REVEGETATION OF ACID FORMING GOLD MINING SPOILS CONTAINING HIGH LEVELS OF ARSENIC¹

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Abstract: This work was conducted at a large gold mine in Paracatu, MG, Brazil, between March 2000 and November 2005. The waste rock studied was a phyllite which contained (in addition to gold) sulfides such as pyrite and arsenopyrite. The objective was to evaluate the survival and growth of plant species on different combinations of plant growth substrate layers deposited over the spoil. Each combination tested consisted of a cover-layer and a final sealing-layer, both deposited over the remaining, slightly-weathered rock spoils. The treatments were as follows: (T1) a cover layer with 50 cm of partially weathered spoil (B1) with lime added over a compacted sealing layer with 30 cm of B1; (T2) a cover layer with 25 cm of limed native soil over 25 cm of limed B1 over a compacted sealing layer of 30 cm of B1; (T3) a cover layer with 50 cm of limed B1 over a compacted sealing layer of 20 cm of limed soil over 20 cm of unlimed B1; and (T4) a cover layer with 25 cm of limed soil over 25 cm of limed B1 over 20 cm of compacted limed soil over 20 cm of unlimed B1. The plant species used were Acacia farnesiana, A. holosericea, A. polyphylla, Albizia lebbeck, Clitoria fairchildiana, Flemingia sp., Mimosa artemisiana, M. bimucronata, and Enterolobium timbauva. Experimental data included diameter of the stem collar (DC), plant height, and survival counts. The greatest survival rate was observed with T4, at 80%. In general, M. bimucronata and A. farnesiana species showed the highest survival rate. The Scott-Knott test a p < 5% indicated that A. holosericea had the greatest growth-response, while A. polyphylla, Flemingia sp., and E. timbauva exhibited lower growth rates. The bioavailable soil As content from (T1), (T2), (T3), and (T4) was 14.69, 0.00, 10.41, and 0.00 mg dm⁻³, respectively. These experimental results support the conclusion that presence of native soil is essential for establishment of plants and minimization of plant toxins like As in mine reclamation efforts, and that appropriate implementation of layer combinations such as studied here allows greater survival rates and biomass gains.

Additional Key Words: Arsenopyrite, land reclamation, legumes

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Introduction

The recovery of areas degraded by mining usually involves the reestablishment of vegetation and in many instances, the process is not monitored systematically (Almeida et al., 2005). There are many parameters that can be used as indicators of the recovery process, but the greatest challenge was to develop or adapt valid criteria for monitoring and evaluating the functionality of the area, as well as discriminating indicators that provide the desired information with accuracy and acceptable cost. Specific indicators must be developed for each individual situation and environment, as it is difficult to develop universal indicators (Rodrigues and Gandolfi, 2001).

Revegetation efforts may be further complicated by the presence of sulfides in mineral rocks, which can lead to the formation of acid rock drainage and the consequent release of heavy metals and metalloids into the environment. This includes arsenic (As), which is currently considered the most hazardous substance to human health (ATSDR, 2008). One of the strategies for *in situ* rehabilitation of mined areas with the presence of significant contamination is phytoremediation, in which vegetation is used for soil decontamination. Decontamination can be facilitated through various processes such as phytostabilization (and immobilization), phytoextraction, phytovolatilization and phytodegradation (Wenzel, 2008).

These techniques usually employ plants along with other practices such as liming (Accioly, 2000), acidification, and application of chelating agents. These processes remove, immobilize, or render the contaminants less harmful to the ecosystem. One of the basic requirements for the success of any revegetation technique is the identification of plants tolerant of the major contaminants present. For example, a study on the feasibility of using tree species for the rehabilitation of areas contaminated by heavy metals received attention due the possibility of uptake of these into plant biomass for long periods of time (Eltrop et al., 1991).

In addition to the selection of tolerant plants in areas with acid spoils and drainage, there is a need for the utilization of additional materials in layers over the phytotoxic mining substrates which minimize their phytotoxic properties and allowing plant establishment (Dias et al., 1999). This study focused on the use of amended sulfidic materials and native soil in layers over a reactive sulfidic substrate and the behavior of several tree and shrub species planted on various combinations of these layers.

