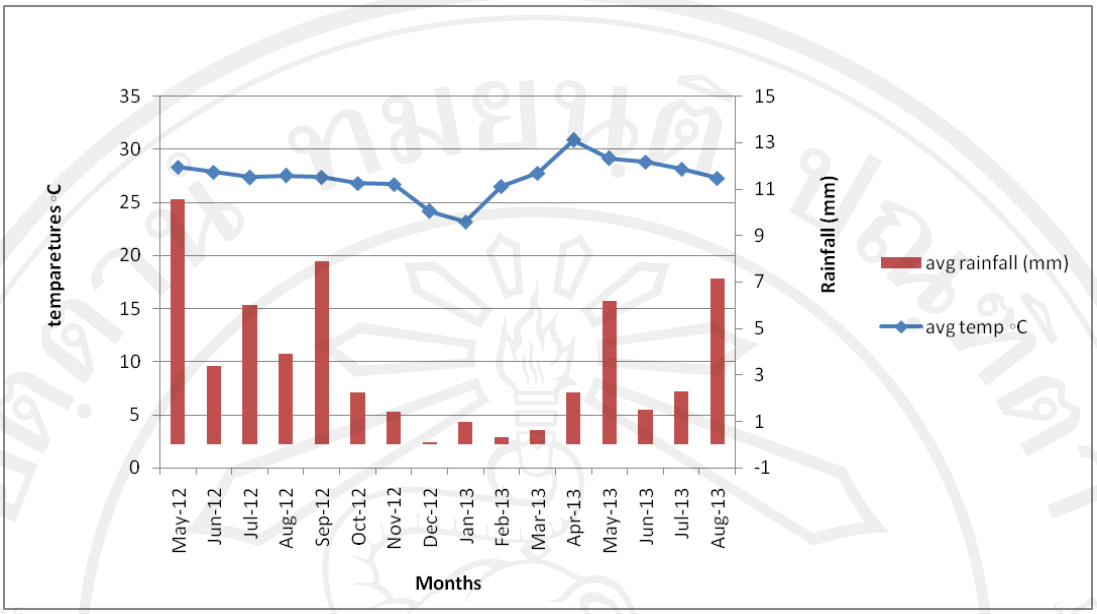

Figure 0-2: Average temperature (◦C) and rainfall (mm) in Lampang. (400m elevation, about20 km distance from the study plots).

3.3.1.1.1 Nursery study Site in Chiang Mai University.

(b) Field study site in Mae than mine, Lampang.

Figure 0-3: (a) & (b) Two of the Study sites in Thailand.

3.4 Species studied:

The tree species selected for this study were those that would have been typical of the lowland deciduous forest type, which would have grown on the study site before mining commenced: *Afzelia xylocarpa* (Kurz) Craib, *Eugenia cumini* (L.) Druce, *Ficus*

racemosa Roxb., *Gmelina arborea* Roxb., *Schleichera oleosa* (Lour.) Oken for the field study and *Afzelia xylocarpa* (Kurz) Craib, *Ficus racemosa* Roxb. , *Gmelina arborea* Roxb., *Schleichera oleosa* (Lour.) Oken, *Adenanthera pavonina* L., *Spondias pinnata* (L.f.) Kurz., *Irvingia malayana* Olive. Ex Benn. and *Catunaregam spathulifolia* Trio. for the nursery study.

3.5 Seed collection and storage:

Seeds were collected from Chiang Mai University campus and surroundings Except for *Eugenia cumini* all fruits were collected from the ground when seeds were properly developed and matured. Good seeds (which sink into the water) of the tree were selected, avoiding those which seem diseased or generally unhealthy. The species selected for this study were expected to be fruiting from May till June i.e. the beginning of the rainy season, which is the optimum time for direct seeding (FORRU, 2008). Since the nursery study could not be set up at the same time as the field study, *Adenanthera pavonina, Spondias pinnata, Irvingia malayana* and *Catunaregam spathulifolia* were collected at the beginning of the cool season; as these seeds available at that time.

