

April 17, 2018

VIA E-MAIL AND FEDERAL EXPRESS

Mr. Darrell Nitschke
Executive Secretary
North Dakota Public Service Commission
600 E. Boulevard, Dept. 408
Bismarck, ND 58505-0480

**RE: Meadowlark Wind I LLC's Application for a Certificate of Site Compatibility
for the New Frontier Wind Energy Project in McHenry County, North Dakota
Case No. PU-11-69**

Dear Mr. Nitschke:

On March 29, 2018, the Commission held an informal hearing in the above-referenced matter to discuss Meadowlark Wind I LLC's ("Meadowlark") planned access road construction methodology in relation to the topsoil segregation requirement in Certification Provision No. 15, attached to the Commission's May 10, 2017 Order of Continuing Suitability ("Order"). As requested during the informal hearing, and in further support of Meadowlark's request to amend the Order, the following information is provided as Late-Filed Exhibit No. 5:

1. Information regarding access road acreage and overall acreage per turbine for wind projects constructed by Blattner Energy in North Dakota (see enclosed Part 1);
2. Information regarding the make-up of 6 percent cement subgrade stabilization used in proposed access road construction methodology (see enclosed Part 2); and
3. Information regarding the effect of cement subgrade stabilization on pH/productivity of soils (see enclosed Part 3).

Additional information regarding topsoil stockpiling is also included in the enclosed Part 3.

An electronic copy of this letter and the enclosed Late-Filed Exhibit No. 5 were filed with the Commission today via e-mail, and an original and 10 copies of each are enclosed.

Attorneys & Advisors / Fredrikson & Byron, P.A.
main 612.492.7000 / 200 South Sixth Street, Suite 4000
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113 PU-11-69 Filed 04/18/2018 Pages: 63

Late-Filed Exhibit 5 in support of request to amend Order
Meadowlark Wind I LLC
Mollie Smith, Fredrikson & Byron

Mr. Darrell Nitschke
April 17, 2018
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If you have any questions, please let me know.

Sincerely,

A handwritten signature in cursive script that reads "Mollie M. Smith". The signature is written in black ink and is positioned to the right of the typed name.

MOLLIE M. SMITH

MMS/ms/63821929

Enclosures

cc: Jerry Lein (via e-mail)
Jack Schuh (via e-mail)
Todd Hartleben (via e-mail)
Chad Tucker (via e-mail)
Bill Behling (via e-mail)
Dan Moller (via e-mail)

Meadowlark Wind I LLC
New Frontier Wind Project
Docket No. PU-11-69

Late-Filed Exhibit No. 5

Part 1: Information regarding access road acreage and overall acreage per turbine for wind projects constructed by Blattner Energy in North Dakota.

Year	Avg. Mile New Road/WTG	Avg. Mile Upgraded County Road/WTG	Avg. Acre Disturbance/WTG
2008	0.48	0.04	0.94
2009	0.44	0.09	0.86
2010	0.17	0.08	0.84
2011	0.44	0.21	0.85
2017	0.39	0.27	0.71

Part 2: Information regarding the make-up of 6 percent cement subgrade stabilization used in proposed access road construction methodology.

See attached document regarding cement stabilization. The cement used is Portland cement, and the chief chemical constituents are as follows:

Lime (CaO)	60 to 67%
Silica (SiO ₂)	17 to 25%
Alumina (Al ₂ O ₃)	3 to 8%
Iron oxide (Fe ₂ O ₃)	0.5 to 6%
Magnesia (MgO)	0.1 to 4%
Sulphur trioxide (SO ₃)	1 to 3%
Soda and/or	0.5 to 1.3%

Potash (Na ₂ O+K ₂ O)	
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Source: <https://theconstructor.org/building/composition-of-portland-cement/5725/>

Part 3: Information regarding the effect of cement subgrade stabilization on pH/productivity of soils.

See attached Blattner Energy Memorandum, dated April 6, 2018, with copies of referenced documents.

TESTING REQUIREMENTS

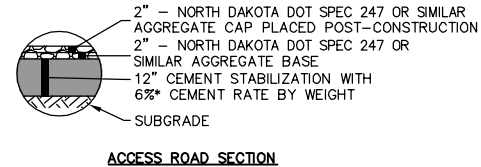
1. ALL TESTING (DCP, PROOF ROLL, SIEVE, DENSITY, ETC.) IS INCIDENTAL TO THE CONTRACT.
2. TESTING SHALL BE PERFORMED BY A DESIGNATED, INDEPENDENT TESTING AGENCY.
3. SUBMIT TESTING AND INSPECTION RECORDS SPECIFIED TO THE ENGINEER FOR REVIEW. THE ENGINEER'S REVIEW DOES NOT RELIEVE THE CONTRACTOR FROM THE RESPONSIBILITY OF CORRECTING DEFECTIVE WORK.
4. PROOF ROLLING SHOULD BE PERFORMED IN THE PRESENCE OF THE GEOTECHNICAL ENGINEER OR QUALIFIED GEOTECHNICAL REPRESENTATIVE USING A FULLY LOADED TANDEM AXLE DUMP TRUCK WITH A MINIMUM GROSS WEIGHT OF 25 TONS, OR A FULLY LOADED WATER TRUCK, OR A FULLY LOADED BELLY DUMP TRUCK WITH AN EQUIVALENT AXLE LOADING. PROOF ROLLING ACCEPTANCE STANDARDS INCLUDE NO RUTTING GREATER THAN 1.5 INCHES AND NO "PUMPING" OF THE SOIL BENEATH/BEHIND THE LOADED TRUCK.
5. SIEVE ANALYSIS:
 - a. SHALL BE CONDUCTED IN ACCORDANCE WITH AASHTO T27 OR
 - b. SHALL BE CONDUCTED IN ACCORDANCE WITH ASTM C136
6. PROCTOR:
 - a. SHALL BE DETERMINED IN ACCORDANCE WITH AASHTO T99 (STANDARD PROCTOR)
 - b. SHALL BE DETERMINED IN ACCORDANCE WITH ASTM D698 (STANDARD PROCTOR)
7. ATTERBERG LIMITS:
 - a. SHALL BE DETERMINED IN ACCORDANCE WITH AASHTO T89 & T90
 - b. SHALL BE DETERMINED IN ACCORDANCE WITH ASTM D4318
8. MOISTURE DENSITY (NUCLEAR DENSITY):
 - a. SHALL BE DETERMINED IN ACCORDANCE WITH AASHTO T310
 - b. SHALL BE DETERMINED IN ACCORDANCE WITH ASTM D6938
9. DYNAMIC CONE PENETROMETER (DCP) TESTING:
 - a. SHALL BE CONDUCTED IN ACCORDANCE WITH ASTM D6951

CEMENT STABILIZATION - PROCEDURE

THE SPECIFICATIONS BELOW ARE GENERAL RECOMMENDATIONS/GUIDELINES FOR CEMENT STABILIZATION OF SUBGRADES FOR THE PROJECT. ACTUAL FIELD CONDITIONS MAY ALTER APPLICATION RATE. CONSULT GEOTECHNICAL ENGINEER FOR FURTHER RECOMMENDATIONS.

1. STRIP THE TOP ±4 INCHES OF TOPSOIL FROM THE AREA TO BE STABILIZED.
 2. PERFORM A PROCTOR (ASTM D 698) TEST TO DETERMINE THE OPTIMUM MOISTURE CONTENT IF THE MATERIAL TO BE STABILIZED DOES NOT MATCH A PREVIOUSLY PERFORMED PROCTOR.
 3. DETERMINE THE IN-SITU MOISTURE CONTENT USING A NUCLEAR DENSITY GAUGE (OR EQUIVALENT MOISTURE CONTENT TEST) IMMEDIATELY IN FRONT OF THE RECLAIMER, PRIOR TO INCORPORATION OF THE CEMENT.
 - 3.1. THE CONTRACTOR WILL BE ADVISED OF IN-SITU MOISTURE CONTENT, AND MOISTURE ADJUSTMENTS SHOULD BE MADE IF IN-SITU MOISTURE IS SIGNIFICANTLY LESS THAN OPTIMUM MOISTURE CONTENT AS DETERMINED BY THE PROCTOR (ASTM D 698).
 4. DURING INITIAL CONSTRUCTION, TEST AREAS USING 6% CEMENT CONTENT BY WEIGHT.
 - 4.1. ADDITIONAL CEMENT CONTENT BY WEIGHT MAY BE REQUIRED TO PROPERLY STABILIZE THE SUBGRADE. FIELD RESULTS WILL DICTATE THE CEMENT APPLICATION RATE.
 - 4.2. DO NOT CONTINUE TO STABILIZE THE SUBGRADE IF THE PERFORMANCE REQUIREMENTS (DCP CRITERIA OUTLINED BELOW) ARE NOT MET. CONSULT THE ENGINEER FOR RECOMMENDATIONS.
 - 4.3. ADDITIONAL CEMENT MAY BE REQUIRED WHEN WETTER SOIL IS ENCOUNTERED.
 5. SOIL AND CEMENT BLENDING:
 - 5.1. COMPLETELY BLEND SUBGRADE SOIL WITH THE APPROPRIATE AMOUNT OF CEMENT BY WEIGHT.
 - 5.2. THE USE OF A RECLAIMER IS AN ADEQUATE BLENDING METHOD. DISCING IS NOT AN ADEQUATE BLENDING METHOD.
 - 5.3. ADHERE TO PROJECT STABILIZATION DEPTH/WIDTH REQUIREMENTS:
 - 5.1.1. 12" DEPTH COMPACTED STABILIZED SUBGRADE
 - 5.1.2. 16.5' WIDTH STABILIZED SUBGRADE
 6. COMPACTION:
 - 6.1. A ROLLING PATTERN WILL BE ESTABLISHED USING A NUCLEAR DENSITY GAUGE DURING COMPACTION.
 - 6.1.1. COMPACTION WILL BE PERFORMED UNTIL THE STABILIZED MATERIAL REACHES THE "BREAK POINT" AFTER WHICH ADDITIONAL PASSES WITH A ROLLER RESULTS IN A LOSS OF DENSITY AS MEASURED BY A NUCLEAR DENSITY GAUGE.
 - 6.1.2. COMPACTION IS TYPICALLY ACHIEVED BY MULTIPLE PASSES OF A PADFOOT ROLLER UNTIL THE ROLLER "WALKS" OUT OF THE RECLAIMED MATERIAL. (SPACE IS VISIBLE BETWEEN THE DRUM AND THE RECLAIMED MATERIAL). THIS IS FOLLOWED BY BLADING AND SHAPING THE RECLAIMED MATERIAL TO FINAL PROFILE AND CROSS SECTION, REMOVING HIGH AND LOW POINTS. SMOOTH DRUM OR RUBBER TIRE ROLLERS ARE THEN USED TO FINISH ROLLING (THIS IS WHEN THE DENSITY TESTS ARE PERFORMED) UNTIL THE DENSITY OF THE MATERIAL REACHES THE "BREAK POINT."
 - 6.1.3. THE NUMBER OF PASSES AND EQUIPMENT USED TO REACH THE "BREAK POINT" IS CONSIDERED THE ROLLING PATTERN.
 - 6.1.4. A NEW ROLLING PATTERN MAY NEED TO BE ESTABLISHED IF THE PERFORMANCE REQUIREMENTS ARE NOT BEING MET. THIS MAY BE DUE TO CHANGES IN THE MOISTURE CONTENT, THE SOIL TYPE, OR THE CEMENT RATE.
 - 6.2. THE TARGET DENSITY IS 98% OF THE MAXIMUM DENSITY ("BREAK POINT") DETERMINED FROM THE ROLLING PATTERN.
 - 6.2.1. DENSITY TESTS SHALL BE TAKEN AT THE RATE OF 1 TEST PER 500 LF IN EACH PASS OF THE RECLAIMER TO CONFIRM THE DENSITY IS MATCHING THE TARGET. ONCE THE ROLLING PATTERN HAS BEEN CONFIRMED IT IS RECOMMENDED THAT TESTING CONTINUE AT 1 TEST PER 1,000 LF IN EACH PASS OF THE RECLAIMER.
 - 6.2.2. IF IT IS NOT POSSIBLE TO ATTAIN 98% OF THE TARGET DENSITY, AND ADDITIONAL APPLICATION OF COMPACTION DOES NOT IMPROVE THE DENSITY, A NEW ROLLING PATTERN SHOULD BE ESTABLISHED.
 - 6.3. THE AIR TEMPERATURE SHALL BE ABOVE 40 DEGREES (F) DURING THE CEMENT STABILIZATION PROCESS. MIXING CEMENT INTO FROZEN GROUND IS NOT ACCEPTABLE.
 7. THE STABILIZED MATERIAL SHALL BE CONTINUOUSLY WET CURED FOR A MINIMUM OF 24 HOURS (WET CURED IS IDENTIFIED VISUALLY AS SURFACE DAMP).
 8. SUBGRADE DCP TESTING AND ACCEPTANCE:
 - 8.1. PERFORM SUBGRADE STRENGTH TESTING BY DYNAMIC CONE PENETROMETER (DCP) PER ASTM D 6951/6951M NO SOONER THAN 24 HOURS AFTER FINAL COMPACTION.
 - 8.2. DCP TESTING FREQUENCY SHALL BE WITH A RANDOM SPACING AND A MINIMUM OF 1 TEST PER 1,000 LF IN EACH PASS OF THE RECLAIMER. A MINIMUM OF 3 TESTS PER ROAD IS REQUIRED.
 - 8.3. THE CBR OF THE STABILIZED SUBGRADE SHOULD BE CALCULATED IN 3" INCREMENTS THROUGH THE ENTIRE DEPTH OF THE STABILIZED LAYER.
 - 8.4. THE MINIMUM REQUIRED CBR PRIOR TO PROOF-ROLLING IS PROVIDED IN THE TABLE BELOW:

TIME ELAPSED BETWEEN FINAL COMPACTION AND DCP TEST	REQUIRED MINIMUM CBR
24 - 48 HOURS	20
3 - 7 DAYS	30
28 DAYS	50
 - 8.5. ONCE THE MINIMUM REQUIRED CBR IS ACHIEVED AT ANY CORRESPONDING TIME PERIOD, THE SUBGRADE IS CONSIDERED ADEQUATE AND THE CONTRACTOR MAY PROCEED WITH PROOF-ROLL TESTING. FUTURE DCP TESTING ON THAT SEGMENT OF SUBGRADE IS NOT REQUIRED.
 - 8.6. IF TESTING DOES NOT INDICATE A CBR OF 30 WITHIN 7 DAYS, ADDITIONAL GRAVEL SURFACING OR RE-STABILIZATION MAY BE REQUIRED AND A NEW ROLLING PATTERN OR ADDITIONAL CEMENT MAY BE REQUIRED FOR FUTURE SUBGRADE STABILIZATION AREAS. CONTACT THE ENGINEER FOR RECOMMENDATIONS.
9. SUBGRADE PROOF-ROLL TESTING AND ACCEPTANCE:
 - 9.1. ONCE THE REQUIRED DCP VALUE HAS BEEN OBTAINED BUT PRIOR TO PLACING AGGREGATE, THE SUBGRADE SHALL BE PROOF-ROLLED. REFER TO THE PROJECT TESTING REQUIREMENTS FOR PROOF-ROLL DEFINITION.
 - 9.2. PROOF-ROLLING SHOULD NOT BE PERFORMED WITHIN 12 INCHES OF THE EDGE OF THE STABILIZED SECTION.
 - 9.3. IF PROOF-ROLLING FAILS, RE-STABILIZATION MAY BE REQUIRED AND A NEW ROLLING PATTERN OR ADDITIONAL CEMENT MAY BE REQUIRED FOR FUTURE SUBGRADE STABILIZATION AREAS. CONTACT THE ENGINEER FOR RECOMMENDATIONS.
10. AGGREGATE PLACEMENT:
 - 10.1. SURFACE AGGREGATE SHALL BE PLACED OVER THE STABILIZED SUBGRADE FOLLOWING PASSING PROOF-ROLL TESTS.
 - 10.2. THE AGGREGATE SURFACING IS INTENDED TO PROTECT THE SUBGRADE FROM WATER BEING PUMPED INTO THE SUBGRADE AND TO PROVIDE A TRACTION COURSE. WESTWOOD RECOMMENDS A MINIMUM OF 2 INCHES OF AGGREGATE INITIALLY PLACED OVER STABILIZED SUBGRADE. MAINTENANCE IS REQUIRED THROUGHOUT CONSTRUCTION AND MAY REQUIRE THE PLACEMENT OF ADDITIONAL AGGREGATE.
 - 10.3. REFER TO THE TESTING SCHEDULE FOR AGGREGATE PLACEMENT CRITERIA.



TESTING SCHEDULE SUMMARY

LOCATION	TEST	FREQUENCY
EMBANKMENT FILL	GRAIN SIZE ANALYSIS, MOISTURE CONTENT, ATTERBERG LIMITS ON FINES CONTENT, & PROCTOR	1 PER MAJOR SOIL TYPE
	MOISTURE DENSITY TEST (NUCLEAR DENSITY)	1 PER LAYER/1000 LF
COMPACTED SUBGRADE	PROOF-ROLL	ENTIRE LENGTH
	MOISTURE DENSITY TEST (NUCLEAR DENSITY)	1 PER 1000 FT OR MIN. 3 PER ROAD
CEMENT STABILIZED SUBGRADE	PROOF-ROLL	ENTIRE LENGTH
	DCP	1 PER 1000 LF
	MOISTURE DENSITY TEST (NUCLEAR DENSITY)	SEE CEMENT STABILIZATION SPECIFICATION
AGGREGATE BASE	PROOF-ROLL	ENTIRE LENGTH
	SIEVE ANALYSIS	1 PER 2500 CY
CRANE PATHS & SHOULDERS	PROOF-ROLL	ENTIRE LENGTH
CRANE PADS	PROOF-ROLL SUBGRADE	ENTIRE PAD
	DCP TEST (NOT REQUIRED UNLESS PROOF-ROLL FAILS)	2 PER PAD
	MOISTURE DENSITY TEST (NUCLEAR DENSITY)	2 PER PAD

392 County Road 50
Avon, MN 56310
320-356-7351



April 6, 2018

Dan Moller
Senior Project Manager
Capital Power

Per last Thursday's informal meeting with the North Dakota Public Service Commission, in Bismarck, two action items arose related to topsoil impacts from cement/lime dust exposure and stockpiling topsoil vs. the renewable industry standard of adjacent spreading. The two points are summarized below with supporting documentation attached hereto.

1. Topsoil impacts from cement/lime dust exposure.

The most conclusive reports we have found to date specific to cement dust mixed with topsoil determined better growth potential with cement than without. Causes are believed to be the presence of calcium (lime) which is known to promote vegetative growth (url:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1182479/>)

Some fertilizers contain cement kiln dust (CKD), which aides plant nutrition (url:

https://www.farmshow.com/a_article.php?aid=11493)

Higher concentrations of lime are known to preserve organic strata (topsoil) (*Agricultural College Survey of North Dakota, by Daniel Willard, page 63*).

2. Stockpiling Topsoil vs. Renewable Industry Standard

To best preserve the topsoil and minimize emissions and cost, Blattner proposes the renewable industry standard to spread the topsoil adjacent to its origin for ease of replacement. In the alternative the following studies suggest stockpiling topsoil have degrading effects

(Ghose, M.K. 2005. *Soil conservation for rehabilitation and revegetation of mine-degraded land.*

TIDEE – TERI Information Digest on Energy and Environment 4(2), 137-150.)