Material and Methods

The field experiment was carried out at a gold mine located in Paracatu, in the state of Minas Gerais, Brazil. It was installed in March 2000 and dismantled in November 2005. Four lanes, five-m wide and twenty-m long, were constructed. These tracks were made of different materials and arranged above the partially weathered sulfidic substrate, constituting the four tested treatments (Fig. 1). The two substrate materials used were a partially-weathered sulfidic spoil (B1) and the Bo horizon of a local Oxisol (soil). Treatments applied were: (T1) a cover layer with 50 cm of B1 with lime added over a compacted sealing layer of 30 cm of B1; (T2) a cover layer with 25 cm of limed soil over 25 cm of limed B1 over a compacted sealing layer of 20 cm of limed B1; and (T4) a cover layer with 25 cm of limed soil over 25 cm of limed B1 over 20 cm of compacted limed soil plus 20 cm of unlimed B1. Liming rates were based on the Al content of the soil and lime was applied at Ca/MgCaCO₃. Monitoring of the experiment was initiated in May of 2000.

The experiment was assembled in a 2 x 2 factorial, completely randomized experimental design, with three replications. Each experimental plot received nine planted species of leguminous tree or shrub (a spacing of $1.0 \times 1.0 \text{ m}$.). Planting was carried out as a sequence of 6 plants of the same species in a row, until the plants of all nine species were planted, and then we followed with a new replication with 6 plants of each species again in a random sequence.

Initial fertilization was carried out in planting holes (30 cm wide by 40 cm deep) with admixtures of 150 g of rock phosphate, 50 g of KCl and 1.0 L of stabilized cattle manure. The following species were used: *Acacia farnesiana, Acacia holosericea, Acacia polyphylla, Albizia lebbeck, fairchildiana Clitoria, Flemingia sp., Artemisiana Mimosa, Mimosa bimucronata* and *Enterolobium timbauva.*

Two evaluations of plant survival and growth were completed, the first in August 2003 (forty months after the beginning of the experiment) and the second in January 2005 (17 months after the first evaluation). Plant growth was evaluated (via biometrics analysis) with values for stem collar diameter (DC) and height of each plant, disregarding those present at the edges. A total of 162 subjects were evaluated in each treatment: 54 per block, with six individuals of each species. Bioavailable soil As levels were measured in samples collected from the substrate cover layer (0-20 cm) using the Mehlich III extractor.

Statistical analysis was performed to verify the homogeneity of variances (via ANOVA) for the different treatments. The degrees of freedom for treatments were deployed in orthogonal contrasts plus additional contrasts, not necessarily orthogonal with all other contrasts (V. Alvarez & Alvarez, 2006).



Figure 1. Diagram of the treatments. B1 is partially-weathered sulfidic substrate. Soil is Bo horizon of Oxisol (clayey). The sealing layer was compacted.

Results and Discussion

In the first evaluation in August 2003, 69 specimens survived in treatment 1 (T1) – a rate of approximately 43%. None of the *Clitoria fairchildiana, Flemingia* and *Acacia sp polyphylla* survived. In the second evaluation in January 2005, no plants were observed to survive in T1. This treatment consisted of B1 as both the rooting substrate and sealing cover layer, with lime in the first layer and compaction the second. The formation of salt crusts was noted on the surface of this treatment along with the highest observed levels of available As, averaging 14.7 mg kg⁻¹. The average levels of As available in samples from treatments 2, 3 and 4 were 0.0, 10.4 and 0.0 mg kg⁻¹, respectively; however the presence of salt crusts was not observed on the surface of treatment 3. Thus, As phytotoxicity was probably not the only cause of death of all individuals in treatment 1.

The overall survival of all species for treatments 2, 3 and 4 in the first evaluation was approximately 66, 83, and 90% respectively. In the second evaluation, these percentages decreased to approximately 58, 72 and 80% for treatments 2, 3 and 4, respectively (Table 1).

Species	Survival							
	Treatr	nent 1	Treatr	ment 2	Treatr	ment 3	Treatr	ment 4
Months	40	57	40	57	40	57	40	57
					%			
A. farnesiana	83.33	0.00	100.0	94.44	100.0	100.0	94.44	88.89
A. holosericea	33.33	0.00	83.33	83.33	100.0	94.44	88.89	88.89
A. polyphylla	0.00	0.00	61.11	61.11	77.78	77.78	88.89	72.22
A. lebbeck	88.89	0.00	88.89	83.33	100.0	100.0	94.44	88.89
C. fairchildiana	0.00	0.00	33.33	0.00	72.22	33.33	94.44	50.00
Flemingia sp.	0.00	0.00	0.00	0.00	11.11	0.00	50.00	50.00
M. artemisiana	44.44	0.00	72.22	50.00	88.89	50.00	94.44	88.89
M. bimucronata	66.67	0.00	94.44	88.89	100.0	100.0	100.0	94.44
E. timbauva	66.67	0.00	61.11	61.11	100.0	88.89	100.0	94.44
Overall Average	42.59	0.00	66.05	58.02	83.33	71.60	89.50	79.63