Fruits were stored at the FORRU nursery in open plastic bags, out of direct sunlight, for one to two days until seeds were extracted. Except for *Afzelia xylocarpa* (which has a hard seed coat) the fruit pulp was removed and the seeds rinsed with water. Seeds were then spread out on paper and air dried in shade overnight, after which the seeds were stored (approximately one and half months) in airtight jars at the FORRU nursery and *Eugenia cumini* was stored in a refrigerator for 30 days, while awaiting field trial. All jars were stored at room temperature (26º-28 ºc) out of direct sunlight.

Table 0-1 : Flowering and fruiting months of the species studied.

Species	Family name	Habitat	Elevation	Flower Month	Fruit Month
Afzelia xylocarpa (Kurz) Craib	Leguminosae	bb/df	350-500	mr-ap	jn-fb
Eugenia cumini (L.) Druce	Myrtaceae	dofbb/df	375-650	mr-ap	jn-jl
Ficus racemosa	Moraceae	mxf, often along stream	350-1000	nv-jl (flowers and fruits in figs)	nv-jl(flowers and fruits in figs)
Gmelina arborea	Verbenaceae	egf,eg/pine	350-1475	fb-mr	mr-jn
Scleichera oleosa	Sapiandacea	bb/df	350-600	mr-my	my-jl
Adenanthera pavonina	Leguminosae	dof bb/ df	350-700	ap-jn	my-nv
Spondias pinnata	Anacardiaceae	dof bb/ df	375-750	mr-my	oc-mr
Irvingia malayana	Irvingiaceae	mxf	350-750	ap-jn	sp-nv
Catunaregam spathulifolia	Rubiaceae	dof bb/ df	450-900	my-jn	ag-ja

(Modified from "The Vegetation and Vascular Flora of Doi Suthep-Pui National Park, Northern Thailand", by Maxwell and Elliott, 2001).

Note:	habitat	bb/df	degraded teak & bamboo+ deciduous forest
		egf	primary evergreen forest
		eg/bb	evergreen forest with bamboo
		eg/pine	evergreen forest with pine
		dof	deciduous dipterocarp-oak seasonal forest
		mxf	mixed evergreen + deciduous seasonal, seasonal forest
		sg	secondary forest
	Month	ja	January
		fb	February
		mr	March
		ap	April
		my	May
		jn	June
		jl	July
		ag	August
		sp	September
		oc	October
		nv	November
		dc	December

Table 0-2 : Collection and sowing dates in the nursery and field.

Species	Collection month	Collection date	Sowing date(field)	Sowing date (nursery)
Afzelia xylocarpa	June	25th June 2012	16th August 2012	16th November 2012
Eugenia cumini	July	3rd July 2012	16th August 2012	Not sown
Ficus racemosa	June	17th June 2012	16th August 2012	16th November 2012
Gmelina arborea	may	28thMay 2012	16th August 2012	16th November 2012
Schleichera oleosa	July	9th July 2012	16th August 2012	16th November 2012
Adenanthera pavonina	November	7th November 2012	16th August 2012	16th November 2012
Spondias pinnata	November	15th November 2012	16th August 2012	16th November 2012
Irvingia malayana	November	12th November 2012	16th August 2012	16th November 2012
Catunaregam spathulifolia	November	10th November 2012	16th August 2012	16th November 2012

Table 0-3: Flowering and fruiting months of the species studied.

Species	Habitat	Elevation	Flower Month	Fruit Month
Afzelia xylocarpa	bb/df	350-500	mr-ap	jn-fb
Eugenia cumini	Dof bb/df	375-650	mr-ap	jn-jl
Ficus racemosa	mxf, often along stream	350-1000	nv-jl(flowers and fruits in figs)	nv-jl(flowers and fruits in figs)
Gmelina arborea	egf,eg/pine	350-1475	fb-mr	mr-jn
Scleichera oleosa	bb/df	350-600	mr-my	my-jl
Adenanthera pavonina	dof bb/ df	350-700	ap-jn	my-nv
Spondias pinnata	dof bb/ df	375-750	mr-my	oc-mr
Irvingia malayana	Mxf	350-750	ap-jn	sp-nv
Catunaregam spathulifolia	dof bb/ df	450-900	my-jn	ag-ja