And

(Sheoran, V.; Sheoran, A. S.; and Poonia, P. (2010) "*Soil Reclamation of Abandoned Mine Land by Revegetation: A Review,*" *International Journal of Soil, Sediment and Water: Vol. 3 : Iss. 2 , Article 13.*)

Please let me know of any questions or concerns related to this matter.

Thank you,

Jason W. Oberg

Jason W. Oberg, PE (ND # 27064)
Civil/Structural Engineer
Blattner Energy, Inc.

HISTORICAL PERSPECTIVE ESSAY

Calcium: A Central Regulator of Plant Growth and Development

Today no one questions the assertion that Ca^{2+} is a crucial regulator of growth and development in plants. The myriad processes in which this ion participates is large and growing and involves nearly all aspects of plant development (recent reviews in Harper et al., 2004; Hetherington and Brownlee, 2004; Hirschi, 2004; Reddy and Reddy, 2004; Bothwell and Ng, 2005). Despite this wealth of research, the concept of Ca^{2+} as an intracellular regulator is relatively recent and within the professional life span of many people who are still active and working on this topic today. The aim of this essay is to identify those lines of thought and research that led to the idea that Ca^{2+} is a second messenger in plant cell growth and development. This essay thus focuses primarily on work starting in the mid sixties and extending to the mid eighties. I do not provide an exhaustive review of the history of Ca^{2+} research, nor do I attempt to treat modern aspects of Ca^{2+} research. However, I do strive to identify the roots of modern Ca^{2+} research and to chart the origin of the current revolution.

EARLY STUDIES ON PLANT CALCIUM

Ca^{2+} is an essential element; however, its role is elusive. When examining total Ca^{2+} in plants, the concentration is quite large (mM), but its requirement is that of a micronutrient (μM). Ca^{2+} is not usually limiting in field conditions, still there are several defects that can be associated with low levels of this ion, including poor root development, leaf necrosis and curling, blossom end rot, bitter pit, fruit cracking, poor fruit storage, and water soaking (Simon, 1978; White and Broadley, 2003). The underlying causes for these effects are not entirely clear; nevertheless, two areas within the cell have been recognized as being important targets. First is the cell wall, where Ca^{2+} plays a key role in cross-linking acidic pectin residues. The second

is the cellular membrane system, where low $[\text{Ca}^{2+}]_e$ increases the permeability of the plasma membrane. These are briefly discussed below.

Ca^{2+} and the Cell Wall

Since the 19th century, it has been appreciated that Ca^{2+} plays a crucial role in determining the structural rigidity of the cell wall (reviewed in Wyn Jones and Lunt, 1967; Burstrom, 1968). During cell wall formation, the acidic pectin residues (e.g., galacturonic acid) are secreted as methyl esters, and only later deesterified by pectin methylsterase, liberating carboxyl groups, which bind Ca^{2+} . It follows that low $[\text{Ca}^{2+}]_e$ should make the cell wall more pliable and easily ruptured, whereas high concentrations should rigidify the wall and make it less plastic. It had become apparent in the mid to late fifties that modifying the $[\text{Ca}^{2+}]_e$ produced a pronounced effect on cell growth. Thus, elevating the $[\text{Ca}^{2+}]_e$ led to an inhibition in shoot or coleoptile growth, whereas reducing its concentration promoted cell and tissue elongation (Bennet-Clark, 1956; Tagawa and Bonner, 1957). Strong support for the Ca^{2+} /pectate interaction came from a quantitative examination of the cation exchange capacity of the coleoptile cell wall, which was shown to be due to the number of free pectic carboxyl groups (Jansen et al., 1960). Still further support came from studies using the cation chelator EDTA, which had been employed to macerate plant tissues without destroying the cell structure (Letham, 1958). The explanation centered around the idea that EDTA, by chelating Ca^{2+} , led to a marked weakening or loss of pectates in the middle lamella, thus removing the agent that cemented cells together.

The importance of the Ca^{2+} /pectate interaction as a regulator of growth encouraged researchers to include a role for auxin in this scheme, particularly because it was becoming evident that Ca^{2+} and auxin

had antagonistic actions. Thus, auxin promoted shoot growth and inhibited root growth, whereas Ca^{2+} inhibited shoot growth and promoted root growth. Working with oat coleoptiles, Bennet-Clark (1956) proposed that there might be a direct antagonism between indoleacetic acid (IAA) and Ca^{2+} . Noting that Ca^{2+} , and the lanthanide praseodymium, inhibited IAA-induced elongation, whereas EDTA reversed the inhibitory activity of Ca^{2+} , and even promoted growth, Bennet-Clark (1956) suggested that IAA acts as a Ca^{2+} / Mg^{2+} chelator. This model proposed that IAA removes Ca^{2+} and leads to a loss of Ca^{2+} pectates, which are replaced by pectate free acids or methyl esters. The latter, because they are not cross-linked, would render the wall plastic and able to elongate (Bennet-Clark, 1956). This idea was challenged by Cleland (1960), who demonstrated that IAA does not enhance the loss of Ca^{2+} from the cell wall, nor does it cause a redistribution of Ca^{2+} between pectin and proto-pectin. Somewhat later, Burling and Jackson (1965) used atomic absorption spectroscopy to show that Ca^{2+} accumulated in the cell walls of elongating coleoptiles and that this accumulation was unaffected by auxin. Further studies by Baker and Ray (1965) and Ray and Baker (1965) established the separation in action between Ca^{2+} and IAA, providing clear evidence that the inhibition of cell elongation by Ca^{2+} does not prevent IAA from stimulating cell wall synthesis. In the presence of Ca^{2+} , and thus the inhibition of cell enlargement, they demonstrated a general promotion of synthesis of matrix polysaccharides in the presence of IAA (Ray and Baker, 1965).

A compelling interaction between Ca^{2+} , the cell wall, and cell growth was also found in pollen tubes. It was shown in 1963 that Ca^{2+} must be present in the medium to support pollen tube growth in vitro (Brewbaker and Kwack, 1963). Using $^{45}\text{Ca}^{2+}$, Kwack (1967) showed that

HISTORICAL PERSPECTIVE ESSAY

incorporation occurred exclusively in the pollen tube wall; some of the autoradiographic images indicated an enhanced accumulation of Ca^{2+} in the apical region. Because the pollen tube cell wall, especially at the tip, is composed almost entirely of pectin, it is reasonable to assume that a Ca^{2+} /pectate interaction dominates the requirement for this ion.

Despite the attractiveness of the idea that cell wall Ca^{2+} achieves its effects through an interaction with pectates, it must be recognized that not all results can be easily accounted for by this explanation (Cleland and Rayle, 1977; Tepfer and Taylor, 1981). The failure to show a close correspondence between the ability of divalent cations to form a pectic gel with their ability to inhibit growth has led to a consideration of other ideas, for example, a direct effect of Ca^{2+} on cell wall modifying enzymes (Cleland and Rayle, 1977). It is important to keep in mind that within the complex framework of carbohydrates and proteins of the cell wall, there could be interactions between Ca^{2+} and molecules other than pectins that could contribute to cell wall structure and extensibility. Nevertheless, a Ca^{2+} /pectate interaction cannot be ignored and deserves attention today as a factor involved in the control of cell growth.

Ca^{2+} and Membrane Permeability

It has also been known for many years that Ca^{2+} plays an important role in controlling membrane structure and function (Wyn Jones and Lunt, 1967; Burstrom, 1968). A general idea is that Ca^{2+} , by binding to phospholipids, stabilizes lipid bilayers and thus provides structural integrity to cellular membranes. From a physiological point of view, a frequent observation has been that Ca^{2+} controls membrane permeability (Epstein, 1972; Hanson, 1984). Thus, when cells are cultured in solutions of low $[\text{Ca}^{2+}]_e$, especially in the presence of EDTA, there is leakage of ions and metabolites (Hanson, 1984). Using roots of soybean and maize, Hanson (1960) showed that a low $[\text{Ca}^{2+}]_e$ caused a marked decline in the ability of these tissues to absorb and retain solutes. A $[\text{Ca}^{2+}]_e$ between

0.1 to 1.0 mM was found to be necessary to maintain the integrity and selective ion transport of the plasma membrane. Epstein (1961) examined the competition between different monovalent cations and reported that Ca^{2+} (0.1 to 1.0 mM), but not Mg^{2+} , promoted the uptake of potassium in the presence of sodium. Thus, Ca^{2+} by some mechanism, imparts selectivity to the ion transport process. In another example, Van Steveninck (1965) found that low $[\text{Ca}^{2+}]_e$ promoted a release of potassium in cultured beet root tissues, which was completely reversed by adding back Ca^{2+} , but not Mg^{2+} . Pollen tubes also showed changes in permeability in response to low $[\text{Ca}^{2+}]_e$, including a significant release of carbohydrates into the medium (Dickinson, 1967).

In a series of studies on leaf abscission and tissue senescence, Poovaiah and Leopold (1973a, 1973b, 1976) reported that Ca^{2+} inhibited or slowed these processes. Recognizing that Ca^{2+} , through cross-linking pectates and cementing cell walls, will directly retard abscission, they noted that several other processes were also affected. During senescence in maize and rumex leaf disks, they showed that Ca^{2+} retarded the loss of chlorophyll, the loss of protein, and the loss of free space, suggesting that the ion plays a regulatory role in maintaining and controlling membrane structure and function (Poovaiah and Leopold, 1973b).

Early ultrastructural studies echoed this refrain. Thus, marked differences were detected at the electron microscope level in the membranes of barley shoot apices cultured in low $[\text{Ca}^{2+}]_e$ relative to the controls (Marinos, 1962). The low Ca^{2+} -induced effect was apparent as relatively gross discontinuities in the nuclear envelope, plasma membrane, and tonoplast, and later in the mitochondria. It is difficult to imagine that such lesions occur in the intact cell because they would immediately lead to cell death. However, they may indicate reduced membrane stability, which leads to breakage and discontinuities during the permanganate fixation process. For that reason, the details of this report must be treated with caution; nevertheless, the differences observed suggest

that membranes cultured in low Ca^{2+} become structurally weakened.

If low Ca^{2+} makes the membrane more permeable, it should follow that elevated concentrations make the membrane less permeable. Using Ca^{2+} itself as the probe, Robinson (1977) showed this to be true in zygotes of the alga *Pelvetia*. Thus, an increase in the $[\text{Ca}^{2+}]_e$ from only 1 to 3 mM reduced the influx of this ion by >10-fold. These results seem counterintuitive and are not well appreciated. Examples certainly exist in which it is evident that an increase in the $[\text{Ca}^{2+}]_e$ causes a corresponding increase in the $[\text{Ca}^{2+}]_i$ (Gilroy et al., 1986), and an extracellular Ca^{2+} sensor recently has been identified in guard cells (Han et al., 2003). However, this situation does not automatically extend to all cell types, as the study by Robinson (1977) shows. In agreement with the studies on *Pelvetia*, we find that increasing the $[\text{Ca}^{2+}]_e$ to 10 mM inhibits lily pollen tube elongation and causes the tip-focused gradient to drop to basal levels (D.A. Callaham and P.K. Hepler, unpublished data). Thus, in experiments in which the $[\text{Ca}^{2+}]_e$ is modulated, the assumption cannot be made that similar changes occur on the cytosol. Rather, an increase in $[\text{Ca}^{2+}]_e$ may generate a decrease in $[\text{Ca}^{2+}]_i$.

Briefly summarizing, early studies on the role of Ca^{2+} in plants focused on the cell wall and on membrane permeability. At that time, there was no widespread appreciation that the $[\text{Ca}^{2+}]_e$ might be very low and that this ion might be acting as a regulator of cytoplasmic processes. Botanists exploring Ca^{2+} effects in the concentration range between 0.1 and 100 mM were unlikely to see changes at the submicromolar level. The concept of Ca^{2+} as a regulator initially derives from studies of animal cells and only later in studies of plant cells. To see how this concept arose, I will focus briefly on Ca^{2+} in animal cell physiology, giving attention to the process of muscle contraction.

CALCIUM AND MUSCLE CONTRACTION

More than 120 years ago, Ringer (1883) showed that the repetitive beating of an

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isolated frog heart was sensitive to different $[Ca^{2+}]_i$ (for review, see Carafoli et al., 2001). When cultured in distilled water, the hearts failed to exhibit the proper contraction; however, when cultured in London city tap water, they exhibited repetitive contractions. Using sequential ion addition to the distilled water, Ringer (1883) discovered that Ca^{2+} was the key factor that supported contraction. Despite these early studies, the idea that Ca^{2+} was a regulator of muscle contraction did not expand at this point. Only considerably later through the efforts of Heilbrunn (1940) was the emphasis again focused on Ca^{2+} . Heilbrunn (1940) showed that muscle contraction could be stimulated through the injection of Ca^{2+} into the frog muscle fiber. Of note, the contraction could take place even when the Ca^{2+} solution was highly diluted. Equally important was the observation that muscle contraction was not supported by injection of other important physiological ions, including sodium, potassium, or Mg^{2+} . Because potassium at that time was considered crucial, the additional observation that massive doses of potassium were ineffective further emphasized the primary role of Ca^{2+} in stimulating contraction (Heilbrunn and Wiercinski, 1947).

As insightful and penetrating as these studies were, Heilbrunn and Wiercinski (1947) were not able to establish the actual $[Ca^{2+}]_i$ in the resting muscle fiber. Indeed, determining the $[Ca^{2+}]_i$ has been difficult for any cell type. Hodgkin and Keynes (1957), using $^{45}Ca^{2+}$ to examine the mobility of this ion in squid axoplasm, made two important observations: first, that the mobility of Ca^{2+} is extremely low; second, that the bulk of the Ca^{2+} is bound, with only 10 μM or less being free and ionized. Further work that established the true $[Ca^{2+}]_i$ depended on two technical developments. The first was the application of cation chelators EDTA and EGTA in physiological studies to carefully control the $[Ca^{2+}]_i$ (Bozler, 1954). Before the availability of effective chelators, it was nearly impossible to construct solutions in the submicromolar range because of the presence of Ca^{2+} as a contaminant, or leaching from glassware. Whereas EDTA has a high affinity for Ca^{2+} , it also has a substantial

affinity for Mg^{2+} . With EGTA, the affinity for Ca^{2+} is not as high as with EDTA, but the relative insensitivity of EGTA to Mg^{2+} means that it is a more efficacious chelator for constructing solutions that are specifically buffered for Ca^{2+} . The second important development was the isolation and characterization of the photoprotein aequorin, a Ca^{2+} sensitive, bioluminescent protein from the jelly fish Aequorea, which provided a means for detecting changes in the $[Ca^{2+}]_i$ in the submicromolar range (Shimomura et al., 1963). At resting $[Ca^{2+}]_i$, the protein generates only a faint glow; Shimomura et al. (1963) initially determined that the resting concentration was between 0.1 and 1.0 μM . However, as the $[Ca^{2+}]_i$ increases, there is an exponential (2.3 power) increase in the amount of light generated, making aequorin a suitable reagent for detecting regions of elevated ion concentration or amplitude modulation.

Despite the favorable properties of aequorin as an indicator of the $[Ca^{2+}]_i$ in living cells, there were substantial problems in its use. First was the need to introduce the protein into cells, and second was the difficulty of detecting and imaging a rather weak signal. The first problem was solved using large cells, which are easy to inject. Of course, more recently, using modern molecular biological methods, it is possible to transfect cells with the aequorin gene and express the protein in virtually any cell (Knight et al., 1991), and even within organelles (Rizzuto et al., 1994). The problems associated with the detection and imaging of the aequorin signal remain with us today. Although detection of a signal without imaging can be done effectively with photomultiplier tubes, imaging, especially from single small cells is difficult due to a low number of Ca^{2+} -dependent photons. Progress has been made in the development of extremely sensitive photon imaging equipment, which has permitted the visualization of these weak signals (Gilkey et al., 1978; Knight et al., 1993).

The determination of the $[Ca^{2+}]_i$ in living muscle cells was performed by studies that involve both of these technologies. In 1964, Portzehl and coworkers used EGTA to produce carefully buffered Ca^{2+} solutions and showed that contraction in an isolated

muscle fiber of the crab *Maia squinado* occurred between 0.3 and 1.5 μM . A few years later in 1967, Ridgway and Ashley injected the giant muscle of the acorn barnacle with aequorin. Within 1 ms after electrical stimulation, they recorded a sharp increase in light, indicating that the $[Ca^{2+}]_i$ had risen (Figure 1). This was followed in 5 ms by an increase in muscle tension. Although the results were not strictly quantitative, Ridgway and Ashley (1967) argued, based on the work of Shimomura et al. (1963), that at rest the $[Ca^{2+}]_i$ would be between 0.1 and 1.0 μM ; therefore, upon stimulation it would be substantially higher. These studies are dramatic and compelling; they clearly demonstrate that the stimulated depolarization of the membrane potential is followed almost immediately by an abrupt increase in bioluminescence (i.e., $[Ca^{2+}]_i$) and with only a further slight lag by the generation of tension (Ridgway and Ashley, 1967). These studies were the first direct demonstration of Ca^{2+} amplitude modulation.

Ca^{2+} AMPLITUDE MODULATION IN NONMUSCLE CELLS

During the next decade, in studies of several different nonmuscle systems, both EGTA and aequorin were used to show that the basal $[Ca^{2+}]_i$ was submicromolar and that through stimulation elevations of the $[Ca^{2+}]_i$ could be elicited. For example, activation of the freshwater protozoans, Spirostomum (Ettienne, 1970), cell cleavage in *Xenopus* (Baker and Warner, 1972), response of the photoreceptor of *Limulus* to light (Brown and Blinks, 1974), oscillations in cytoplasmic streaming in the plasmodial slime mold, *Physarum* (Ridgway and Durham, 1976), and egg activation in the medaka fish, *Oryzias latipes* (Ridgway et al., 1977), and sea urchin, *Lytechinus pictus* (Steinhardt et al., 1977) were shown to be anticipated by an increase in the $[Ca^{2+}]_i$. The examples of egg activation are especially efficacious in establishing a primary role for Ca^{2+} amplitude modulation in development. Whereas Ridgway et al. (1977) employed eggs from medaka, a fresh water fish, Steinhardt and coworkers (1977) used eggs from a marine sea urchin.

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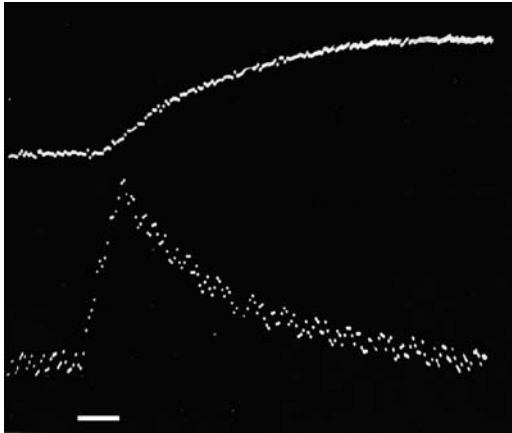


Figure 1. A $[Ca^{2+}]_i$ Increase Precedes Muscle Contraction.

After an electrical stimulus, the giant muscle of the acorn barnacle, which had been injected with aequorin, exhibits an abrupt rise in the $[Ca^{2+}]_i$ (bottom trace). Soon thereafter, an increase in muscle tension begins (top trace), which continues even though the Ca^{2+} quickly returns to basal level. The Ca^{2+} -dependent light emission from aequorin is measured with a photomultiplier tube. Bar = 20 ms. (Figure courtesy of Ridgway and Ashley, 1967, Figure 1a, with permission of Elsevier.)