Table 1. Survival percentage of species used in the experiment for evaluations conducted at 40 and 57 months after planting

Regardless of treatment, in the first evaluation *Albizia lebbeck* and *Acacia farnesiana* exhibited the highest survival rate, at 93 and 94% respectively. The lowest survival rates were observed in *Flemingia sp.* and *Clitoria fairchildiana*, at 15 and 50%, respectively. In the second evaluation, the lowest survivals were for the same species, but the *Mimosa bimucronata* and *Acacia farnesiana* species stood out with the highest survival rates (Table 2).

Table 2. Survival percentage of the	species in the two	conducted evalu	ations related to	all of the
examined treatments				

Species	1 st evaluation (40 months)	2^{nd} evaluation (57 months)
	%	%
Acacia farnesiana	94.44	70.83
A. holosericea	76.39	66.67
A. polyphylla	56.94	52.78
Albizia lebbeck	93.06	68.06
Clitoria fairchildiana	50.00	20.83
Flemingia sp.	15.28	13.89
Mimosa artemisiana	75.00	47.22
M. bimucronata	90.28	70.83
Enterolobium timbauva	81.94	61.11
Overall Average	70.37	52.47

To analyze for overall treatment effects on the growth of the species, statistical analysis of contrasts for the two assessments was used, with no orthogonality between them. For both assessments, the average values of the collar diameter (DC) and plant height (HP) of all species were taken. In the second evaluation, since some treatments showed 100% mortality, it was possible to establish only two contrasts.

First Evaluation (40 months after planting)

In Table 3 the coefficients of contrasts compared and the average values of contrasts to the collar diameter (DC) and plant height are shown. The variation coefficients for DC and plant height were 8.46 and 11.04, respectively. According to the C₁ contrast, there was greater growth of plants, regardless of species, in Treatment 2 compared with Treatment 1. The DC was, on average, 7.10 mm larger (p <0.05) and the median plant height was 34.61 cm higher (p <0.1). There was, therefore, a positive overall effect of soil + liming, providing better conditions for the growth of plants of different species.

The positive effect of the soil can also be observed in the sealing layer, where its presence produced greater growth of plants, regardless of the cover layer combination. When compacted soil + liming were used together with the B1 substrate in the sealing layer (contrast C_2), the DC was on average 3.72 mm larger (p <0.1) and the plant height was 41.62 cm higher (p <0.01). According to the C_3 contrast, even when the cover layer was composed of only B1 + lime, the presence of compacted soil + lime in the sealing layer provided better conditions for plant growth. Here the DC was, on average, 5.40 mm larger (p <0.1) and the height was 46.87 cm higher (p <0.05).

Source	C ₁	C_2	C ₃	C_4	C ₅	C_6
т1	_1	_1	_1	0	0	_1
T2	-1	-1 -1	0	0	-1	-1
T3	0	1	1	-1	0	-1
T4	0	1	0	1	1	1
DC (mm)	7.10*	3.72°	5.40°	3.73	2.03	5.41*
Height (cm)	34.61°	41.62**	46.87*	24.11	36.37°	29.36*

Table 3. Average contrasts for collar diameter (DC) and plant height.

°, *, **: Significant at 10, 5 and 1% respectively, by the F test.

When compacted soil + liming were used together with the B1 substrate in the sealing layer, and compared to its presence/absence in the cover layer (C_4 contrast), there was no significant difference between treatments for DC and plant height. This shows that the presence of soil compacted + liming in the sealing layer seems to have more of an effect on plant growth than their presence in the cover layer. When soil + liming was present in the cover layer and the presence of compacted soil + liming in the sealing layer was varied, there was no significant difference between treatments for DC, but the average plant height was 36.37 cm (p <0.1) higher when the soil was present in the sealing layer (C_5 contrast).