Seed characteristics:

Seed size category based on seed weight and they are divided into three groups: a) S small (<0.01–0.099 g), b) I intermediate (0.1–4.99 g), c) L Large (>5.0 g) (seed size category determined from mean of a random sample of no less than 20 seeds per species) (Doust *et al*. 2006).Seed coat thickness also divided into three groups: a) Thin (<0.01–0.099 mm), b) moderate (0.1–0.49 mm), c) Tk Thick (>0.5 mm) (Tunjai & Elliott, 2011). Before germination types have been divided into two types; epigeal or hypogeal; but nowadays hypogeal or epigeal type has been criticized on etymological ground. As the cotyledons can be divided on cryptocotylar (cotyledons enclosed in seed coverage) or phanerocotylar (cotyledons free from seed coverage). On the basis of cotyledons characteristics Gardwood, 1996 described five types of seedlings, which are showed in the figure3-4. The studied species seedling types are enlisted in Table

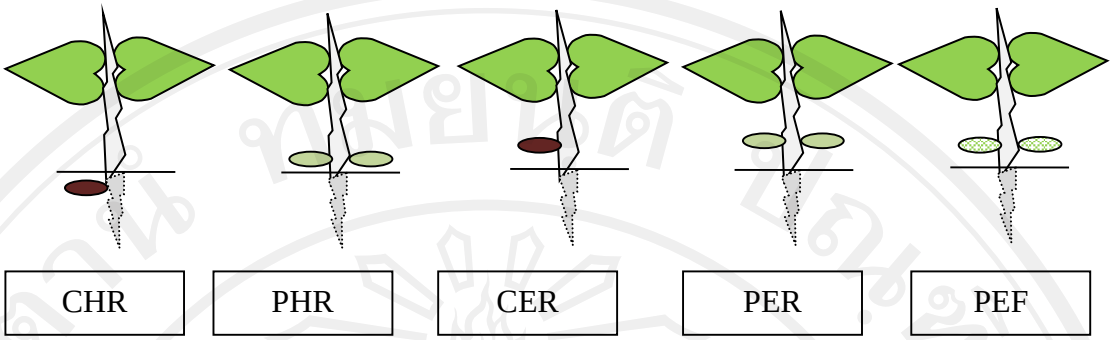

Figure 0-4: Schematic diagram of seedlings type: CHR- cryptocotylar hypogeal reserves, PHR- phanerocotylar hypogeal reserves, CER- cryptocotylar epigeal reserves, PER- phanerocotylar epigeal reserves, PEF- phanerocotylar epigeal reserves.

Table 0-4: Seed characteristics.

Species	Seed size	Seed coat	Orthodox/ Recalcitrant	Pre-sowing treatment	Germination type
Afzelia xylocarpa	Large	Hard	Orthodox	Scarification	CER
Eugenia cumini	Small	Soft	Recalcitrant	Ringed the seeds with water and dry under shade	CHR
Ficus racemosa	Small	Hard	Orthodox	nothing	PEF
Gmelina arborea	Medium	Hard	Intermediate	Ringed with water and dry under sun	PEF
Schleichera oleosa	Medium	Hard	Orthodox/ Intermediate	Ringed with water and dry under sun	PEF
Adenanthera pavonina	Medium	Hard	Orthodox	Scarification	CER
Spondias pinnata	Large	Hard	Orthodox	Clipping	CHR
Irvingia malayana	Large	Hard	Orthodox	Clipping	CHR
Catunaregam spathulifolia	Small	Hard	Orthodox	Nothing	CHR

3.6 Treatment used in the experiment:

3.6.1 Biosolids:

Biosolids are municipal treatment plant solids, determined to be safe for land application. The word sludge is a generic term that most people use to refer to some type of unprocessed waste material. There is still some interchange of these two words,

but biosolids always refers to processed wastewater solids that have met specific criteria and are suitable for land application**.**

Table 0-5: Analysis of biosolids component.

ph	7.72
OM (g/100)	19.75
NH4+-N(mg/kg)	2461.72
NO3—(mg/kg)	317.81
Total N(g/kg)	1.57
P(mg/kg)	68.72
K(mg/kg)	777.7
Moistures (%) (Wet wt.-dry wt.)/dry wt. of soil*100	212.22

*Pepsi cola-Lays Companies waste water plant (Basically waste comes from Potato chips production process).