In both instances, the eggs had been injected with aequorin, and in both examples, clear documentation of a $[Ca^{2+}]_i$ increase was noted after fertilization. In an extension of the studies on medaka eggs, Gilkey et al. (1978), using sensitive imaging equipment, were able to observe the spatial and temporal dynamics of the Ca^{2+} -dependent light emission. Their results reveal that the $[Ca^{2+}]_i$ rises at the point of sperm entry (the micropyle), reaching $\sim 30 \mu M$, and propagates as a wave that travels at the rate of $12 \mu m/s$ through the cortex of the egg. By the late seventies, therefore, it had been established in several cell types that the basal $[Ca^{2+}]_i$ is $\sim 0.1 \mu M$ and, importantly, that a variety of different events can be activated through a change or amplitude modulation of the $[Ca^{2+}]_i$ up to $1 \mu M$ or higher.

Ca^{2+} AMPLITUDE MODULATION IN PLANTS

Although plants do not possess muscles as such, it can be viewed as an interesting example of parallelism that our understanding of Ca^{2+} regulation in plant cells in part originated from studies on the control actomyosin in cytoplasmic stream-

ing. In the sixties, it had been recognized that the action potential in large internode cells of the Characean algae would induce a very rapid but reversible inhibition of cytoplasmic streaming (Barry, 1968; Tazawa and Kishimoto, 1968). Tazawa and Kishimoto (1968) showed that it was not the formation of a gel or the coagulation of the cytoplasm that led to streaming cessation but rather an inhibition of the driving force. Realizing that there were substantial ion changes during the action potential, they focused primarily on chloride and potassium but nevertheless suggested that Ca^{2+} might also be involved. At the same time, Barry (1968), working with *Nitella* and using ion replacements, provided clear evidence that the presence of Ca^{2+} , but not Mg^{2+} , in the extracellular medium caused the cessation of streaming during the action potential. These studies further emphasized that it was not the action potential per se that led to streaming inhibition but rather the presumed influx of Ca^{2+} . Barry (1968) also directed attention to the actomyosin system as the focus for Ca^{2+} activity. Further work, involving the perfusion of the large internode cells of *Nitella* and *Chara*, produced a system that could be readily manipulated experimentally. Williamson (1975) established that

streaming, in addition to requiring ATP, was dependent on a very low $[Ca^{2+}]_i$ ($0.1 \mu M$). If the concentration was elevated to $1.0 \mu M$, there was a decrease in cytoplasmic streaming by 20%, and if the $[Ca^{2+}]_i$ was increased to $10 \mu M$, the streaming would be inhibited by a $>80\%$. Similar results reported by Tazawa et al. (1976) further emphasized the conclusion that elevated $[Ca^{2+}]_i$ inhibited cytoplasmic streaming. At the time these studies were published, they may not have enjoyed widespread acknowledgment because there were questions whether findings from the Characean algae were relevant to equivalent processes in higher plants. The subsequent studies on *Vallisneria* dispelled this concern, showing that cytoplasmic streaming, as in *Nitella* and *Chara*, was regulated by the $[Ca^{2+}]_i$ (Yamaguchi and Nagai, 1981; Takagi and Nagai, 1983). Today, it is widely recognized for non-flowering and flowering plants alike that low $[Ca^{2+}]_i$ ($0.1 \mu M$) permits streaming, whereas elevated $[Ca^{2+}]_i$ ($1.0 \mu M$) inhibits the process.

The major breakthrough that established the relationship between the action potential, Ca^{2+} , and the inhibition of streaming came from the pioneering studies of Williamson and Ashley (1982). Using internode cells of *Nitella* and *Chara*, into which the photoprotein aequorin had been microinjected, they showed that the action potential elicited an abrupt rise in the $[Ca^{2+}]_i$ (Figure 2) together with a parallel decrease in cytoplasmic streaming. The system also showed impressive recovery with a relatively rapid return to basal $[Ca^{2+}]_i$, followed by a resumption in cytoplasmic streaming. Williamson and Ashley (1982) further established that the basal $[Ca^{2+}]_i$ in *Chara* was $\sim 0.1 \mu M$, whereas in *Nitella*, it was $0.4 \mu M$. When stimulated, the $[Ca^{2+}]_i$ in *Chara* rose to $6.7 \mu M$, whereas in *Nitella*, it rose to $43 \mu M$. A closely following study by Kikuyama and Tazawa (1983) provided results in agreement with Williamson and Ashley (1982), firmly establishing the change of $[Ca^{2+}]_i$ during the action potential in *Nitella* and *Chara*. These studies were the first and for several years remained the most convincing example of Ca^{2+} amplitude modulation in plants.

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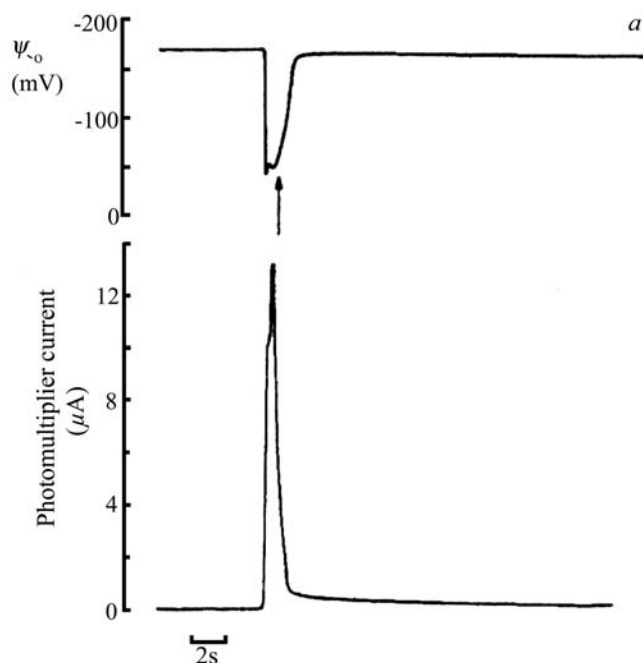


Figure 2. The Action Potential in Chara Elicits a $[Ca^{2+}]_i$ Increase.

A Chara internode cell, which had been injected with aequorin, is stimulated electrically to induce an action potential (top trace). Following closely is a sharp increase in the photomultiplier current indicating Ca^{2+} -dependent light emission from aequorin (bottom trace). Bar = 2 s. (Figure courtesy of Williamson and Ashley, 1982, Figure 2a, with permission of Nature Publishing Group <http://www.nature.com/>).

After these pioneering studies on Nitella and Chara, there have been additional studies in plants showing that the basal $[Ca^{2+}]_i$ is low and that increases can occur following different stimuli. Gilroy et al. (1986) used the permeant acetoxymethyl-ester of quin2 to show that the $[Ca^{2+}]_i$ in mung bean root protoplasts was 171 nM. This study is important because it was the first to use a fluorescent indicator. Although quin2 is no longer used, the second-generation fluorescent dyes developed by R.Y. Tsien and colleagues, for example fura-2 and indo-1 (Grynkiewicz et al., 1985), and especially in their dextranated forms, have proved extremely effective in allowing us to assay $[Ca^{2+}]_i$ in plants. Other methods have also provided compelling results. For example, Miller and Sanders (1987), using a Ca^{2+} selective intracellular microelectrode, found that the alga Nitellopsis had a basal $[Ca^{2+}]_i$ of 400 nM in the dark. However, when cultured in light, the $[Ca^{2+}]_i$ dropped to 150 nM. The interpretation put

forth was that the process of photosynthesis, together with ion uptake by chloroplasts, caused the reduction of the $[Ca^{2+}]_i$. Also using Ca^{2+} selective microelectrodes, Felle (1988) showed that auxin induced Ca^{2+} oscillations in maize coleoptiles. Here, the basal $[Ca^{2+}]_i$ was 119 nM, which in the presence of auxin rose in an oscillatory fashion to 300 nM. Yet another example was the induction of stomatal closure by ABA, which was shown to be accompanied by an increase in the $[Ca^{2+}]_i$ to 600 nM in Commelina guard cells that had been injected with the fluorescent indicator dye fura-2 (McAinsh et al., 1990). Note is also made of the dramatic tip-focused Ca^{2+} gradient observed in pollen tubes (Obermeyer and Weisenseel, 1991; Rathore et al., 1991; Miller et al., 1992), a result that was anticipated given the earlier demonstration of $^{45}Ca^{2+}$ influx in these cells (Jaffe et al., 1975). However, the fluorescent dyes allowed direct visualization of free Ca^{2+} . Also, the use of fura-2 covalently

linked to a 10-kD dextran provided a means for avoiding dye sequestration (e.g., into vacuoles) and for permitting long term recording of the $[Ca^{2+}]_i$ (Miller et al., 1992). Finally, in a dramatic development that fused molecular methods to Ca^{2+} cell biology, Knight et al. (1991) introduced the aequorin gene into tobacco plants and were able to show that different agents, including touch, cold shock, and fungal elicitors, induced Ca^{2+} stimulated luminescence. Suffice it to say that by the late eighties and early nineties several studies, using different techniques, had documented a low basal $[Ca^{2+}]_i$ and demonstrated amplitude modulation in plant cells.

CONCEPT OF Ca^{2+} AS A REGULATOR

The studies discussed above make it abundantly clear that the $[Ca^{2+}]_i$ in plant cells, as in animal cells, is low and that plants are able to respond to various stimuli by eliciting a change in the $[Ca^{2+}]_i$. However, just as a professional orchestra does not need the oboist to sound them the appropriate A, neither did the plant biologists need these data to suggest that Ca^{2+} was a potential signal transducer. By the early to mid seventies, the ideas were in the air, and thus well before the actual documentation of the $[Ca^{2+}]_i$, many scientists working on different aspects of plant growth and development were coming to recognize the potential importance of Ca^{2+} as an intracellular signaling agent. Although there were probably several paths that were responsible for focusing attention on the regulatory function of Ca^{2+} , I will mention a few lines of thought and research that I think were important in shaping the ideas of plant biologists.

Ca^{2+} and Cyclic AMP: The Discovery of Calmodulin and Calcium-Dependent Protein Kinases

In the late fifties, Sutherland and Rall (1958) discovered that adenosine 3',5'-monophosphate (cyclic AMP) levels increased in liver tissues in response to epinephrine and furthermore that this small nucleotide was implicated as a second messenger in a wide variety of cellular reactions frequently

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involved in the phosphorylation of proteins (reviewed in Rasmussen, 1970). It soon became apparent that Ca^{2+} was also involved in many of these reactions, where it was recognized that a stimulus that caused an increase in cyclic AMP also generated an increase in Ca^{2+} ion uptake. The confluence of the activities of these two agents led Rasmussen (1970) to speculate that, "The basic elements in this widespread biochemical control mechanism are: calcium ions, adenosine 3', 5'-monophosphate (cyclic AMP), intracellular microtubules, microfilaments, secretory vesicles, and a class of enzymes known as protein kinases which phosphorylate specific proteins with adenosine triphosphate (ATP) as substrate." I can clearly remember reading this article (Rasmussen, 1970) and being struck by its bold vision. At the time it was not possible to make a case for the participation of cyclic AMP in plant development (reviewed in Trewavas, 1976), but all the other components could be recognized as possible contributors, including notably Ca^{2+} , the cytoskeleton, directed secretion, and protein kinases.

The continuing studies in different animal systems on the regulation of cyclic AMP led to discovery of calmodulin. In 1970, it was reported that 3',5' nucleotide phosphodiesterase (PDE), the enzyme that degrades cyclic AMP to 5'-AMP, was in part regulated by a heat stable protein (Cheung, 1970; Kakiuchi and Yamazaki, 1970), which itself did not show enzymatic activity. An important further observation revealed that PDE was controlled by Ca^{2+} with basal rates under 1 μM and maximal activation at 20 μM (Kakiuchi and Yamazaki, 1970). Subsequent biochemical investigations established that the heat stable factor was a protein that was dependent on Ca^{2+} , with half-maximal activation at 2.3 μM (Teo and Wang, 1973). Initially, this was called the "calcium-dependent regulator," a name that was changed to "calmodulin" (Cheung, 1980). It was also soon realized that calmodulin was extremely common, being found in virtually all tissues that were tested, and that it was very similar to troponin C, the Ca^{2+} switch for striated muscle (Cheung, 1980).

The discovery of calmodulin did not escape the attention of the plant biologists. Muto and Miyachi (1977) first showed that NAD kinase isolated from pea seedlings required an activator protein, which was sensitive to acid and alkali conditions, and also heat stable. These properties, together with its relatively low molecular mass (28 kD), led Muto and Miyachi (1977) to draw a tentative connection of the protein they had identified with the PDE activator in animal systems. However, it was Anderson and Cormier (1978), using appropriate metal chelators, who first discovered the Ca^{2+} requirement of the NAD kinase activator protein in plants. Their results firmly established the close relationship between it and the Ca^{2+} -dependent regulator protein or calmodulin. It soon became apparent that calmodulin was widely present in plants (Watterson et al., 1980). These results truly made the case for Ca^{2+} as an intracellular regulator of plant processes.

Continued investigations by many researchers revealed the existence of different kinases, in addition to NAD kinase, that were Ca^{2+} /calmodulin dependent. For example, Ca^{2+} /calmodulin regulation was demonstrated for an unspecified protein kinase (Polya and Davies, 1982) and for plant quinate:NAD⁺ 3-oxidoreductase (Ranjewa et al., 1983). Of particular interest and excitement was the discovery at this time of kinases that were Ca^{2+} dependent but calmodulin independent (Hetherington and Trewavas, 1982). This line of research led eventually to the discovery of calcium-dependent protein kinases (CDPKs) (Putnam-Evans et al., 1986; Harmon et al., 1987), which are now recognized as members of a large family of kinases that does not exist in animals. In animals, there are no kinases that are directly regulated by Ca^{2+} ; rather, their Ca^{2+} -stimulated kinases work through a relay system, often involving calmodulin (for review, see Carafoli et al., 2001). By contrast, plants have the calmodulin pathway (for review, see Snedden and Fromm, 2001; Zhang and Lu, 2003) and a separate system involving the CDPKs (for review, see Harmon et al., 2001; Cheng et al., 2002; Harper et al., 2004); this marks a significant and unique

difference in the mechanism of Ca^{2+} regulation between plant and animal cells.

Ca^{2+} and Cell Division

For me, there was a distinct awakening in the early seventies about Ca^{2+} as an intracellular regulator. First, the article by Rasmussen (1970), mentioned above, was enormously stimulating and provided a broad sweep about a role for Ca^{2+} in many different signal transduction processes, perhaps especially including a relationship between Ca^{2+} and the cytoskeleton. But the truly defining moment occurred with the publication by Weisenberg (1972), which showed that a low [Ca^{2+}] (<1.0 μM) was necessary to achieve microtubule polymerization in vitro. Before this, numerous unsuccessful attempts had been made to solve this vexing problem. Weisenberg (1972) had been using phosphate buffers with only limited success. He then switched to the newly introduced family of zwitterionic buffers and found one, *N*-(2-acetamido)-iminodiacetic acid, which dramatically supported microtubule polymerization. Further work indicated that the unique property of this buffer was its ability to chelate Ca^{2+} . Thus, in the presence of 100 mM *N*-(2-acetamido)-iminodiacetic acid and 10 mM calcium, the free [Ca^{2+}] would only be 6 μM , yet this concentration was sufficient to block polymerization of microtubules. Further studies with EDTA, and especially EGTA, clearly established that it was Ca^{2+} and not Mg^{2+} that was responsible for the depolymerization of microtubules (Weisenberg, 1972).

At that time, I was working on mitosis and cytokinesis in plants, focusing on the formation, organization, and function of the mitotic apparatus and phragmoplast. Considerable attention was directed toward microtubules but also to associated structures, including elements of the endoplasmic reticulum, which could be seen to form close structural appositions with microtubules in both the mitotic apparatus and phragmoplast. I was teaching cell biology, which included muscle physiology as a topic, and was aware of the central role that Ca^{2+} played in muscle contraction. By that time, troponin had been discovered,

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and it was well known that contraction was exquisitely attuned to the $[Ca^{2+}]_i$ from resting (0.1 μM) to those that activated contraction (0.3 to 1.5 μM). In addition, studies on the sarcoplasmic reticulum from striated muscle had led to the isolation and partial characterization of the Ca^{2+} pump (MacLennan and Wong, 1971).

Putting these lines of inquiry together switched on the proverbial light in my mind. It seemed plausible that microtubules in the mitotic apparatus might be controlled by the local $[Ca^{2+}]_i$, which in turn would be regulated by the nearby endoplasmic reticulum (Figure 3) (Hepler et al., 1981). This general idea, which Barry Palevitz and I articulated in our review on the cytoskeleton (Hepler and Palevitz, 1974), guided research in my laboratory for several years thereafter. Spindle-associated endoplasmic reticulum was found to contain deposits of Ca^{2+} (Wick and Hepler, 1980; Wolniak et al., 1983), and spindle microtubules were shown to be sensitive to elevations in $[Ca^{2+}]_i$ with depolymerization occurring when the concentration was raised to 1.0 μM or more (Zhang et al., 1992). Restriction of the $[Ca^{2+}]_i$ was seen to affect progress through mitosis (Hepler, 1985), whereas stimulation of Ca^{2+} entry was found to promote bud initial formation in mosses (Saunders and Hepler, 1982) and

red light-stimulated spore development in ferns (Wayne and Hepler, 1984). Nevertheless, evidence for the occurrence of Ca^{2+} amplitude modulation during division was and still is decidedly mixed (Hepler, 1989); indeed, a compelling example of Ca^{2+} amplitude modulation in plant cell division has not been established.

Ca^{2+} and Polarized Cell Growth

An area of endeavor that drew early interest toward Ca^{2+} concerned the regulation of polarized plant cell development. Through the pioneering efforts of Jaffe and coworkers (Jaffe et al., 1974; Weisenseel et al., 1975), it was discovered that polarized cells (e.g., *Fucus* zygotes, pollen tubes, and root hairs) drove substantial ion currents through themselves. The bulk of the ion current appeared to consist of a polarized influx of potassium, focused at the growing point (i.e., the rhizoid of *Fucus* or the growing tip of the pollen tube). However, it was soon appreciated that Ca^{2+} influx constituted a small but potentially important component of the total current (Jaffe et al., 1975; Robinson and Jaffe, 1975; Weisenseel and Jaffe, 1976). Robinson and Jaffe (1975), using *Pelvetia* eggs that were polarized through unilateral illumination, showed that approximately five times more $^{45}Ca^{2+}$ entered the shaded rhizoid pole than

entered the illuminated thallus pole. A subsequent study in which the rhizoids grew toward an experimentally imposed gradient of the Ca^{2+} -ionophore, A-23187, added strong support for the idea of localized Ca^{2+} influx as a regulator of polarized development (Robinson and Cone, 1980).

Studies on growing pollen tubes also provided persuasive support for localized ion fluxes and for a specific role for Ca^{2+} in the regulation of polarized growth. Application of the vibrating electrode revealed strong currents in which an influx of potassium at the apex appeared to be balanced by an outward flux of protons in the region of the grain (Weisenseel et al., 1975; Weisenseel and Jaffe, 1976). Importantly, Ca^{2+} , as noted earlier (Brewbaker and Kwack, 1963), was essential for tube growth (Weisenseel and Jaffe, 1976). Additionally, autoradiography revealed an accumulation of $^{45}Ca^{2+}$ in the apical domain, providing support for the idea that ion flow into the tip created an intracellular gradient (Jaffe et al., 1975). A certain amount of the autoradiographic signal observed by Jaffe et al. (1975) may be due to Ca^{2+} binding in the cell wall as noted by Kwack (1967). Nevertheless, when considered with the other studies on current flow, these data are consistent with a small but significant influx of Ca^{2+} across the plasma membrane.