Regardless of the combination used in the sealing layer, the presence of soil + liming in the cover layer, compared with the use of the B1 substrate + liming only (C_6 contrast), provided for greater growth of plants. The relatively minor differences observed confirm the greater importance of soil in the sealing than the layer cover layer. The related data on the growth of the species studied in each treatment, for the 2003 evaluation, are shown in Table 4. Figure 2 shows the average values of all species analyzed for the four treatments.

Species	Treatr	ment 1	Treatr	ment 2	Treatr	ment 3	Treatr	ment 4
	DC	HP	DC	HP	DC	HP	DC	HP
	mm	cm	mm	cm	mm	cm	mm	cm
A. farnesiana	18.59	129.1	29.93	182.2	28.20	174.6	36.76	222.5
A. holosericea	56.38	234.2	68.48	299.7	75.92	298.3	87.21	346.5
A. polyphylla	-	-	14.52	113.9	13.93	128.8	9.44	87.56
A. lebbeck	26.44	98.6	28.89	127.8	48.20	206.8	43.24	192.0
C. fairchildiana	-	-	39.47	60.2	33.95	92.0	35.34	118.5
Flemingia sp.	-	-	-	-	9.45	90.0	15.62	171.0
M. artemisiana	19.53	91.0	20.93	127.2	21.82	140.9	35.65	209.8
M. bimucronata	48.66	199.2	53.35	239.5	41.05	242.3	53.48	286.3
E. timbauva	13.04	91.6	22.37	119.7	21.84	165.2	21.57	157.6

Table 4. Average collar diameter (DC) and average height of plants (HP) for the nine species at 40 months after planting.



Figure 2. Average collar diameter - DC (a) and average plant height (b) for the 4 treatments at 40 months after planting.

Second Evaluation (at 57 months after planting)

In this evaluation, since no plants in treatment 1 survived, only the effect of the compacted soil with liming in the sealing layer could be observed by the contrasts. Considering the other treatments, the presence of soil in the sealing layer continued to produce a significant biomass increase; the average DC was 11.25 mm larger (p < 0.05) and the plant height was 30.98 cm higher (p < 0.05) in the presence of the compacted B1 substrate.

The same effect was observed in the cover layer, where there was significant biomass increase in the soil plus limed B1 substrate in the cover layer mixture when compared with the B1 substrate only. In this case, the sealing layer was composed of more B1 + compacted limed soil. In Table 5, the coefficients of contrasts set and the average values of contrasts for the collar diameter (DC) and plant height are shown. The variation coefficients for DC and plant height were 5.78 and 6.47, respectively.

Source	C_1	C_2
ΤĴ	0	1
12	0	-1
Т3	-1	0
Τ4	1	1
DC (mm)	6.08*	11.25**
Height (cm)	25.14°	30.98^*

Table 5. Average contrasts for collar diameter (DC) and plant height.

°, *, **: Significant at 10, 5 e 1 %, respectively, by the F test.

The data related to the growth of the species for each treatment for the 2003 evaluation are presented in Table 6. The average values of all species analyzed for the four treatments are shown in Fig. 3. The presence of limed soil in the cover layer and compacted limed soil in the sealing layer (T4) produced the best results for overall plant growth, regardless of the species used. However, a more dramatic effect was observed when limed soil was present in the sealing layer, suggesting a potentially important new practice for revegetation in these environments.

Specie	Treatn	nent 1	Treatr	ment 2	Treatr	ment 3	Treatr	ment 4
	DC	HP	DC	HP	DC	HP	DC	HP
	mm	cm	mm	cm	mm	cm	mm	cm
A. farnesiana	-	-	29.25	188.4	30.05	173.6	42.19	237.1
A. holosericea	-	-	75.85	383.5	87.45	360.2	107.93	390.6
A. polyphylla	-	-	13.22	137.8	11.68	128.6	9.91	96.9
A. lebbeck	-	-	28.97	129.0	49.23	199.7	43.21	202.9
C. fairchildiana	-	-	-	-	29.20	95.5	47.02	189.2
<i>Flemingia</i> sp.	-	-	-	-	-	-	23.57	177.0
M. artemisiana	-	-	21.50	117.8	27.60	218.0	42.24	278.9
M. bimucronata	-	-	43.64	274.1	39.54	256.6	60.00	301.9
E. timbauva	-	-	18.37	126.4	21.66	167.7	20.45	169.7

Table 6. Average collar diameter (DC) and average height of plants (HP) for the nine species studied in the field experiment.