3.6.2 Compost:

Compost used in this study was made from urban organic wastes and cow feces in the ratio 3:1.Organic matter was used > 20%, also NPK used in it where N proportion was higher.(ref: cmgreencity network, collaborate with Chiang Mai municipality).

3.6.3 Fertilizer:

The fertilizer used in this experiment was N: P: K which proportion was 1:2:1(keaw suay homm organic fertilizer).

3.7 Germination trials in the nursery:

For each of the 8 selected species, 324 seeds were sown in germination trays in the nursery, using a randomized complete block design (RCBD) consisting of 3 treatments and 3 replicates, 36 seeds per replicate. The 3 treatments were different germination media: i) biosolids + mine substrate (1/1), ii) compost + mine substrate (1/1) and iii) control (mine substrate) – with one germination tray per replicate.

Seeds of each species were subjected to the best pre-sowing treatment needed to accelerate and maximize germination, according previous results obtained by the Forest

Restoration Research Unit (FORRU-CMU). Seed germination was monitored every week for 2 months, to determine which substrate results in the highest seed germination rate. Once seedlings developed one pair of fully expanded true leaves, they were transferred to plastic bags (9 x 2 ½ inches) with mine substrate and the treatments listed above. Height, RCD (root collar diameter) and health score (0-3) of every seedling were recorded every month for 3 months. Median length of seed dormancy (the time between sowing and germination of half of the seeds which eventually germinate (FORRU, 2008)) was calculated for each replicate.

3.8 Germination trials in the field:

The field trial was an RCBD with 5 substrate amelioration treatments and 3 replicates. The treatments were: i) biosolids+fertilizer, ii) biosolids, iii) compost + fertilizer, iv) compost and v) control (no substrate amelioration treatments).

Each triplicate sub-plot consisted of 36 seed sowing points, each marked by a bamboo pole (color coded by treatments), about 1.2 m apart, laid out in a square grid pattern 6 x 6 points. At each sowing point, one seed of each of the five species was sown into PVC tubes (5.5 cm in diameter) about 40 cm apart (color coded to match the species).

Each triplicate sub-plot covered an area of 7.2×7.2 m^2 with a total area of 843.75 m^2.

The treatments were selected to improve germination success in two stages: 1. Soil preparation with biosolids, Compost and fertilizer - in every hole biosolids and compost were mixed with around 1kg of soil and 50 g of fertilizer (N: P: K 12:24:12) added; 2. Seed germination was monitored every week for 3 months. Germination data were collected every one week, to calculate per cent germination and length of dormancy. For each seedling germinated in the field; height, RCD (root collar diameter) and health score (0=dead to 3= good health) were recorded for 5 months (FORRU, 2008).