Ca^{2+} in pollen tubes was subsequently examined for gradients in membrane-associated Ca^{2+} (chlortetracycline) (Reiss and Herth, 1979) and total Ca^{2+} (proton-induced x-ray emission) (Reiss et al., 1983), both of which provide results consistent with the apex being elevated in the ion. However, the demonstration of the steep, tip-focused gradient, using fluorescent dyes (Obermeyer and Weisenseel, 1991; Rathore et al., 1991; Miller et al., 1992), finally provided the necessary proof for the postulated asymmetric distribution of intracellular free Ca^{2+} . Based on the studies on polarized ion currents, the suggestion was made that Ca^{2+} gradients might create an electrical field across the cytoplasm that would be sufficient to segregate cytoplasmic components by electrophoresis (Jaffe et al., 1974; Robinson and Jaffe, 1975). Robinson and Jaffe (1975) also suggested that Ca^{2+} might affect intracellular motility

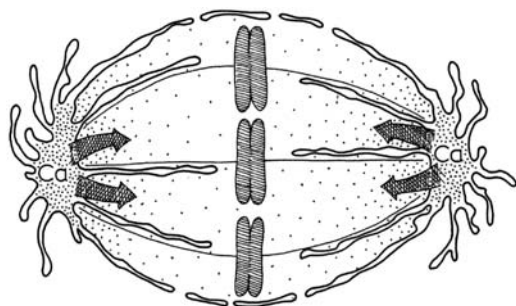


Figure 3. Diagram of a Dividing Plant Cell in Late Metaphase.

This figure depicts a system of Ca^{2+} -containing endoplasmic reticulum that extends from the spindle poles to the chromosomes along kinetochore microtubules. It was suggested that during anaphase, Ca^{2+} release from the endoplasmic reticulum activates motile processes (e.g., microtubule depolymerization) and thus facilitates movement of the chromosomes to the spindle poles. In support of this model, Ca^{2+} -stimulated depolymerization of microtubules and facilitation of chromosome motion have been observed (Zhang et al., 1992). Although an endogenous increase in $[Ca^{2+}]_i$ during anaphase has been reported using the absorbance indicator arsenazo III (Hepler and Callahan, 1987), this has not been repeated with a more efficacious fluorescent dye. (Figure courtesy of Hepler et al., 1981, Figure 7, with kind permission of Springer Science and Business Media.)

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and in particular the formation and function of microtubules. In broad terms, these ideas can be appreciated as antecedents for the view that the Ca^{2+} gradient in polarized cells contributes to the control of secretion (Holdaway-Clarke and Hepler, 2003).

Ca^{2+} and Secretion

The substantial body of work showing that Ca^{2+} affected the permeability properties of the cell membrane, while interesting in itself, did not provide a very compelling or complete understanding of the mechanism of action of the ion. Although usually not explicitly stated, there are different studies where you can almost hear the authors struggling with this rather vague concept and where they are attempting to formulate a more specific model. An example is the induction of α -amylase release in barley aleurone cells by gibberellic acid. Studies on this model system had gained attention because it seemed that the molecular basis for gibberellic acid might emerge. An early and influential observation was that of Chrispeels and Varner (1967), who showed that the presence of Ca^{2+}_e (mM) greatly facilitated the appearance of gibberellic acid-induced α -amylase in the medium. The increase was not trivial; as shown by Jones (1973), 20 mM CaCl_2 stimulated an 18-fold increase over the water control. If an increase in the $[\text{Ca}^{2+}]_e$ renders the membrane less permeable, why then would there be an increased release of α -amylase? It is my supposition that this conundrum puzzled different researchers leading eventually to the realization that Ca^{2+} specifically stimulated the process of enzyme secretion (Jones and Jacobsen, 1983), an idea that stands as a paradigm today in both plant and animal systems (Zorec and Tester, 1992).

Ca^{2+} and Plant Growth Regulators

In addition to a potential interaction between gibberellic acid and Ca^{2+} , connections between this ion and other plant growth regulators were emerging. Thus, Ca^{2+} was seen to enhance the ability of cytokinin to retard senescence (Poovaiah and Leopold, 1973b) and leaf abscission (Poovaiah and Leopold, 1973a) and to

promote cotyledon expansion (Leopold et al., 1974). Ca^{2+} was also found to inhibit cytokinin stimulation of anthocyanin (Elliott, 1977) and betacyanin synthesis (Elliott, 1979). Yet other studies identified a Ca^{2+} /cytokinin/ethylene connection, although there was disagreement between published reports on the nature of the interaction. Lau and Yang (1975), in studies on mung bean hypocotyl segments, reported that kinetin greatly stimulated uptake of $^{45}\text{Ca}^{2+}$, and Ca^{2+} stimulated the uptake and metabolism of kinetin ^{14}C . Of particular note, both Ca^{2+} and kinetin caused a striking increase in ethylene (Lau and Yang, 1975). By contrast, Poovaiah and Leopold (1973a), in studies of leaf abscission in bean petiole explants, found that Ca^{2+} inhibited ethylene production. Quite apart from studies of higher plants, LeJohn and coworkers (1973) identified a Ca^{2+} binding cell surface glycoprotein in the oomycetes, *Achlya* and *Blastocladiella*, which released Ca^{2+} when challenged with cytokinin. They postulated that cytokinin stimulates the availability and uptake of Ca^{2+} , thus promoting metabolism (LeJohn et al., 1973).

Although an early idea concerning a Ca^{2+} /auxin interaction has already been discussed and largely dismissed, especially to the extent that these agents affect wall structure and expansion, nevertheless, there were physiological processes suggesting a possible coordination in their activity. For example, auxin transport was reduced by low $[\text{Ca}^{2+}]_e$ (EDTA) (DeLa Fuente and Leopold, 1973), whereas auxin-induced proton secretion was stimulated by Ca^{2+} (Rubinstein et al., 1977). The pervasiveness of Ca^{2+} activity led Leopold (1977) to open a summary article with the statement that, "Actions of each of the plant growth hormones can be altered by calcium salts...." Despite these many encouraging leads, the idea of Ca^{2+} as a signaling agent does not directly emerge from these studies. With stimulatory effects being caused by millimolar changes in the $[\text{Ca}^{2+}]_e$, it would be nearly impossible to derive a sense about the Ca^{2+} status in the cytosol. Nevertheless, an awakening was taking place, and one that emphasized the importance of Ca^{2+} over Mg^{2+} , and the

monovalent cations, as a regulator of plant growth and development.

Ca^{2+} and Light

Well before the demonstration that photosynthesis lowers the $[\text{Ca}^{2+}]_i$ in *Nitellopsis* (Miller and Sanders, 1987), a Ca^{2+} /light interaction had been noted in different organisms. The red/far-red reversible phytochrome pigment system was a common focus of attention. Among the early studies, note is made of phytochrome stimulated nyctinastic leaf movement in *Albizia*, which was shown to be inhibited by low $[\text{Ca}^{2+}]$ as generated by culture in EDTA (McEvoy and Koukkari, 1972). Ca^{2+} was found to markedly enhance the photoreversible red light-induced depolarization of the membrane potential in *Nitella* (Weisenseel and Ruppert, 1977). A photoreversible red light-induced increase in Ca^{2+} efflux was noted in oat coleoptiles (Hale and Roux, 1980). Red light, provided as a microbeam to filaments of *Mougeotia*, was also shown to stimulate a 2- to 10-fold increase in the uptake of $^{45}\text{Ca}^{2+}$ (Dreyer and Weisenseel, 1979). Finally, red light-stimulated chloroplast rotation in *Mougeotia* was reduced simply through elimination of Ca^{2+} from the culture medium (Wagner and Klein, 1978). Based on these early studies and also influenced by Rasmussen (1970), Haupt and Weisenseel (1976) suggested that phytochrome molecules located in the plasma membrane function as Ca^{2+} carriers and facilitate an increase in the $[\text{Ca}^{2+}]_i$ when irradiated with red light. Their results supported the idea that Ca^{2+} would control contractile proteins within the cell, and thus the movement of chloroplasts, as demonstrated in *Mougeotia* (Haupt and Weisenseel, 1976). Although most current research on phytochrome is not directed toward Ca^{2+} , a potential role remains likely, given the identification of SUB1, a Ca^{2+} binding protein involved with both cryptochrome and phytochrome (Guo et al., 2001).

Ca^{2+} HOMEOSTASIS: PUMPS AND CHANNELS IN PLANTS

Ca^{2+} and Mitochondria

Given the enormous disparity in $[\text{Ca}^{2+}]$ between the cytosol (0.1 μM) and the

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outside medium or storage compartments (0.1 to 10 mM), it became obvious that the cell must exert extremely close control over the movement of the ion. However, even before the disparity in $[Ca^{2+}]_i$ was fully appreciated, early studies revealed that certain organelles, in particular mitochondria, exhibited the ability to take up large quantities of Ca^{2+} . Initial studies conducted in the late fifties and early sixties on mitochondria derived from different animal cells (e.g., liver and kidney) revealed that Ca^{2+} sequestration depended on respiration, was sensitive to uncouplers, and required inorganic phosphate (for review, see Carafoli et al., 2001). Also in the sixties, Hodges and Hanson (1965) demonstrated that maize mitochondria, like their animal cell counterparts, actively participate in the uptake and accumulation of Ca^{2+} . These pioneering studies revealed that Ca^{2+} uptake requires respiration or the addition of ATP and is blocked by uncoupling agents. That the process of Ca^{2+} uptake by plant mitochondria was broadly expressed was shown by Chen and Lehninger (1973), who examined and compared the activity of these organelles from 14 different species of higher plants and fungi. Although all mitochondria actively sequestered Ca^{2+} , those from sweet potato and white potato tubers were particularly active and comparable to those from rat liver. Additional work also established that plant mitochondria, like those from animal sources, exhibited greater uptake capacity but lower affinity than the similar process in microsomes (Dieter and Marmé, 1980, 1983). These observations led to the conclusion that mitochondria, while capable of taking up large amounts of ion, would not be the organelle involved in establishing the low basal $[Ca^{2+}]_i$.

A further intriguing observation was the sensitivity of mitochondrial Ca^{2+} uptake to light. Roux et al. (1981) noted that the $[Ca^{2+}]_i$ in the culture medium surrounding isolated oat mitochondria increased when the preparation was irradiated with red light. Because the change in the $[Ca^{2+}]_i$ was prevented by subsequent exposure to far-red light, phytochrome was implicated. Additional studies with ruthenium red, which blocks sequestration, led them to

favor the idea that red light, rather than blocking influx, stimulated efflux of Ca^{2+} (Roux et al., 1981). Although further studies at this time (Dieter and Marmé, 1983; Yamaya et al., 1984) failed to agree with the details provided by Roux et al. (1981), they nevertheless supported the basic tenet that Ca^{2+} uptake by plant mitochondria is in part regulated by light.

An important finding concerning basic mitochondrial metabolism was the discovery that at least one associated enzyme, namely NADH dehydrogenase, was regulated by Ca^{2+} . The early studies of Miller et al. (1970) and Coleman and Palmer (1971), using EDTA, directed attention toward Ca^{2+} , but the later study of Møller et al. (1981), using both EDTA and EGTA, definitively established a role for Ca^{2+} and not for Mg^{2+} . It appeared from these studies that plant mitochondria not only were able to sequester Ca^{2+} , but that the ion, through the control of NADH oxidation, participated in regulation of mitochondrial function. In light of these various issues, the mitochondrial/ Ca^{2+} connection in plants deserves renewed attention as emphasized recently by Hetherington and Brownlee (2004). The idea of both plant and animal mitochondria being viewed only as a safety valve capable of responding to a vast overload of Ca^{2+} is being challenged in studies of animal cell mitochondria. Evidence is emerging that mitochondria respond to relatively small changes in the $[Ca^{2+}]_i$ through spatial juxtaposition with the endoplasmic reticulum (Rizzuto et al., 1994; Carafoli et al., 2001). Similarly, plant mitochondria may be playing a more central role in cytosolic Ca^{2+} regulation than has been appreciated heretofore.

Ca^{2+} and Chloroplasts

Chloroplasts also were seen to participate in the uptake of Ca^{2+} . In 1964, Nobel and Packer, drawing parallels with studies on mitochondria, demonstrated that isolated spinach chloroplasts sequestered Ca^{2+} when irradiated with light and supplemented with ATP. Several years later, when it was apparent that plant cells maintained very low $[Ca^{2+}]_i$, the role of the chloroplast in the regulation of this ion

received further attention. Different laboratories using chloroplasts from wheat (Muto et al., 1982) and spinach (Kreimer et al., 1985a, 1985b) confirmed the dramatic light-dependent uptake of Ca^{2+} . Kreimer et al. (1985a, 1985b) further concluded that photosynthetic electron transport was essential and that it was the membrane potential rather than pH that drove Ca^{2+} uptake.

A particular interest in the role of Ca^{2+} in the chloroplast centered on the regulation of NAD kinase. Previous discussion has emphasized the role these studies played in the discovery of calmodulin in plants (Muto and Miyachi, 1977; Anderson and Cormier, 1978). Considerable complexity surrounded this problem because reports arose showing both calmodulin-dependent and -independent forms of the enzyme and both cytoplasmic and organellar location (Moore and Åkerman, 1984). Nevertheless, it seemed apparent that at least some of the NAD kinase, which was associated with chloroplasts, was Ca^{2+} dependent and light activated (Muto et al., 1982). With the further finding that calmodulin was localized in chloroplast stroma, Jarrett et al. (1982) suggested that light stimulated the uptake of Ca^{2+} and that the Ca^{2+} /calmodulin complex activated NAD kinase. The product, NADP, then served its important function as the terminal electron acceptor for photosystem I.

Ca^{2+} and Microsomes

From a historical point of view, our appreciation of a microsomal Ca^{2+} sequestration system is the most recent, although in many ways it emerges as the most important in regulating the basal $[Ca^{2+}]_i$ (Evans, 1998; Sze et al., 2000). Researchers examining animal cells, in particular those working on muscle, made early and important progress on the identification of an ATPase on the sarcoplasmic reticulum that drove the uptake of Ca^{2+} against a concentration gradient (MacLennan and Wong, 1971). Plants may not have such a hypertrophied system for Ca^{2+} uptake, nevertheless early studies provided evidence that microsomal vesicles were capable of Ca^{2+} uptake. Already in their landmark study on

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mitochondria, Hodges and Hanson (1965) made reference to preliminary work showing Ca^{2+} uptake in a microsome preparation from etiolated maize seedlings. However, it was Gross and Marmé (1978) who demonstrated the presence of Mg^{2+} /ATP-dependent Ca^{2+} uptake in microsomes of maize, squash, oats, and mustard, suspension cells of parsley, and the alga *Cryptomonas*. Although the membrane fraction was not fully identified, the studies were consistent with it being derived from the plasma membrane. A more definitive identification of plasma membrane activity derives from studies of *Neurospora*, in which Ca^{2+} accumulation in inverted vesicles depended on a Ca^{2+} / H^{+} antiporter (Stroobant and Scarborough, 1979).

A major problem in these early studies concerned the identification of the source of the membrane vesicles. For example, Dieter and Marmé (1981) provided evidence for a calmodulin-dependent Ca^{2+} -ATPase; however, they were not able to identify the membrane. Rasi-Caldogno et al. (1982) isolated two nonmitochondrial membrane fractions, the heavier of which appeared to be a Ca^{2+} -ATPase and the lighter one a Ca^{2+} / H^{+} -antiporter. Studies in which the source of the membrane system was defined included those of Kubowicz et al. (1982), who isolated a plasma membrane enriched fraction that was active in the sequestration of Ca^{2+} . Of further note here was the observation that auxin promoted Ca^{2+} uptake, whereas cytokinin was inhibitory. Endoplasmic reticulum vesicles were isolated by Buckhout (1984), who demonstrated high affinity Ca^{2+} accumulation that was not dependent on calmodulin. Finally, attention is directed toward the study of Schumaker and Sze (1985), who carefully isolated and identified two membrane fractions from oat roots. The more prominent one derived from the vacuole and the less prominent one from the endoplasmic reticulum. Both fractions sequestered Ca^{2+} , with vacuolar membranes appearing to use a Ca^{2+} / H^{+} -antiporter and the endoplasmic reticulum membranes employing a Ca^{2+} -ATPase. In conclusion, it can be seen that studies conducted between the mid sixties and the mid

eighties and to the present day (Sze et al., 2000) established that plants possess a rich and multifaceted mechanism for Ca^{2+} sequestration.

Ca^{2+} Influx

In parallel with the concept of uptake and sequestration, which lowers $[\text{Ca}^{2+}]_i$, is the equally important matter of entry or release, which raises the $[\text{Ca}^{2+}]_i$. Given the huge concentration gradient, which may be in the order of 1000- to 10,000-fold, together with a substantial charge gradient, which may be -100 mV or more, there is an enormous combined force that will drive Ca^{2+} into the cell. It follows that only a few Ca^{2+} channels may be required to impart a rapid increase in the $[\text{Ca}^{2+}]_i$. The activity of Ca^{2+} channels at the whole cell level is shown directly in the previously cited work by Williamson and Ashley (1982), who recorded a sharp $[\text{Ca}^{2+}]_i$ rise immediately after the action potential in *Nitella* and *Chara* (Figure 2). The Characean algae were also used to demonstrate the activity of single Ca^{2+} channels (Berestovsky et al., 1976); however, this is a topic that has exploded in more recent years (for review, see Tester, 1990; Hetherington and Brownlee, 2004).

CONCLUSIONS

Beginning in the sixties and extending through the seventies to the early eighties, several lines of investigation were being followed by plant biologists that were consistent with the notion that Ca^{2+} is a crucial cellular regulator. The stage was set, and as a result of these pioneering efforts there was a virtual explosion of work in the eighties (Hepler and Wayne, 1985; Trewavas, 1986; Kauss, 1987) (Figure 4) and to the present day that continues to define and characterize the enormous role that Ca^{2+} plays in the regulation of plant growth and development. To some degree, studies on plants were impeded by the presence of a cell wall, which provides an enormous reservoir for Ca^{2+} . With its own requirements being very high ($10 \mu\text{M}$ to 10mM), the $[\text{Ca}^{2+}]_e$ in the wall would easily swamp the relatively trivial amounts seen in the cytosol (0.1 to $10 \mu\text{M}$). But as small as the concentration is, Ca^{2+}_i clearly has a powerful impact on a host of growth and developmental processes.

The extensive involvement of Ca^{2+} frequently leads to the vexing question: how can one ion control so many events? The answer is not entirely known, but the broad framework for its solution can be drawn. In

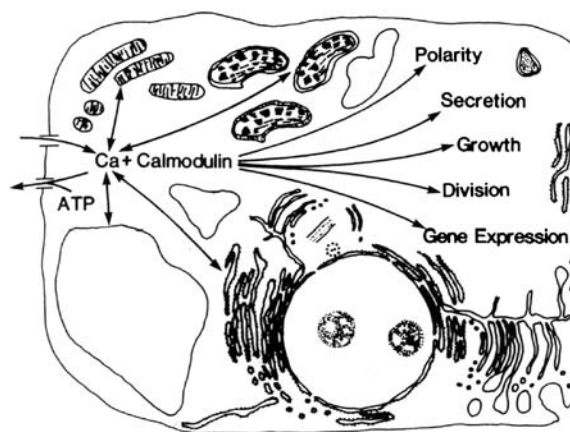


Figure 4. Diagram Depicting the Role of Ca^{2+} as a Signaling Agent in Plant Cells Published in 1985/86 (Trewavas, 1986).

Although some important features of Ca^{2+} signaling were not known at this time (e.g., the existence of CDPKs), the figure nevertheless represents and anticipates the central role that Ca^{2+} plays in many aspects of plant growth and development. (Figure courtesy of Trewavas, 1986, frontispiece figure, with permission of Springer Science and Business Media.)