Figure 3. Average collar diameter - DC (a) and height of plant (b) for each treatment at 57 months after planting.

Evaluation of Species

For overall evaluation of the species tested, they were grouped by the Scott - Knott test of 5% probability and results of both evaluations are presented in Tables 7 and 8. Those species most able to produce large quantities of biomass are the most interesting for the revegetation program at this mine. From this perspective, *Acacia holosericea* stands out with the highest values for DC and plant height, forming a separate grouping by the Scott - Knott test.

Specie	DC	Height
	mm	cm
Acacia holosericea	69.10a	290.67a
Mimosa bimucronata	51.47b	247.92b
Albizia lebbeck	35.82c	156.04c
Clitoria fairchildiana	32.90c	60.37d
Acacia farnesiana	28.04d	173.14c
Mimosa artemisiana	23.27d	139.35c
Enterolobium timbauva	18.97e	133.53c
Acacia polyphylla	8.87f	85.71d
Flemingia sp.	6.15f	104.97d
CV (%)	10.35	14.24

Table 7. Average collar diameter (DC) and average plant height for the nine species at 40 months after planting, grouped by the Scott - Knott test at $p \le 0.05$.

Averages followed by the same letter do not differ from each other, by the Scott-Knott test group at 5% probability.

In the first evaluation, large differences in plant growth were observed between species, which were divided into six separate groups. In the second evaluation, the species were divided into four groups for DC and three groups for plant height. *Acacia holosericea* stood out again, with relatively high biomass production. In both evaluations, lower biomass production was observed in the *Enterolobium timbauva, Flemingia sp.* and *Acacia polyphylla* species. Regardless, these species could be used to improve the biological diversity of the revegetation program.

Espécie	DC ^{1/}	Altura ^{1/}
	mm	cm
Acacia holosericea	88.43a	377.26a
Mimosa bimucronata	49.88b	285.44b
Albizia lebbeck	40.04c	174.41c
Acacia farnesiana	33.91c	194.58c
Clitoria fairchildiana	33.32c	120.88c
Mimosa artemisiana	29.38c	189.16c
Enterolobium timbauva	19.34d	159.69c
Flemingia sp.	15.86d	153.84c
Acacia polyphylla	11.48d	123.98c
CV (%)	10.11	9.72

Table 8. Collar diameter (DC) and height of plants for the different species to the nine species at57 months after planting, grouped by the Scott - Knott test

Averages followed by the same letter do not differ from each other, by the Scott-Knott test group at 5% probability.

Acacia holosericea responded strongly to treatment differences, with average DC values n the first evaluation of 56.38, 68.48, 75.90 and 87.21 mm, and average heights of 234.17, 299.67, 298.33 and 346.50 cm for treatments 1, 2, 3 and 4, respectively (Table 8). The same behavior was observed in the second evaluation. The survival rate for this species did not vary significantly by treatment (Table 2).

Generally, the *Clitoria fairchildiana, Acacia polyphylla* and *Enterolobium timbauva* species did not respond to the treatments, for either growth or survival. The other species did show differential responses to the treatments and appear to be sensitive to changes in the substrates. Specifically, *Flemingia* sp. proved to be very sensitive. Also, the largest growth of *Acacia farnesiana* and *Mimosa artemisiana* was observed with T4 and growth was significantly lower on other treatments.

The use of layers of sulfidic materials for vegetation establishment was also studied by Dias et al. (1999, 2000). According to those studies, the absence of these layers made the process of revegetation of these areas very difficult. However, in their preliminary studies, the planting of crops directly into the sulfidic substrate lead directly the death of all individuals.

Conclusions

The use of a cover layer over a compacted sealing layer over sulfidic spoil materials allowed for successful growth of plants. The addition of native soil in at least one of these layers provided the greatest survival rates and the highest growth for all tested species. The use of soil as a sealing layer showed the best results compared to other sealing materials, both for mitigation of acid drainage (not reported here) and for revegetation of areas containing metal sulfides. The presence of soil provided higher growth rates and survival rates of the studied species.

Among the species studied, *Acacia holosericea* had the highest biomass production. In contrast, the lowest production was observed in the *Enterolobium timbauva*, *Flemingia sp.* and *Acacia polyphylla* species. Although the *Acacia holosericea* had higher biomass production, it was more sensitive to environment changes (varying cover and sealing layers).

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