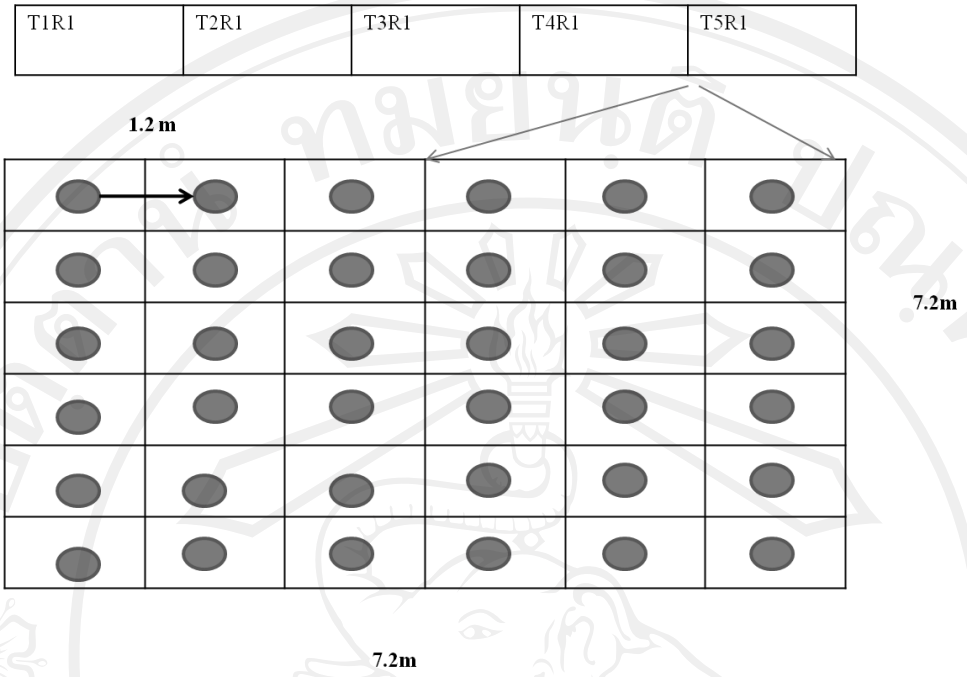

Figure 0-1: Diagram of experimental plot in field site.

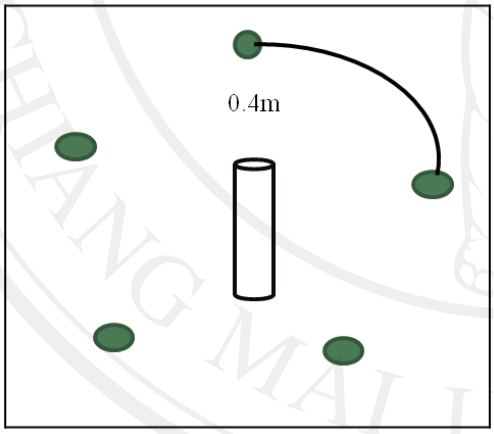

Figure 0-2: Seeds sowing point.

3.9 Data analysis:

Data were analyzed by Microsoft Excel 2007 Data Analysis Tool pak. The median length of dormancy (MLD) and percent germination were calculated at the end of the germination trial (when no further germination had occurred for 11weeks).A two way ANOVA without replication was performed to detect any significant differences in germination percentages among species and treatments in the field. A two way ANOVA was performed to detect significant differences in percent germination, Percent establishment, (Percent establishment = Seedlings that established in the field study after seed sown, which still alive after 12 months; expressed from, percent of the seeds sown & no. of seedlings that alive after certain time), and growth performances of surviving seedlings in field conditions and have to see the effect of applying treatments. Student's t-test (paired two samples for means) was performed when ANOVA results showed any significant differences on Percent germination, percent establishment, and growth performances of surviving seedlings in field conditions. The relative growth rates (RGR) were calculated using the formula:

$RGR = \ln(G2) - \ln(G1)\ T2 - T1 \times 365 \times 100$ where G1 and G2 are the growth parameters (root collar diameter, height, and canopy width) at the beginning (T1) and end (T2) of the sampling period.

In the nursery, median length of dormancy (MLD) and percent germination were calculated at the end of the germination trial (when no further germination had occurred after 8 months later). A two way ANOVA, without replication was performed to detect any significant differences in germination percentages among species and treatments in germinated seeds the nursery trial. If any significant result was found, Student's t-test (paired two samples for means) was performed to determine significant differences between treatments or species.

A suitability score was also calculated, based on a combination of performance parameters to determine which species are good for direct seeding in degraded area. The suitability score or percentage (0-100) of maximum possible score with available data, based on species field performance (establishment and growth) at the middle of 2nd rainy season after sowing, was calculated to predict which tree species are suitable for

restoration by direct seeding. First, species with survival percentages less than 10% by the middle of 2nd rainy season (expressed as a percentage of seed sown) was rejected.

Then for all those species which exceed this threshold, suitability index was calculated using the formula:

Suitability Score: $establishment * RCD$

Where establishment is the percentage of seedlings remaining alive at the middle of 2nd rainy season, and height was selected to represent the size in the formula. Importantly, size should have equal weight as survival in the calculation to get the raw suitability score. All raw scores were then divided by the highest value and multiplied by 100 to get a rank score.