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brief, Ca^{2+} regulation in plants is richly endowed with many components that can define and adjust responses in both time and space (Reddy and Reddy, 2004). Influx channels on the plasma membrane and release channels from internal stores (endoplasmic reticulum, vacuole, and mitochondria) provide several ways to generate rapid ion elevations or create local gradients. Once the $[\text{Ca}^{2+}]_i$ has risen, there are then a wide variety of response factors, including both calmodulin-dependent kinases and notably the CDPKs, which will phosphorylate a response element and thus stimulate or inhibit an event or process. Finally, plants exhibit frequency as well as amplitude modulation, providing yet another means of generating signals that have unique properties (Evans et al., 2001; Holdaway-Clarke and Hepler, 2003). Because these different pathways can interact, the number of individual signatures multiplies. As noted by Reddy and Reddy (2004) from their analysis of the Arabidopsis proteome, there are ~700 known protein components that function at various stages of Ca^{2+} signaling. Plants thus possess myriad ways in which Ca^{2+} can operate as the intermediary in transducing the stimulus into the appropriate response. The challenge for the future lies in identifying and characterizing these many different events and processes.

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Photo courtesy Minnesota Agricultural Utilization Research Institute
Steve Lorentz has used corn stalks ground in a tub grinder to mulch 20,000 trees.

Tub Grinder Used To Make Cornstalk Bale Mulch

"I've used it on 15,000 of my trees and about 5,000 others and they're doing great," says Steve Lorentz who uses a tub grinder to lay down a mat of mulched cornstalks around the base of his young trees to keep down weeds and limit rodent damage.

The Stanton, Minn., tree farmer is finding cornstalk mulch works as well as commercial plastic mulching material but costs much less, as little as \$1.50 per tree. He gets 5 by 6-ft. cornstalk bales from local farmers.

He uses a Patz tub grinder to shred cornstalks into 1 to 3-in. pieces, then blows the material around the base of the trees in an 8-in. deep mat through the machine's polyurethane hose. Lorentz equipped the tractor-pulled grinder with a hose swivel that pivots 270 degrees so he can blow the mulch up to

15 ft. in any direction from the grinder.

Using the tub grinder, Lorentz can mulch up to 400 trees per hour.

The mulch lasts about two seasons before it degrades and that's about ideal for the young trees that are hardy enough to do without it after that length of time, he says.

Lorentz also planted 200 tomato plants into a 6-in. thick cornstalk mulch this year and uses it on flowers planted around his house with good results.

He wants to make the system more mobile, perhaps by mounting the grinder on a truck, to make it more practical, he says.

Contact: FARM SHOW Followup, Steve Lorentz, 33249 35th Ave., Stanton, Minn. 55018 (ph/fax 507 263-4021).



Unit mounts on a four-wheel cart built out of 2-in. sq. tubing. An oversized radiator out of a 3/4-ton pickup cools the engine.



The gearbox is at a right angle to the engine crankshaft instead of in-line so it doesn't take up much room.

Power Cart Fitted With Portable PTO, Air Compressor

By C.F. Marley

"It's cheaper than using the 40 hp tractor I used before and it's a lot more maneuverable. If you have a flat tire out in the field, you can pull this rig right out to it," says B.L. "Butch" Uhnken who built a portable power cart fitted with a pto and an air compressor.

The Jacksonville, Ill., farmer primarily uses the portable rig to power an 8-in. dia., 72-ft. long auger at bin sites.

Mounted on a four-wheel cart built out of 2-in. sq. steel tubing, the unit is powered by a 30 hp Mercedes diesel engine off a semi trailer refrigerator unit.

It's fitted with a pto and right angle gear box that came off an old Mayrath auger.

"The gearbox is at a right angle to the engine crankshaft instead of in-line so it doesn't take up as much room," Uhnken explains. "It's pretty cramped quarters around some of the bins."

An oversized radiator out of a 3/4-ton 4-WD Ford pickup cools the engine.

Since the big radiator has a tendency to collect bees wings, the air compressor comes in handy for cleaning it off.

The air compressor is an upright air conditioning unit off an old Ford car. It's used to power a 1 1/2-in. dia. cylinder with 4-in. stroke which Uhnken uses to tighten the belts on the engine to activate the pto. The cylinder has a capacity of 125 psi's, but Uhnken used regulators to step down the compressor to 60 psi's, all that's needed to tighten the belts.

"I also put restrictors on the air line to keep from shearing auger pins when the pto starts up," he says.

The cart rides on four Deere riding mower wheels, with the front wheels set close together for easier maneuvering around bin sites.

Uhnken uses the tongue mounted on front of the cart to transport it from farm to farm behind his pickup. He positions it at sites by hand.

Out-of-pocket expense was about \$400, including \$200 for the Mercedes engine.

Contact: FARM SHOW Followup, B.L. Uhnken, 25 Westfair, Jacksonville, Ill. 62650 (ph 217 472-3851 or 245-4359).

"Cement Dust" Fertilizer Achieves "Amazing Results"

A new pelletized fertilizer containing cement dust is achieving "amazing results" in field tests on a variety of crops, according to a Missouri farmer who says the product boosts yields and reduces nitrogen leaching.

"First year test plots at a Midwest research farm produced amazing results," says Gregory Wommack of Silex, Mo., who's been working on the new fertilizer for years. "Soybeans treated with the product produced 6.6 more bushels per acre, a 17 percent increase. Alfalfa treated with it produced 1.9 more tons per acre."

Similar results were reported in 16 vegetable crops, four cereal grains and corn, Wommack says.

The product, Potassa-Cal, is a blend of potassium, ground calcium carbonate, calcium sulfate, and a calcium-rich by-product of cement manufacturing called cement kiln dust (CKD). Calcium and other trace minerals contained in CKD are important to plant nutrition and productivity, Wommack explains, and he and scientists have come up with a way to make a soil soluble pellet out of the fine, powdery product.

The pellets are brown in color and about the same size as urea pellets, with the same blendability as other dry fertilizers.

The fertilizer is broadcast at 300 to 400 lbs. per acre with a truck spreader at planting. Or banded with fertilizer discs on the

planter at 220 to 250 lbs. per acre, with placement 3 to 4 in. from the row and 2 in. deep.

In soybeans, the product results in taller soybeans with more pods per plant. For example, in Wommack's 30-in. soybeans, plants averaged 55 to 60 in. tall with 100 pods per plant, compared with a typical height of 40 to 45 in. and bearing pods of 70 to 80 per plant.

What's more, in leaching tests on corn treated with a blend of Potassa-Cal and urea, nitrate leaching in the soil was cut by up to 50 percent.

"That's particularly impressive considering that the urea added an additional 180 lbs. of N per acre," Wommack notes. "Based on what we've seen, we expect Potassa-Cal could reduce leaching potential by 45 percent and could cut N use by 10 to 15 percent in broadcast applications."

Full benefits of Potassa-Cal may not be realized for a couple years after it's first applied, as indicated in independent tests by Arise Research, Casey, Ill., and Louisiana State University, Baton Rouge, Wommack notes.

The product is expected to be on the market this year and sell for about \$130 per ton.

Contact: FARM SHOW Followup, Magic Green Corporation, 4598 Hwy. H, Silex, Mo. 63377 (ph 573 384-6300; fax 6305).

Photo courtesy: Carl Ferencz, Oshawa, Ontario
Kerry's lock-in-a-tube consists of a lock inside a 5-in. dia. piece of pipe. Keeps thieves from using a bolt cutter on lock.

Simple, Crook-Proof Lock-In-A-Tube

None of the high-tech crime-fighting gadgets money can buy work as well as this simple-to-build "lock-in-a-tube" that a North American farm equipment dealer came up with.

"We tried motion detectors, cameras and security lights and were still getting broken into every six months," says Arnold Kerry, Utica Farm Equipment, Port Perry, Ontario. "We haven't lost a single lock since we came up with this idea last year."

Kerry welded lengths of 5-in. dia. pipe to the top of his gates, which are made of 4-in. dia. pipe, to completely enclose padlocks so that bolt cutters can't be used on them.

The 15-in. long pipe sections weld at 90 degree angles to strap iron on top of the gates. The strap iron forms a clevis that attaches

the gates together with the pin running up through the clevis. The padlock fits inside the pipe and secures the gates.

"The only way to get at the locks now is to use a torch or hammer and chisel and that evidently attracts too much attention for crooks," Kerry says. "The real beauty of this idea is that it can be adapted to any situation, including the farm."

Cost to install the guards on both the front and back gate at his dealership was less than \$100 (Canadian).

Contact: FARM SHOW Followup, Utica Farm Equipment, P.O. Box 717, Port Perry, Ontario, Canada L9L 1A6 (ph 905 985-9701; fax 9704).

THE THIRD BIENNIAL REPORT

OF THE DIRECTOR OF THE

Agricultural College Survey
of North Dakota

TO THE

Governor of North Dakota

ADMINISTRATIVE REPORT AND ACCOMPANYING
PAPERS FOR THE YEARS
1905-6

DANIEL E. WILLARD
Director

BISMARCK:
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SOIL SURVEY OF THE CANDO AREA.

BY ELMER O. FIPPIN AND JAMES L. BURGESS.

(By permission of the Bureau of Soils, Department of Agriculture, Washington, D. C.)

LOCATION AND BOUNDARIES OF THE AREA.

Towner county is located in the northeastern quarter of the state, and is bounded on the north by the Canadian line. The greatest dimension of the county is from north to south, measuring forty-three and one-half miles, or a little more than seven tiers of townships, while its width from east to west is twenty-four miles, covering four townships. The area surveyed comprises the two southern tiers of townships and has an extent of 288 square miles, being twenty-four miles east and west by twelve miles north and south.

The main line of the Great Northern Railway crosses the state about eight miles south of the county, and from Churchs Ferry a branch line extends in a northerly direction through the center of the area surveyed to the town of St. John, near the Canadian line, in Rolette county. Cando and Maza are the only shipping points in the area. Cando is a town of approximately 2,000 population, contains a number of substantial business houses engaged in handling the various supplies and products of the region, and is the site of large elevators belonging to six different companies. Maza is a small village, being important chiefly as a grain-shipping center.

In the prosecution of the field work of the survey the bureau had the co-operation of the state agricultural and economic geological survey, to the extent of furnishing two men and paying their expenses for a period of nearly a month and a half.

HISTORY OF SETTLEMENT AND AGRICULTURAL DEVELOPMENT.

The area, like all of the surrounding region, is a comparatively new one agriculturally. It was first opened for settlement in 1883, and under the homestead and other similar federal enactments, such as the tree-claim and pre-emption acts, all of the land has been taken up. No land was sold by the government or granted to trans-

A further peculiarity is the way in which the various coulees are connected by these lines of drainage, and also the parallel position and near approach of several of the systems to each other. A study of the accompanying map will show two large coulees coming down from the north, one on each side of the Big Coulee, which they gradually approach and into which they finally empty. These, in turn, are formed by the union of streams, which continue parallel and near to each other for distances of several miles.

The surface geology of the area and of all the surrounding country is of the glacial type. The Dakota lobe of the ice pushed down over this country, forming a continuous mantle of glacial till, and during its recession and temporary advances formed lines of gravelly hills called terminal moraines. Associated with these is the roughest topography of the area, in which are found the small glacial lakes.

The melting of the ice during the period of recession produced large volumes of water that drained off to the south, and it was this water derived from the glacier in the immediate vicinity and from regions many miles to the north, beyond the Turtle Mountains, that produced the system of coulees mentioned above. In *The Story of the Prairies*, Professor Willard describes the Big Coulee as forming an outlet from large bodies of glacial water far to the northward into Devils Lake and thence into the Red river valley. The passage of this large volume of water through the area, as floods laden with sediment, and with varying currents, is responsible for the deposit of the large masses of material giving rise to the soils of the area, other than those formed directly from the boulder clay. The various grades of sand and gravel, mostly stratified in the form of bars and ridges, and of the huge mass of silty clay in the vicinity of Cando, were probably formed by this agency of the glacial waters. All of the surface forms are the result, directly or indirectly, of the action of ice, and the depth of the glacial material ranges in different parts of the area from thirty or forty feet to more than 100 feet.

SOILS.

The most prominent characteristic of the soils of this area is the large amount of organic matter and lime they contain, as compared with soils of similar texture farther east and south.

The preponderance of organic matter is not due to a large original amount produced in the soil, but, as was intimated in the discussion of climate, it is a result of the meteorological conditions. The source of all the humus is the plant roots and stems that were left in the soil from season to season by the prairie grass before cultivation began and by the cultivated crops now. All processes of decomposition are hastened by a high temperature, and the longer it continues the more thorough they will be. In this region the summers are comparatively short, the atmospheric temperature is not high on the average, and the soil temperature is comparatively low. This condition hinders the bacterial and fungus growths that are essential to rapid decomposition of organic matter, and a gradual accumulation of humus is the result.

The amount of humus present is the cause of the dark color of the soil of the area—a color that tends to increase the amount of heat absorbed during sunshine, thus maintaining a higher soil temperature. This is an important fact when associated with areas having as short growing seasons as here, and it is probable that the crop adaptation of the region would be quite different if the soils were a very light color, even though they contained the same amount of plant food as at present.

A large amount of lime is distributed through all types of soil, but is most abundant in the loams and gravelly loams. It has been derived from the grinding up by the glacier of lime-bearing crystalline rocks and also of considerable amount of limestone. This has been subsequently redistributed and in some places concentrated through the agency of the soil water. The largest accumulations are between the twelfth and thirtieth inches, where lime sometimes appears to form more than a third of the bulk of the soil. In gravel beds covered to a depth of from fifteen to thirty inches by silty material all of the pebbles in the upper part of the gravel are covered with lime, or the whole mass may be cemented together by depositions from the water leached from the soil above.

This large amount of lime is very useful in the soil, since it tends to increase the granulations of the fine-textured types, prevents acidity from the decomposition of the organic matter, which process it hastens, and is favorable to the growth of the grains most abundantly produced in the region. For the growth of flax, however, less lime is desirable.

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Soil Reclamation of Abandoned Mine Land by Revegetation: A Review

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SOIL RECLAMATION OF ABANDONED MINE LAND BY REVEGETATION: A REVIEW

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ABSTRACT

Mining of mineral resources results in extensive soil damage, altering microbial communities and affecting vegetation leading to destruction of vast amounts of land. Reclamation is the process to restore the ecological integrity of these disturbed mine land areas. It includes the management of all types of physical, chemical and biological disturbances of soils such as soil pH, fertility, microbial community and various soil nutrient cycles that makes the degraded land soil productive. Productivity of soil can be increased by adding various natural amendments such as saw dust, wood residues, sewage sludge, animal manures, as these amendments stimulate the microbial activity which provides the nutrients (N, P) and organic carbon to the soil. The top soil gets seriously damaged during mineral extraction. The consequences of physical disturbance to the top soil during stripping, stockpiling, and reinstatement cause unusually large N transformations and movements with eventually substantial loss. Management of top soil is important for reclamation plan to reduce the N losses and to increase soil nutrients and microbes. Revegetation constitutes the most widely accepted and useful way to reduce erosion and protect soils against degradation during reclamation. Mine restoration efforts have focused on N-fixing species of legumes, grasses, herbs, and trees. Metal tolerant plants can be effective for acidic and heavy metals bearing soils. Reclamation of abandoned mine land is a very complex process. Once the reclamation plan is complete and vegetation has established, the assessment of the reclaimed site is necessary to evaluate the success of reclamation. Evaluation of reclamation success focuses on measuring the occurrence and distribution of soil microflora community which is regulated by interactions between C and nutrient availabilities. Reclamation success also measures the structure and functioning of mycorrhizal symbiosis and various enzymatic activities in soil. This paper includes physical, chemical and biological mine soil properties, their management to make soil productive, top soil management, vegetation of various species and assessment of effectiveness of reclamation.

Keywords: mining, soil, reclamation, revegetation

1. INTRODUCTION

Land is one of the most important resources on which human beings depend. The rate of consumption of mineral resources is continuously increasing with the advancement of science and technology, economic development, industrial expansion, acceleration of

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urbanization and growth of population. Growth of our society and civilization thus heavily rely upon the mining industry to operate and maintain comfort. The end result for mining activities on the surface is mining wastes and alteration of land forms which is a concern to the society and it is desired that the pristine conditions are restored. Mine wasteland generally comprises the bare stripped area, loose soil piles, waste rock and overburden surfaces, subsided land areas, other degraded land by mining facilities, among which the waste rocks often pose extreme stressful conditions for restoration. The mining disrupts the aesthetics of the landscape along with it disrupts soil components such as soil horizons and structure, soil microbe populations, and nutrient cycles those are crucial for sustaining a healthy ecosystem and hence results in the destruction of existing vegetation and soil profile (Kundu and Ghose, 1997). The overburden dumps include adverse factors such as elevated bioavailability of metals; elevated sand content; lack of moisture; increased compaction; and relatively low organic matter content. Acidic dumps may release salt or contain sulphidic material, which can generate acid-mine-drainage (Ghose, 2005). The effects of mine wastes can be multiple, such as soil erosion, air and water pollution, toxicity, geo-environmental disasters, loss of biodiversity, and ultimately loss of economic wealth (Wong, 2003; Sheoran et al., 2008).

It is imperative from the above that the mineral extraction process must ensure return of productivity of the affected land. An increase in the concerns for environment has made concurrent post-mining reclamation of the degraded land as an integral feature of the whole mining spectrum (Ghose, 1989). Conservation and reclamation efforts to ensure continued beneficial use of land resources are essential. Reclamation is the process by which derelict or highly degraded lands are returned to productivity, and by which some measures of biotic function and productivity is restored. Long term mine spoil reclamation requires the establishment of stable nutrient cycles from plant growth and microbial processes (Singh et al., 2002, Lone et al., 2008; Kavamura and Esposito, 2010). Soil provides the foundation for this process, so its composition and density directly affect the future stability of the restored plant community. Restoration of vegetation cover on overburden dumps can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Wong, 2003). Reclamation strategies must address soil structure, soil fertility, microbe populations, top soil management and nutrient cycling in order to return the land as closely as possible to its pristine condition and continue as a self-sustaining ecosystem.

Ecological restoration and mine reclamation have become important parts of the sustainable development strategy in many countries. Good planning and environmental management will minimize the impacts of mining on the environment and will help in preserving eco-diversity. This article assesses the deterioration of chemical, physical and biological soil properties due to surface mining and also their management with a purpose to get productive mine soil. The article also assesses effectiveness of soil from mining waste in reclamation of mined degraded land for its sustainable and beneficial use. Discussion on post mining land use forms and control of soil pollution through acid-mine-drainage is beyond the scope of this paper. Readers are encouraged to refer studies presented elsewhere on these subjects, e.g. Sharma et al., (1996) for post mining land use and, Saharan et al., (1995) and Sheoran and Sheoran, (2006) for acid-mine-drainage management issues.

2. SOIL PROPERTIES IMPORTANT FOR PLANT GROWTH

2.1 Chemical Property

2.1.1 Soil pH

Soil pH is a measure of active soil acidity and is the most commonly used indicator of mine soil quality. The pH of a given mine soil can change rapidly as the rock fragments weather and oxidize. Pyritic minerals (FeS_2), when present, oxidized to sulfuric acid and drastically lower the pH, while carbonate (Ca/MgCO_3) bearing minerals and rocks tend to increase the pH as they weather and dissolve. Unweathered (or unoxidized) mine soils those contain a significant amount of pyritic-S in excess of their neutralizers (carbonates) will rapidly drop the pH to a range of 2.2 - 3.5 after exposure to water and oxygen. Vegetation achieves optimal growth in soil at a neutral pH. When the soil pH drops below to 5.5, reduced legume and forage growth occur due to metal toxicities such as aluminum or manganese, phosphorus fixation, and reduced population of N-fixing bacteria. This growth hence inhibits plant root growth and many other metabolic processes. A mine soil pH range in the range of 6.0 to 7.5 is ideal for forages and other agronomic or horticultural uses (Gitt and Dollhopf, 1991; Gould et al., 1996). Maiti and Ghose, (2005) reported that the pH vary from 4.9 to 5.3 in a mining dump site situated in Central Coalfield Limited's (CCL), North Karanpura area in the Ranchi district of Jharkhand State of India and thus indicated the acidic nature of the dumps. This acidic nature arose due to the geology of the rock presented in the area. It has been reported earlier that at pH less than 5, along with Fe, the bioavailability of toxic metal such as nickel, lead and cadmium also increases (Maiti, 2003).

2.1.2 Soil Fertility

The three major macronutrients, namely nitrogen, phosphorus and potassium are generally found to be deficient in overburden dumps (Coppin and Bradshaw, 1982; Sheoran et al., 2008). All newly created mine soils, and many older ones, will require significant fertilizer element applications for the establishment and maintenance of any plant community. Organic matter is the major source of nutrients such as nitrogen, and available P and K in unfertilized soils (Donahue et al., 1990). A level of organic carbon greater than 0.75% indicates good fertility (Ghosh et al., 1983). The level of organic carbon in overburden was found to be 0.35% to 0.85%. Organic carbon is positively correlated with available N and K and negatively correlated with Fe, Mn, Cu, and Zn (Maiti and Ghose, 2005). Initial applications of fertilizers have shown to increase the specific numbers, plants co-density and growth rates of vegetation.

Some of the important metallic micronutrients that are essential for plant growth are Fe, Mn, Cu, and Zn. These micronutrients are available in the soil due to continuous weathering of minerals mixed with primary minerals. These metals are more soluble in acidic solution, and they dissolve to form toxic concentrations that may actually hinder plant growth (Donahue et al., 1990; Barcelo and Poshenrieder, 2003; Das and Maiti, 2006). Maiti and Ghose, (2005) while working on restoration of acidic coal overburden reported that it is essential to increase the pH and organic matter content for sustainable reclamation of mining overburdens. During investigation it was found that locally available drought resistant, fast-growing trees able to grow in acidic nutrient deficient

soils increased the concentration of available Fe in all reclaimed dumps higher than 4.5 mg kg⁻¹, Mn with average value of 13 mg kg⁻¹, 9 to 42 mg kg⁻¹ for Zn, 0.32 to 1.22 mg kg⁻¹ for Cu. According to Lindsay and Norvell, (1978) if the concentration of micronutrients in the soil was higher than 4.5 mg kg⁻¹ for Fe, 1.0 mg kg⁻¹ for Mn, 1.0 mg kg⁻¹ for Zn and 0.4 mg kg⁻¹ for Cu, the values are rated as highly sufficient for ecological sustainable reclamation.

Type of fertilizer and its application rate will vary according to the site, soil type, and post mining land use (Kenny and Bremner, 1966). Care to be taken while preparing fertilizer prescription and applying on the rehabilitated areas. Roots of seedling can be damaged if the fertilizer is placed too close to the plant (Schmidt, 2003; Ghose, 2005).

2.2 Physical Properties

2.2.1 Rock Content

Soil particles those smaller than 2 mm are responsible for majority of water and nutrient holding capacity in the mine soils. Particles larger than 2 mm are referred to as "coarse fragments". Soils constituting high coarse fragments have larger pores that cannot hold enough plant available water against leaching to sustain vigorous growth over the summer months. The coarse fragment contents in a typical mine spoil vary (< 30- > 70%) due to differences in rock hardness, blasting techniques, and spoil handling. Particle size distribution of mine soils is directly inherited from their parent rocks or spoils. The rock content in the surface of a reclaimed bench or outcrops will decrease overtime due to weathering of rock fragments to soil sized particles. Top soil materials, when they can be salvaged, are typically much lower in rock content than spoils and therefore have better water retention characteristics (Nicolau, 2002; Moreno-de las Heras et al., 2008) Hu et al., (1992) are of the opinion that soil with stone content greater than 50% should be rated as poor quality. Stone content of coal mine overburden dumps has been reported to be as high as 80-85% (Maiti and Saxena, 1998). Maiti and Ghose, (2005) reported stone content in overburden dumps in range of 35%-65%, with an average value of 55%.

2.2.2 Soil Texture

Relative amount of sand (2.0 - 0.05 mm), silt (0.05 - 0.002 mm), and clay (< 0.002 mm) sized particles determine the texture of soil. Mine soils with sandy textures cannot hold as much water or nutrients as finer textured soils like loams and silts. The silts are finer textured soils and have a tendency to form surface crusts, often contain high level of soluble salts, and have a poor "tilth" or consistence. The particle size distribution of the soils with loamy textures is generally ideal. Silt loam textures are common where spoils are dominated by siltstones (Ghose, 2005). Ghose, (2005) reported the maximum sand content of 66% and clay only 8.6% in mined soil. Singh et al., (2004) and Singh and Singh, (2006) also reported maximum content of sand (80%) and least content of clay (11%) at the Singrauli Coal field India.

2.2.3 Soil Aggregation

Soil aggregation controls soil hydrology, affect soil diffusion and the degree of nutrient availability to the soil (Lindemann et al., 1984; Heras, 2009), and may reduce erosion

potential (Elkins et al., 1984), and constitutes a pathway of organic carbon stabilization and long term sequestration (Six et al., 2004). Aggregate structure breaks down as successive layers of soil are removed and stockpiled elsewhere on the site when mining begins. The resulting compaction reduces water holding capacity and aeration. Macro aggregate stability is largely responsible for macro porosity, which determines soil drainage rate and aeration; it changes seasonally and is often affected by cultivation and cropping regime (Kay, 1990). Micro-aggregate stability is more resilient than macro-aggregate stability as the organic matters responsible for binding the soil particles together reside in pores too small for microorganisms to occupy (Gregorich et al., 1989). Micro-aggregates are less sensitive to cropping practices than macro-aggregates (Dexter, 1988) and are responsible for crumb porosity which controls the amount of available water for vegetation (Davies and Younger, 1994).

2.2.4 Moisture, Bulk Density, Compaction and Available Rooting Depth

Moisture content in a dump is a fluctuating parameter which is influenced by the time of sampling, height of dump, stone content, amount of organic carbon, and the texture and thickness of litter layers on the dump surface (Donahue et al., 1990). During the winter, the average moisture content of 5% was found to be sufficient for the plant growth. During high summer (May-June), moisture content in overburden dumps was reported to be as low as 2-3% (Maiti et al., 2002). Maiti and Ghose, (2005) reported average field moisture content of all the dumps was 5%.

Bulk density of productive natural soils generally ranges from 1.1 to 1.5 g/cm³. High bulk density limits rooting depth in mine soils. In seven year old overburden dumps, the bulk density was found to be as high as 1.91 Mg m⁻³ (Maiti and Ghose, 2005). Bulk density in the soil under a grass sward in the UK has been found to be as high as 1.8 Mg m⁻³ (Rimmer and Younger, 1997). Soil compaction directly limits plant growth, as most species are unable to extend roots effectively through high bulk-density mine soils. Severely compacted (bulk density > 1.7 g/cc) mine soils, particularly those with less than two feet of effective rooting depth, shallow intact bedrock and the presence of large boulders in the soil simply cannot hold enough plant-available water to sustain vigorous plant communities through protracted drought. Three to four feet of loose non-compacted soil material is required to hold enough water to sustain plants through prolonged droughts. Compacted zones may also perch water tables during wet weather conditions, causing saturation and anaerobic conditions within the rooting zone. Repeated traffic of wheeled mining machineries (loaders and haulers), and bulldozers to a lesser extent, form compacted zones in the mining dumps.

2.2.5 Slope, Topography and Stability

Mine soils with slopes greater than 15% are generally unsuitable for intensive land uses such as vegetable or crop production, but they may be suitable for grazing and reforestation. Broad flat benches and fills with slopes less than 2% often have seasonal wetness problems. Many benches with an overall gentle slope contain areas of extreme rockiness, pits, hummocks, and ditches. Average slope of most reclaimed modern mines is quite a bit steeper than the older benches, but the newer landforms are considerably smoother and more uniform in final grade. Bench areas directly above intact bedrock on older mined lands are usually fairly stable but may be subject to slumping, especially

when near the edge of the out slope. Tension cracks running roughly parallel to the out slope indicate that the area is unstable and likely to slump. Decreased soil stability can lead to increase in bulk density because the matrix does not resist slaking, dispersion by water and the forces imparted by wheels, hooves and rainfall (Daniels, 1999). This, in turn, leads to decreased aeration and water infiltration rate and the development of anaerobic conditions. N losses by denitrification may follow under such environment (Davies and Younger, 1994).

2.2.6 Mine Spoil/Soil Color

Mining activities remove surface earth, piling it over unmined land and forming chains of external dumps i.e. mine spoil/ wasteland. Mine spoils possess very rigorous conditions for both plants and the microorganism culture. Biological functionality along with the nutrient cycle is disturbed leading to a non- functional soil system. This is mainly due to low organic matter contents and other unfavorable physico-chemical and microbiological characteristics (Singh and Singh, 1999; Jha and Singh, 1993; Singh and Singh, 2006). The color of a mine spoils or weathered mine soil can tell us much about its weathering history, chemical properties, and physical make up. Bright red and brown colors in spoils and soils generally indicate that the material has been oxidized and leached to some degree. These materials tend to be lower in pH and free salts, less fertile, low in pyrites, and more susceptible to physical weathering than darker colored materials. Gray colors in rocks, spoils and soils usually indicate a lack of oxidation and leaching and these materials tend to be higher in pH and fertility. Very dark gray and black rocks, spoils, and mine soils contain significant amounts of organic materials and are often quite acidic. Dark colored spoils are also difficult to re-vegetate during the summer months because they absorb a great deal of solar energy and become quite hot (Daniels, 1999). Natural succession process to recover this spoil may take hundreds of years.

2.2.7 Top soil

Top soil is used to cover poor substrate and to provide improved growth conditions for plants. Stockpiling of top soil in mounds during mineral extraction has been shown to affect the biological, chemical and physical properties of soil (Hunter and Currie, 1956; Barkworth and Bateson, 1964; Harris et al., 1989; Johnson et al., 1991; Davies et al., 1995). Top soil is a scarce commodity, and it is never stored in the majority of potential sources. Also, in a tropical climate where 90% of rainfall is precipitated within three months of the rainy season, storing of the top soil and preservation of soil quality remains problematic. Top soil is never stored for reuse; instead it is borrowed from nearby areas for the reclamation of the degraded mined-out areas. At a depth about 1m in the stockpile, the number of anaerobic bacteria increases where as those of aerobic bacteria decreases (Harris et al., 1989). This inhibits nitrification due to poor aeration within the stockpile leading to an accumulation of ammonia in the anaerobic zones. Once the soil is removed from the stockpile and reinstated, aerobic microbial population rapidly re-establishes, usually higher than the normal level (Williamson and Johnson, 1991) and nitrification restarts at higher than the normal rates. If high level of ammonia is present in a reinstated soil, the amount of nitrate generated is likely to be much greater than the normal. Consequently there is high potential for N loss to the environment via leaching or/and denitrification (Johnson and Williamson, 1994). Nitrate leached to water courses

is not only a threat to aquatic environment and drinking water supplies (Addiscott et al., 1991) but if nitrogen is lost from soil in the form of gaseous nitrogen or nitrous oxides; this will contribute the degradation of ozone layer (Isermann, 1994; Davies et al., 1995). The period between the initial removal of top soil and final laying of the same over the reclaimed area might have a long time lapse. Hence, properties of stockpiled soil continually deteriorate and ultimately become biologically non-productive if it is not preserved properly (Ghose, 2005).

2.3 Biological Properties

2.3.1 Soil Microbe

Soil microbe populations must be addressed deliberately as another soil component. It plays a major role in aggregate stabilization, which is important for maintaining suitable structural conditions for cultivation and porosity for crop growth (Ghose, 2005). Their activity declines when soil layers are disrupted and is slow to resume independently. Soil microbes include several bacterial species active in decomposition of plant material as well as fungal species whose symbiotic relationship with many plants facilitates uptake of nitrogen and phosphorus in exchange of carbon. They produce polysaccharides that improve soil aggregation and positively affect plant growth (Williamson and Johnson, 1991). Sites with an active soil microbe community exhibit stable soil aggregation, whereas sites with decreased microbial activity have compacted soil and poor aggregation (Edgerton et al., 1995). Microbial activity decreases with depth and time as topsoil continues to be stored during mining operations (Harris et al., 1989). Microbial activity, measured in ATP (adenosine tri phosphatase) concentrations, plummets to very low levels within a few months. Response to glucose is slower by microbes at all depths, suggesting that metabolic rates decrease with time (Visser et al., 1984).

2.3.2 Bacteria

Bacteria play an important role in decomposition of organic materials, especially in the early stages of decomposition when moisture levels are high. In the later stages of decomposition fungi tend to dominate. Rhizobia are single celled bacteria, belongs to family of bacteria Rhizobiacea, form a mutually beneficial association, or symbiosis with legume plants. These bacteria take nitrogen from air (which plant cannot use) and convert it into a form of nitrogen called ammonia (NH_4^+) used by plants (Gil-Sotres et al., 2005). Free living as well as symbiotic plant growth promoting rhizo-bacteria can enhance plant growth directly by providing bioavailable P for plant uptake, fixing N for plant use, sequestering trace elements like iron for plants by siderophores, producing plant hormone like auxins, cytokinins and gibberlins, and lowering of plant ethylene levels (Glick et al., 1999; Khan, 2005).

When soil layers are removed and stockpiled, the bacteria inhabiting the original upper layers end up on the bottom of the pile under compacted soil. A flush of activity occurs in the new upper layer during the first year as bacteria are exposed to atmospheric oxygen. After two years of storage there is little change in the bacterial numbers at the surface, but less than one half the initial populations persist at depths below 50 cm (Williamson and Johnson, 1991).

2.3.3 Mycorrhizal Fungi

Arbuscular mycorrhiza fungi are ubiquitous soil microbe occurring in almost all habitats and climates. The hypha network established by mycorrhizal fungi breaks when soils are initially moved and stockpiled (Gould et al., 1996). It is well documented that mycorrhizal associations are essential for survival and growth of plants and plant uptake of nutrient such as phosphorus and nitrogen, especially P deficient derelict soils (Khan, 2005). An important arbuscular mycorrhiza genus is *Glomus*, which colonize a variety of host species, including sunflower (Marschner, 1995). Dual inoculation with *Trichoderma koningii* and AM fungi increased plant growth of *Eucalyptus globulus* under heavy metal contamination conditions (Arriagada et al., 2004, 2005).

There is a little decrease in viable mycorrhizal inoculum potential during the first two years of storage (Miller et al., 1985). Viability of mycorrhizas in stored soils decreases considerably and possibly to the levels 1/10 those of the undisturbed soil (Rives et al., 1980). Miller et al., (1985) indicate that soil water potential is a significant factor affecting mycorrhizal viability. When soil water potential is less than -2 MPa (drier soil), mycorrhizal propagules can survive for greater lengths of storage time; when soil water potential is greater than -2 MPa, length of storage time becomes more important. In drier climates, deep stockpiles may not threaten mycorrhizal propagule survival. In wetter climates, shallow stockpiles are more important to maximize surface-to-volume ratios with regard to moisture evaporation.

3. MANAGEMENT OF THE PRODUCTIVE MINE SPOIL

3.1 Rebuilding Soil Structure

The first soil component addressed during reclamation is the structure of the soil itself as it is replaced onto the reclamation site. Soil structure includes soil aggregation, or the way in which soil particles are held together, and the size of the particles comprising the layers at different depths. The degree to which soil is loosely constructed versus compacted can be altered during reclamation by the method of replacement adopted (Visser et al., 1984). Using a tyre mounted mining machine (scrapers) than crawler mounted (dozers) to dig stored soil can minimize compaction. Transporting soil from the stockpile to the reclamation site on a conveyor belt with trundling action improves soil structure by breaking up massive aggregates. As smaller aggregates continue to tumble, they tend to acquire an agglomerative skin of fine particles, which promotes loose soil structure. Loosely constructed, or "fritted", subsoil is very important to plant root systems. The extent of the root system determines a plant's ability to maximize its surface area and access a greater volume of water and soil nutrients. Plants grown in fritted subsoil have root patterns with extensive vertical and lateral penetration. Rock contents in the surface of a reclaimed bench or out-slope will decrease over time due to weathering of rock fragments to soil sized particles and therefore have better water retention characteristics. Gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) has traditionally been used to improve sodic media for plant growth (Richards, 1954). It can be used to improve the structure of poorly structured sodic soils. Gypsum is normally incorporated into soil at about 5-10 tonnes/ha. Application of gypsum results in replacement of sodium with calcium on the soil exchange surfaces, which can improve the soil structure, reduce surface crusting and

increase water infiltration. It may also reduce the pH of sodic soils (soil with $\text{pH} > 8.5$) (Ghose, 2005). An exchangeable sodium proportion of greater than 6% can indicate an unstable soil structure.

3.2 Management of Soil pH

Acidic mine soils can be effectively neutralized once they have been again spread at the reclamation site by applying either cement kiln dust (CaO) or limestone (CaCO_3). Lime application rates must account for both past and future pyrite oxidation in order to maintain neutral soil pH levels over time. Lime addition is a common method to decrease the heavy metal mobility in soils and their accumulation in the plant as it increases the pH of soil. Plants like *Gravellia robusta*, can be planted at acidic dumps (pH 3.6-3.9), which increases the soil pH (Gitt and Dollhopf, 1991). Organic amendments such as woodchips, composted green waste or manure, biosolids etc also increases the soil pH, in addition improves soil structure, water holding capacity, cation exchange capacity, provide a slow-release fertilizer and serve as a microbial inoculum (Tordoff et al., 2000; Jordan et al., 2002).

3.3 Increase Soil Fertility

Areas reclaimed for agriculture or other intensive use will normally require maintenance of the fertilizer programmed. There are also certain amendments which have shown promise for improving spoil as a plant growth medium. Saw dust has been shown to increase the survival rates of certain trees, forbs and shrubs (Uresk and Yamamoto, 1986). Smith et al., (1985) observed that the addition of woodchips to bare spoils was second only to topsoil application for increasing plant establishing and their growth. Gitt and Dollhopf, (1991) observed similar results when wood residue had been used as a spoil amendment. Amendment with wood residue with N increases the effects of fertilizers such as N, P, K or gypsum while amendments with gypsum increases the level of soluble salts (Voorhees and Uresk, 1990; Sheoran et al., 2009).

Majority of N needed to supply plant/soil community comes from N-fixation and subsequent mineralization of organically combined N. Therefore, maintenance of a vigorous legume component within the plant community is critical for reclamation success. Most mine soils do not contain native populations of the essential N-fixing Rhizobium bacteria those enable legumes to capture atmospheric N, so care must be taken to carefully inoculate all legume seeds used in new plantings. Since N is primarily combined in organic matter in soils, the addition of organic amendments to the soil can greatly enhance total soil N and its availability over time. Sewage sludge has been shown to be an effective mine soil amendment in numerous studies, but it may not always be available in sufficient quantities for use on remote sites. Local and state regulations and community attitudes frequently complicate the use of sewage wastes on disturbed lands. Sawdust and bark mulch are also helpful in increasing the initial mine soil organic matter contents but are generally low in N content. Saw dust and sewage sludge have been widely recognized as effective short-term fertilizers and sources of long term slow release nitrogen (Sydnor and Redente, 2002; Munshower, 1994; Hall, 1984), besides serving as microbial inoculums. In addition, organic matter improves soil structure, reduces erosion, and increases infiltration. Furthermore, organic wastes can increase the water holding capacity of minespoils. Therefore, use of these materials as soil amendments will also

require heavy fertilization with N- fertilizer. The maintenance of plant available phosphorus (P) in mine soils over time is hindered by two factors: (i) fresh mine spoils are generally low in readily plant available (water soluble) P; (ii) as mine soils weather and oxidize they become enriched in Fe-oxides that adsorb water soluble P which is then "fixed" into unavailable forms. The tendency of mine soils to fix P increases over time. Because organic bound P is not subject to P-fixation, it is critical to establish and build an organic-P reservoir in the soil to supply long-term plant needs through P-mineralization. Large fertilizer applications of P during reclamation will insure that sufficient P will be available over several years to support plant growth and to build the organic-P pool. Some P will also become available to the plant community as native calcium phosphates in the rocks decompose, but this P is not sufficient to meet the needs of a vigorous plant community. Some species, particularly from the family Protease, are reported to be adversely affected by application of P-fertilizers. These adverse affects are likely to be seen principally on sandy soils, and are less likely to occur on finer soils with a greater capacity to adsorb P. The long term productivity of the plant/soil system is dependent upon several major factors: (i) accumulation of soil organic matter and N; (ii) maintaining N-fixing legumes in the sand; and (iii) establishment of an organic-P pool and avoidance of P-fixation (Daniels, 1999; Ghose, 2005).

3.4 Recharging Soil Microbes

3.4.1 Bacteria

In one study, amending replaced topsoil with hay and processed sewage sludge was more effective than topsoil inoculation in stimulating bacterial growth and activity, particularly for bacteria that oxidize ammonia (Lindemann et al., 1984). Bacteria present in the soil require a source of readily oxidizable carbon provided by the hay and sludge to fuel metabolic activity and stimulate nitrogen cycling. Topsoil contains carbon, but it is often in the form of coal or other humic material mixed during soil replacement and is not readily usable (Moynahan et al., 2002).

3.4.2 Mycorrhiza

Mycorrhizal propagule densities remain low immediately after reclamation on uninoculated sites, but re-establish themselves after couple of years (Williamson and Johnson, 1991). This coincides with the appearance of host plants, such as tall fescue, that are more conducive to mycorrhizal colonization than those first appearing on the site (winter wheat) (Gould et al., 1996; Gould and Hendrix, 1998). Mycorrhizal propagules existing in the topsoil may be stimulated by the presence of suitable host plants. Lindemann et al., (1984) found that covering re-spread soils with 30 cm of topsoil (without mycorrhizal inoculum) also stimulated host colonization by mycorrhizal fungi whereas using hay, topsoil with inoculum, or sewage sludge had no effect. Sewage sludge may suppress mycorrhizal development by increasing the phosphorus available to host plants (Daft and HacsKaylo, 1976). Soil microbe population persists in stored soil and can be stimulated during reclamation by charging the system with a source of organic carbon or by adding suitable host plants. Many plant species, particularly those that are mycorrhizal (e.g. *Sericea lespedeza*), are able to draw P from difficultly available sources.

Managing the microbial population in the rhizosphere - by using an inoculum consisting of a consortium of plant growth promoting rhizobacteria, mycorrhiza-helping bacteria, N-fixing rhizobacteria, and arbuscular mycorrhizal fungus as allied colonizers and biofertilizers; could provide plants with benefits crucial for ecosystem restoration. It is important to use indigenous arbuscular mycorrhizal fungus strains which are best adapted to actual soil and climatic conditions to produce site-specific arbuscular mycorrhizal fungus inocula (Mummey et al., 2002b; Khan, 2004).

3.5 Re-establishing Nutrient Cycles

Nutrient cycling is very closely linked to soil microbe activity. It is the process by which carbon, nitrogen, and phosphorus are reused within an ecosystem due to the metabolic activity of plants and soil microbes. Carbon and nitrogen cycles in particular are disrupted as soil microbe populations decline and must be re-established during reclamation.

3.5.1 Carbon Cycle

Organic carbon fuels the metabolic activity of many soil microbes. Microbes obtain carbon through their symbiotic relationships with suitable host plants or from organic carbon available in the soil resulting from decomposition of plant and animal matter. Removal of topsoil from a mining site and mixing it with underlying soil considerably reduces the relative proportion of organic carbon (Visser et al., 1984). Little additional change in this proportion results from extended storage of soil.

Researchers frequently found the amount of organic carbon to be the limiting factor in stimulating microbial metabolic activity (Williamson and Johnson, 1991). Amending soil with bark (Elkins et al., 1984) or fertilizing and planting ryegrass (Williamson and Johnson, 1991) provides bacteria with enough organic carbon to stimulate metabolic activity, which can be measured by increased microbial carbon. Plant like *Dalbergia sissoo* improves the field moisture content (7%), pH (5.5), organic carbon (85%), and NPK. The increase in organic carbon level is due to the accumulation of leaf litter and its decomposition to form humus (Maiti and Ghose, 2005).

3.5.2 Nitrogen Cycle

Soil organic matter has a very important influence on soil physical and chemical properties, on biological activities, and as a source of plant nutrients, especially nitrogen. Nitrogen in organic form is converted by microorganisms into ammonium (NH_4^+). Under certain conditions specific microbes in the soil use ammonium N in the soil for energy and in doing so oxidize ammonium $\text{N}(\text{NH}_4^+)$ first into nitrite $\text{N}(\text{NO}_2^-)$ and then into nitrate $\text{N}(\text{NO}_3^-)$ which plants can then use to grow, a process referred to as nitrification. Some of that nitrogen is taken in by plants in that area, and some of it escapes into the atmosphere. Free-floating atmospheric nitrogen can in turn be “fixed” by plants which will eventually be eaten or die, starting the cycle all over again. Amending the stockpiled soils with 15 cm topsoil during re-spreading stimulates nitrification and reduces leaching. Davies et al., (1995) reported that during the first two years after reclamation, nitrification rates in reclaimed sites were less than those in undisturbed sites, but approached levels similar to undisturbed sites after two years.

Nutrient recycling and availability on reclaimed sites is reflected in part by the rate of decomposition of plant material. Litter decomposition in mined land versus unmined land is often retarded during the initial months after reclamation (Lawrey, 1977). Presence of heavy metals which reduce soil pH and lack of an existing litter layer create an unfavorable microclimate for soil microbes responsible for breaking down organic matter. Decomposition rates begin to equalize after six months suggesting increased microbial activity, but the initial death of recycled nutrients could impede establishment of new plants. Elkins et al., (1984) demonstrated that amending mine spoils with bark rather than topsoil significantly increases soil microbe activity and consequently decomposition rates but results in less available NO₃ than in the spoil which is not amended. Oxidation of soil nitrogen to NO₃ may be impeded by acidic soils or by the time length required by certain bacteria to become established.

3.6 Top Soil Management

The top soil is severely damaged if it is not mined out separately in the beginning with a view to replace it on the filled void surface area for reclamation in order to protect the primary root medium from contamination and erosion and hence its productivity (Kundu and Ghose, 1998a, b). Sendlein et al., (1983), however, indicate that systematic handling and storage practice can protect the physical and chemical characteristics of top soil while in storage and also after it has been redistributed into the regarded area. Ghose, (2005) advised to avoid topsoil storage, especially in long term, for a time length by which the mine spoil can not maintain its sustainability for suitable plant growth without biological reclamation and also, maintenance of growth of aerobic bacteria. The following steps are; however, need to be followed for keeping the soil in good condition if storage is unavoidable:

(a) The surface of the stockpile should be thoroughly ripped with suitable sub-soiling machinery for the purpose of

- Relieving surface compaction caused by the passage of scrapers and other machines.
- Aeration of soil.
- Encouragement of deep-rooting plants by introduced vegetation.

(b) Following ripping, the heap should be cultivated with suitable low-maintenance species (like dwarf grasses) immediately to prevent erosion and gully formation.

(c) The surface vegetation should be actively maintained with seedling and weed control operations.

After final grading and before replacement of the top soil, slippage surface should be eliminated to promote root penetration. Top soil should be redistributed in a manner that achieve an approximate uniform and stable thickness consistent with the approved post mining land uses, contours, and surface water drainage system. It prevents excess compaction of top soil and protects it from wind and water erosion. It is of greater importance than any other factor in achieving successful reclamation of surface mined land. The top soil must be uniformly redistributed in a manner which assures placement and compaction compatible with the needs of the species those will be used to restore the distributed area to its pre-mined potential (Ghose, 2005). Nitrogen losses can be reduced

by preventing the development of anaerobic conditions in the soil mound. Soil storage is for very short periods, periodically opening up and aerating the soil while stockpiled or permanently aerating, allowing drainage with a network of pipes and use of nitrification inhibitors after restoration are the operations that may in part ameliorate the problem (Davies et al., 1995). Vast majority of surface mines today employ some form of controlled overburden placement techniques and utilize top soil substitutes derived from blasted mine spoil materials. This occurs because natural soils tend to be thin, rocky, acidic, and infertile often making it impractical to salvage and re-spread topsoil on surface mined areas. The plant species used in active reclamation therefore are grown in mine spoils composed of freshly blasted overburden materials. The properties of these mine spoils are directly controlled by the physical and geochemical properties of the rock strata from which they are derived (Nagle et al., 1996; Daniels, 1999). Sydnor and Redente, (2002) reported that topsoil if amended with addition of organic wastes increased above ground biomass influence trace element uptake. Even waste rock if properly neutralized, fertilized and amended with organic matter could also be directly revegetated.

4. RE-VEGETATION AT ABANDONED MINE LAND

Vegetation has an important role in protecting the soil surface from erosion and allowing accumulation of fine particles (Tordoff et al., 2000; Conesa et al., 2007b). They can reverse degradation process by stabilizing soils through development of extensive root systems. Once they are established, plants increase soil organic matter, lower soil bulk density, and moderate soil pH and bring mineral nutrients to the surface and accumulate them in available form. Their root systems allow them to act as scavengers of nutrients not readily available. The plants accumulate these nutrients redeposit them on the soil surface in organic matter from which nutrients are much more readily available by microbial breakdown (Li, 2006; Conesa et al., 2007a; Mendez and Maier, 2008a).

The revegetation of eroded ecosystems must be carried out with plants selected on the basis of their ability to survive and regenerate or reproduce under severe conditions provided both by the nature of the dump material, the exposed situation on the dump surface and on their ability to stabilize the soil structure (Madejon et al., 2006). Normal practice for revegetation is to choose drought-resistant, fast growing crops or fodder which can grow in nutrient deficient soils. Selected plants should be easy to establish, grow quickly, and have dense canopies and root systems. In certain areas, the main factor in preventing vegetation is acidity. Plants must be tolerant of metal contaminants for such sites (Caravaca et al., 2002; Mendez and Maier, 2008b).

Role of exotic or native species in reclamation needs careful consideration as newly introduced exotic species may become pests in other situations. Therefore, candidate species for vegetation should be screened carefully to avoid becoming problematic weeds in relation to local to regional floristic. For artificial introduction, selection of species that are well adapted to the local environment should be emphasized. Indigenous species are preferable to exotics because they are most likely to fit into fully functional ecosystem and are climatically adapted (Li et al., 2003; Chaney et al., 2007).

Grasses are considered as a nurse crop for an early vegetation purpose. Grasses have both positive and negative effects on mine lands. They are frequently needed to stabilize

soils but they may compete with woody regeneration. Grasses, particularly C4 ones, can offer superior tolerance to drought, low soil nutrients and other climatic stresses. Roots of grasses are fibrous that can slow erosion and their soil forming tendencies eventually produce a layer of organic soil, stabilize soil, conserve soil moisture and may compete with weedy species. The initial cover must allow the development of diverse self-sustaining plant communities (Shu et al., 2002; Singh et al., 2002; Hao et al., 2004).

Trees can potentially improve soils through numerous processes, including - maintenance or increase of soil organic matter, biological nitrogen fixation, uptake of nutrients from below and reach of roots of under storey herbaceous vegetation, increase water infiltration and storage, reduce loss of nutrients by erosion and leaching, improve soil physical properties, reduce soil acidity and improve physical properties, reduce soil acidity and improve soil biological activity. Also, new self-sustaining top soils are created by trees. Plant litter and root exudates provide nutrient-cycling to soil (Pulford and Watson, 2003; Coates, 2005; Padmavathiamma and Li, 2007; Mertens et al., 2007).

On mine spoils, nitrogen is a major limiting nutrient and regular addition of fertilizer nitrogen may be required to maintain healthy growth and persistence of vegetation (Yang et al., 2003; Song et al., 2004). An alternative approach might be to introduce legumes and other nitrogen-fixing species. Nitrogen fixing species have a dramatic effect on soil fertility through production of readily decomposable nutrient rich litter and turnover of fine roots and nodules. Mineralization of N-rich litter from these species allow substantial transfer to companion species and subsequent cycling, thus enabling the development of a self-sustaining ecosystem (Zhang et al., 2001). Singh et al., (2002) reported that native leguminous species show greater improvement in soil fertility parameters in comparison to native non-leguminous species. Also, native legumes are more efficient in bringing out differences in soil properties than exotic legumes in the short term.

5. DETERMINING EFFECTIVENESS OF SOIL RECLAMATION

Some assessment should be made to determine once the reclamation plan is complete and vegetation has established. It is to determine how closely the reclaimed site functions, in comparison to similar undisturbed sites, as an ecosystem. Reclamation of abandoned mine land is a very complex process. Most researchers agree that reclamation success must be measured by more than the presence of vegetation on the site. Several parameters must be considered in order to determine the state and functionality of the soil system since no individual parameter provides sufficient information for ecosystem reclamation. Both the activation of basic soil biological processes and the rearrangement of soil particles into stable aggregates are key factors related to the soil functionality (Filip, 2002; Sourkova et al., 2005; Heras, 2009).

Bentham et al., (1992) developed a three-dimensional system measuring ATP, dehydrogenase activity, and ergosterol to classify habitats based on microbiological and physico-chemical characteristics. While their entire dataset include other factors, such as soil moisture content, type of ecosystem, restored versus undisturbed site, they found that using the selected three-dimensional system allowed distinction of different habitats. The results can then be used in conjunction with reference databases of undisturbed sites to evaluate success of restoration.

Microbial activity is a key factor affecting the functioning of all terrestrial systems. It has an important role in decomposition and nutrient cycling. Measurement of process rates governed by the soil microflora and general metabolic activities of these organisms is used to evaluate the reclamation efforts (Mummey et al., 2002a; Izquierdo et al., 2005). Edgerton et al., (1995) found a positive linear correlation between soil aggregate stability and microbial biomass carbon suggesting that measuring the productivity of the microbial community leads to reasonable assumptions about the quality of soil structure. Further, it was suggested that evaluating soil microbe populations and their metabolic activity may be utilized to determine the stability of a restored ecosystem.

A mycorrhiza is a mutualistic association between plants and fungi that affects all terrestrial communities. By affecting the success of individual plants, the association may play a role in the success of reclamation efforts by their presence (improving the growth and fitness of desirable species) or in failure by their absence. Several methods currently are used to assess mycorrhizal activity. These include both direct and indirect methods. Bioassays of soils for mycorrhizal fungi have been commonly used for a long time. There are two indirect techniques for quantifying mycorrhizal activity based on bioassays for mycorrhizal fungi. These have primarily been used to test soils prior to planting to estimate the potential for recovery of mycorrhizae. Mycorrhizal inoculum potential (MIP) is used as a mean to determine the potential for mycorrhizae to reestablish following a disturbance. Another procedure is called most probable numbers estimate (MPN) of mycorrhizal fungal densities. In both types of procedures known amount of test soil is mixed into a standard, sterile soil and seeded with a given mycorrhizal plant. After a known period, the plant is harvested and the number of propagules (MPN) or mycorrhizal inoculum potential (MIP) estimated by the percentage of root length infected by mycorrhizal fungi (for VA mycorrhizal) or by the proportions of root tips infected (ectomycorrhizae). Direct method involves determining the percentage of the root length containing VA mycorrhizae or the percentage of root tips that are ectomycorrhizae using plants collected from the field at different times following the replacement of the growth medium (Allen and Friese, 1992).

Soil enzymes activities have been used as sensitive indicators for reflecting the degree of quality reached by a soil in the reclamation process (Caravaca et al., 2003). A direct measurement of the microbial population is the dehydrogenase activity. Dehydrogenase is an oxidoreductase, which is only present in viable cells. This enzyme has been considered as a sensitive indicator of soil quality in degraded soils and it has been proposed as a valid biomarker to indicate the changes in soil management under different agronomic practices and climates. Measurement of soil hydrolases provides an early indication of changes in soil fertility since they are related to the mineralization of such important nutrient elements as N, P and organic carbon (Ceccanti et al., 1994).

6. CONCLUSION

Reclamation is an essential part in developing mineral resources in accordance with the principles of ecologically sustainable development. The goal of surface mine reclamation is to restore the ecological integrity of disturbed areas. Revegetation constitutes the most widely accepted and useful way of reclamation of mine spoils to reduce erosion and protect soils against degradation. The revegetation must be carried out with the plants

selected on the basis of their ability to survive and regenerate in the local environment, and on their ability to stabilize the soil structure. Revegetation facilitates the development of N-fixing bacteria and mycorrhizal association, which are fundamental for maintaining the soil quality by mediating the processes of organic matter turnover and nutrient cycling.

Reclamation of overburden dumps can be managed effectively once the chemical, physical and biological properties of soil have been correctly determined. Compaction, low water holding capacity, bulk density, deficiency of micro and macro nutrients and associated rooting restrictions are the major factors limiting the productivity of mine soils. High level of potential acidity (low pH) severely restricts the productivity of some mine soils but this problem is much more limited in extent than mine soil compaction. Stockpiling of top soil not only decreases the microbial activity but also disturbs the structure of soil. Top soil is an essential component for land reclamation in mining areas. Stockpiling should systematically handle and store the top soil so that its physical and biological characteristics can be protected. Productive topsoil substitutes can be generated from hard rock overburden or fresh soil, but care must be taken in selection and placement. Productivity of soil can also be increased by adding various amendments such as hay, saw dust, bark mulch, wood chips, wood residues, sewage sludge, animal manures as they stimulate the microbial activity (bacteria and mycorrhiza), which provides the nutrients (N, P) and organic carbon to soil. Acidic dumps can be restored by planting the metal tolerant plants, which can grow in nutrient deficient soil with elevated metal content. Planting of different grass, trees species, rotating with legumes and native species because of their adaptation to deficiency of nutrients and fast growing traits, shall be able to restore the soil fertility and accelerate ecological succession. Once the abandoned mine lands have vegetation growing on the surface, the regeneration of these areas for productive use will begin and offsite damages will be minimized. In addition, establishment of vegetation also improves the aesthetics of the area.

Reclamation of overburden dumps is not an operation, which should be considered only at, or just before mine closure. Rather, it should be a part of an integrated program of an effective environmental management through all phases of resource development - from exploration to construction, operation, and closure. Mining organizations are developing the expertise to reassemble the species that have chance to grow, develop, and rebuild the local biodiversity. They are achieving this through careful attention to all aspects of reclamation and revegetation: from initial planning, clearing, soil removal, storage and replacement through species selection and re-establishment of vegetation with its associated organism; to maintenance of areas for future. The initial vegetation efforts must establish the building blocks for a self-sustaining system so that successive processes lead to the desired vegetation complex. The best time to establish vegetation is determined by the seasonal distribution and reliability of rainfall. All preparatory work must be completed before time when seeds are most likely to experience the conditions, which are needed for germination and survival, that is, reliable rainfall and suitable temperature.

Reclamation must go beyond planting a new landscape by considering the land as an integrated system that function above and below the ground. Researchers have demonstrated techniques that appear successful over periods of several years and have indicated that there is much more to learn about their long-range effects.

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Restoration and revegetation strategies for degraded mine land for sustainable mine closure

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Abstract

Several changes occur in the physical, chemical and microbiological properties of soil as a result of mining and storage. The inability to preserve topsoil is one of the basic obstacles to the restoration and revegetation of degraded mined land. This paper examines the problem of preserving mine soil, and focuses on the restoration and revegetation of degraded land for effective mine closure. Systematic handling and storage practices can protect topsoil while in storage, and after it has been redistributed on to the regraded area. Large areas of land in India remain unproductive, despite efforts to grow vegetation on degraded mined land. The reasons for this are not completely understood. A fact-finding survey was conducted and the significance of the shelf-life concept is discussed. The study reveals that soils show a continuous decrease in quality, and ultimately become biologically sterile. This paper concludes that biological reclamation is essential if the soil is to be stored beyond the shelf-life period. A strategy for the restoration and revegetation of mine-degraded land has been proposed for sustainable mine closure, and the preservation of stockpiled topsoil, its redistribution on the regraded areas, and the nutrients and amendments to be added, are discussed in this paper.

Key words: topsoil, preservation, shelf-life, stockpiling, sterility, reclamation

INTRODUCTION

The mining industry in India has embraced the concept of sustainable development. Sustainable development aims to meet the needs of society today, while conserving ecosystems for the benefit of future generations. Restoration is the process by which the impacts of mining on the environment are repaired. It is an essential part of developing mineral resources in accordance with the principles of sustainable development. Increasingly, today, the environmental protection sought by mining operators includes the maintenance of biodiversity. Biodiversity, or biological diversity, is the full variety of living forms – plants, animals and micro-organisms – their genetic make-up, the different species and the ecosystems of which they are part. Good plan-

ning and environmental management will minimise the impacts of mining on the environment and will help to preserve this diversity. This is particularly important where there may be potential impacts on rare or endangered species of plants or animals. Mining is a temporary land use, which should be integrated with, or followed by, other forms of land use. Restoration of mines should be directed towards a clearly defined future land use for the area.

The mineral extraction process must ensure the return of the productivity of the affected land. With rising environmentalism, concurrent post-mining reclamation of the degraded land has become an integrated feature of the whole mining spectrum (Ghose 2001). Conservation and reclamation efforts to ensure continued beneficial use of land resources are essential. Open-cast mining severely disturbs land in and around mining areas. In this respect the Indian coal mining scenario is poor. The situation becomes clearer when the cumulative effects of coal production are taken into consideration. Ghosh (1990) reported that every million tonnes of coal extracted by surface mining in India damages a surface area of about 4 ha. It has been estimated that in

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1994–95 the coal industry was responsible for rendering an area of about 500 ha a year biologically unproductive, and that this had risen to 1400 ha a year by 2000 (Chari *et al.* 1989).

In the process of opencast mining, several changes occur in the physical, chemical and microbiological properties of soils (Kundu and Ghose 1998). Some are caused by the construction of the storage rather than during the course of storage (Sendlein *et al.* 1983). Topsoil is an essential component in abandoned mines for vegetation growth, and has to be preserved for post-mining land reclamation (Kundu and Ghose 1994). It should be noted that there might be a long period between the initial removal of the topsoil and its final laying on the reclaimed area (Kundu and Ghose 1997a). Therefore, the stockpiled soil may deteriorate and ultimately become biologically sterile. As these soils will be utilised for the renewal of degraded land, it is essential to evaluate the continual degradation of the soil properties over time. The issue is the preservation of topsoil (stockpiling) in the restoration of mined land. This problem is very acute in India, and large areas are continually being rendered unproductive every year (Kundu and Ghose 2000). Renewal of degraded land has become a great problem for the Indian mining industry. Efforts to grow vegetation on damaged land as a part of biological reclamation have so far been unsuccessful. Degradation of soil quality depends on climatic conditions and various other factors. If the biological reclamation is not done in good time, leaching will remove the nutrients released by microbiological activity and rainwater erosion, the nutrient cycle will break down, and the soil will ultimately become bio-

logically unproductive. The objective of the study is to assess the deterioration of soil properties due to mining and stockpiling, and also to evaluate the restoration techniques for the renewal of the damaged land for its sustainable and beneficial use.

This paper examines the revegetation stage of restoration and focuses on the strategy for restoration and revegetation for sustainable mine closure. Successful restoration to a low-maintenance land use such as a native ecosystem, which is sustainable in the long term, requires an understanding of the basic concepts of soil development, plant succession and species diversity. Restoration aims to accelerate the natural successional processes so that the plant community develops in the desired way. The vegetation must be resilient to disturbance, especially fire, and nutrient cycling, and natural inputs must meet the demand for nutrients. Planning is the key to successful restoration. The restoration plan is an integral part of the mine plan, and, where possible, progressive restoration is preferable to rehabilitating the whole area only after mining is completed.

DAMAGE TO LAND CAUSED BY COAL MINING

Coal mining started in the Raniganj Coalfield (RCF) area in 1774 during the British East India period. In fact it was the first source of coal in India. Until the 1970s, mining was mainly underground, with no significant opencast operations. The underground mining did not involve the use of much machinery. Mechanised opencast mining began during the 1970s, particularly after

Table 1. Present use of damaged land

Cause of damage	Nature of damage	Area affected (ha)	Present use of land	Possibility of reclamation
Subsidence due to underground working	Lowering of land up to 0.7 m	4582	Afforestation, fallow or wasteland	4102 ha
Open excavation due to opencast mining	Remaining as void	402	Wasteland, water reservoir	Will be reclaimed
Waste dump from opencast mining	—	200–350	Wasteland	Major part will be reclaimed
Surface fires	—	1000	Wasteland	Can be reclaimed
Unstable ground due to underground collapse	—	408	Afforestation, fallow and wasteland	—

Source: Anon. 1988

Table 2. Pre-mining land use patterns of the project and land affected by mining activities

Pre-mining land-use pattern Class of land	Total area (ha) A = A ₁ + A ₂	Land (ha) affected by mining activities	
		Directly affected (mining only) A ₁	Indirectly affected (industrial site, overburden dump, township, etc.) A ₂
1. Agriculture	1873.81	1171.67	702.14
2. Forest	110.56	109.91	0.65
3. Danga (cultivable waste)	113.05	85.25	27.80
4. Village	46.15	24.38	21.77
5. Water body	13.70	5.87	7.83
6. Others (road, nullah, etc.)	19.73	9.70	10.03
Total	2177.00	1406.78	770.22

Source: Anon. 1987

nationalisation. There has been degradation of land due to subsidence, surface collapse and fires caused by hazardous mining activities.

The Raniganj coalfield covers an area of 1530 sq. km, containing about 1306 sq. km of coal-bearing land. There are about 117 coal mines and about 60 sq. km of the coalfield have been damaged by mining operations. The total estimated coal production is 950 Mt. Of these mines, twelve are opencast, and 105 underground. In 1987–88 these mines produced 12.17 Mt and 15.84 Mt of coal respectively. Some 61.66 Mt/y of coal was produced by 2000, by which time 14 000 ha of land in the RCF had been damaged. Of the 60 sq. km of damaged land, not all is damaged beyond reclamation. This may be seen from Table 1.

Several large mechanised opencast mines are due to come into operation within two to three years. Also, future extraction of coal deposits from the underground mines will cause subsidence. Furthermore, the RCF contains about ten seams lying to a depth of 1200 m and over. Currently, mining is largely confined to depths of 300–700 m, and confined to the exploitation of the top few seams. In the eastern and central parts of the coalfield, the underlying seams are mostly virgin. Therefore it may not be possible to restore the entire coal belt to its pre-mining condition, although in all such coal projects land reclamation has been provided for to restore the land to this condition. Of 45–92 sq. km, about 42.02 sq. km, chiefly in the western part of the coalfield, may be restored.

Opencast mining severely disturbs land in and around mining areas. Opencast mining is progressively accounting for a larger share of India's coal production. Coal production from opencast mining increased from 14 Mt (20% of the total) at the time of coal mine nationalisation in 1971–72, to 170 Mt (68%) in 1994–95, and increased further to about 250 Mt (70%) by 2000 (Ghose 1996). The area damaged by opencast mining depends on the seam thickness, stripping ratio and quarry depth (Kundu and Ghose 1997b). The total land requirement during the plan period (1990–95) of Coal India Limited (CIL) was about 0.12 million ha, of which 30% are in forest areas (Baliga 1990). Ghosh (1990) reported that every million tonnes of coal extracted by surface mining methods damages an area of 4 hectares in India. The coal industry has been responsible for rendering biologically unproductive an area of about 500 ha a year, rising to about 1400 ha a year by 2000 (Chari *et al.* 1989). Indian coal production, which is in the order of 320 Mt/y, is currently supporting some 70 000 MW of thermal power generation, and, with further developments being planned for 2010, this is expected to increase to 150 000 MW. A developing country like India must continue to promote industrial development if it is to achieve its target of establishing a 150 000 MW power generation capacity by 2010 (Ghose 2003a). This will require increased mineral fuel production. More specifically, to meet its proposed energy needs, India must produce nearly double the quantity of coal that it is mining at present, as its

needs for the fuel will be in the range of 550 Mt/y by 2010 (Ghose 2003b).

Opencast mining leads to a variety of environmental problems, the most serious being land degradation. Virtually all surface mining methods produce dramatic changes in the landscape. They result in the formation of large overburden dumps and huge voids in the mining sites (Namdeo 1989). Today, India has thousands of hectares of this barren land. By 2000, 500 Mm³ of overburden had been removed from coal mines alone. This has led to serious problems in respect of solid waste disposal (Ghose 1997).

IMPACTS ON SOIL QUALITY

By far the greatest impact of mining on the nation's soil resources is due to opencast mining, which has a much greater potential to cause deterioration of soil quality than underground operations. Topsoil is an essential component for land reclamation in coal-mining areas (Kundu and Ghose 1994). The topsoil is seriously damaged if it is not mined out separately in the beginning, with a view to replacement for subsequent reclamation of the area (Ghose and Kundu 1998). This is particularly important due to the scarcity of topsoil in coalfield areas. It is necessary to save topsoil for later use in a way that protects the primary root medium from contamination and erosion, and hence preserves its productivity (Kundu and Ghose 1998). Sendlein *et al.* (1983) indicate, however, that systematic handling and storage practice can protect the physical and chemical characteristics of topsoil while in storage and also after it has been redistributed into the regraded area. Monitoring and implementation of these steps in accordance with site-specific modern technology will minimise the deterioration and provide a medium for plant growth. Thus, it is clear that the problems of topsoil management are diverse, and require well-directed applied research. The present paper attempts to contribute to this by comparing soil sample characteristics from unmined soil with those of excavated mine soil. A fact-finding survey was conducted on these two groups of soil samples by laboratory analysis, and the impact of mining on land resources has been evaluated.

RESTORATION STRATEGY

The type of mining and the characteristics of a particular mineral deposit both affect the degree to which mining disturbs the landscape. Underground mining usually causes little surface disturbance and restoration is restricted to tailings dumps, removal of buildings and

equipment, and making the area safe. Surface mining results in the destruction of the existing vegetation and soil profile (Kundu and Ghose 1997c). Removal of overburden and waste rock, and their placing in waste dumps or the mined-out pit can significantly change the topography and stability of the landscape. Some overburdens may release salt, or contain sulphidic material which can generate acid mine drainage. These materials can sometimes be selectively placed so that they do not cause problems, or they may require special restoration treatments. These are the basic principles of restoration (Plass 1978).

Despite the wide range of climate and soil conditions, and different types of mining operations in India, the basic restoration procedures employed have many similarities. Research before mining can be an important factor in preparing a successful restoration strategy. The more that is understood of the structure and function of the pre-mining ecosystem, the greater the chance of successful restoration (McCormack 1976). Data on the pre-mining vegetation and fauna are a baseline against which the restoration can be assessed. Depending on the agreed appropriate post-mining land use, research may be necessary to define species selection, the ecology of native species and seed biology, plant establishment techniques, plant symbioses, and a range of other issues that ensure the success of the restoration strategy.

The objectives of the restoration include a statement of the final land use planned for the area. This use takes into account the capabilities of the restored area and the level of management that is required to maintain this land use. Restoration plans are to be drawn up as early as possible in the development of a project. Sufficient resources are to be allocated to enable the restoration aims to be met. Comprehensive and accurate records are to be kept of all restoration activities.

Clearing

The area cleared should always be the minimum necessary for the safe operation of the mine. Where possible, a use should be found for the cleared vegetation. It can often be used during restoration as a source of seed, as a mulch to protect soil from erosion, or as a habitat for fauna. When the restoration objective is to restore the original ecosystem, the optimum time for clearing may be determined by the season when the important plant species set seed.

Topsoil removal

Preservation of topsoil quality is often the most important factor in successful restoration, particularly where the objective is to restore a native ecosystem. If the topsoil contains large numbers of seeds of undesirable spe-

cies, then it may be better to use the subsoil as a substrate for restoration (Biswas and Mukherjee 1989). However, in most situations, the topsoil from all areas being cleared should be retained for subsequent restoration. The topsoil contains the majority of the seeds and other plant propagates (rhizomes, lignotubers, roots, etc.), soil microorganisms, organic matter and much of the more labile (more readily cycled) plant nutrients. The term 'topsoil' generally refers to the A soil horizon which is usually darker than the underlying soil because of the accumulation of organic matter (Braddy 1988). Topsoil should be removed after clearing the vegetative cover from the areas to be disturbed and before any drilling, blasting, mining, or other surface disturbance. It is essential that stripping should be carried out when the soil is as dry as possible. This will reduce to a minimum the risk of compaction and damage to the soil structure by smearing and remoulding. A prolonged rainfall would make conditions unsuitable for stripping. Topsoil should normally be stripped from a site by scrapers. The routing of scrapers during this operation must be planned to minimise machine movement, which causes compaction and damage to the soil structure. Further, carefully controlled operation is necessary to ensure planned stripping depths for the topsoil and subsoil. These soils should be stripped and stored separately. Inter-mixing of these soils during the stripping operation is not a good practice. All topsoil should be removed in separate layers from the areas to be disturbed. If the topsoil is less than 15 cm thick, then a 15 cm layer that includes the 'A' horizon, and all the consolidated material (if the total available is less than 15 cm), should be removed and the mixture segregated and redistributed as the surface soil. The 'B' horizon

and portion of the 'C' horizon (or other underlying layers demonstrated to have qualities for comparable root development) should be segregated and replaced as subsoil (Ghose and Kundu 1991).

Timing of topsoil stripping

The timing of topsoil stripping can be important for subsequent restoration. Soils should not be stripped or replaced when they are too wet or too dry, as this can lead to compaction, loss of structure, and a loss of viability of seeds and mycorrhizal inocula (a natural ecosystem component that increases uptake of plant nutrients from the soil). The ideal moisture content to enable soil to resist damage will vary with different soils. Local knowledge and experience will be required to determine when soils can be handled without damage. In most areas there is a distinct period when many native species flower and set seed. Clearing and soil stripping should take place after seed set, where possible, to maximise the stores of seed in the soil. If the objective is to maximise the contribution of other plant propagates (e.g. lignotubers, corms, bulbs, rhizomes and roots), then the optimum time to strip the soil may be when the soils are cool and wet. However, this may jeopardise other soil values and increase the risk of spreading plant pathogens.

Replacement and stockpiling

Topsoil should be replaced along the contour where possible. This will help in erosion control by reducing water flow downslope and increasing water storage. Wherever possible, the topsoil should be immediately replaced on an area where the landform reconstruction is complete (this is known as 'direct return'). Direct

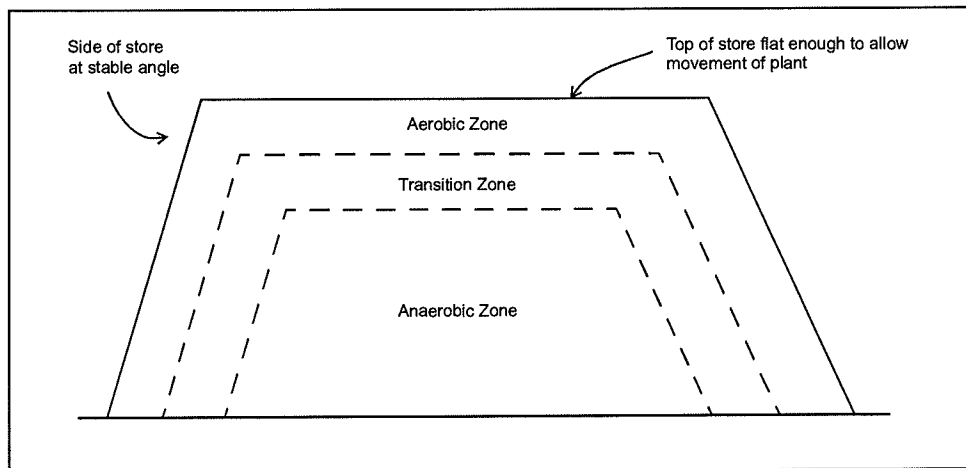


FIGURE 1. SCHEMATIC DIAGRAM OF A SOIL STORE CROSS-SECTION

return has several advantages compared with placing the topsoil in stockpiles and storing it for later restoration. First, it avoids double handling. Second, the need to create stockpiles may mean that extra land must be cleared. Third, and most importantly, stockpiling reduces the quality of the soil resource. Stockpiles become anaerobic, soil structure deteriorates, organic matter and nutrients may be lost, seeds deteriorate, other plant propagates die and populations of beneficial soil microorganisms are reduced significantly. Species which do not have hard seed coats are particularly susceptible to loss during stockpiling. These species are often more difficult to re-establish on restored areas by seeding than hard-seeded species, and so their loss is especially significant. Other research has shown that both the density and numbers of species of native plants are significantly decreased when an area is restored with stockpiled rather than direct-returned topsoil.

The principles of stockpiling of topsoil and the rationale behind it are as follows:

- (i) topsoil and other materials removed should be stockpiled only when it is impractical to promptly redistribute such materials on regraded areas;
- (ii) stockpiled materials should be selectively placed on a stable area (not disturbed), and protected from wind and water erosion, unnecessary compaction, and contaminants which lessen the capability of the materials to support vegetation when redistributed.

Geometry of topsoil heap

To maintain a maximum level of biological activity in the topsoil heaps, they should be constructed as follows:

- (i) to provide the maximum surface area; and
- (ii) to have slopes capable of avoiding erosion and gully formation.

Space constraints imposed by the site factors and topsoil texture would generally dictate the overall size and shape of the heaps, but, if possible, the maximum height should be 5 m, with a slope of 1 in 3 (i.e. 18.5° to the horizontal) (Figure 1). A stockpile of heavier soils should be as shallow as possible, ideally less than 1.0 m in height.

As a rule of thumb, the following stock geometry should be maintained as far as possible to preserve the topsoil:

- (a) Height
 - (i) 5.0 m (max.) for sandy soil

- (ii) 2.0–3.0 m for loamy soil
- (iii) 1.0 m for heavy clayey soil
- (iv) 0.5–1.0 m (max.) for intermediate soil texture.

- (b) Slope

As above, for all soil types.

Topsoil redistribution

After the final grading, and before the replacement of topsoil, slippage surfaces should be eliminated to promote root penetration. Topsoil should be redistributed in a manner that achieves an approximately uniform, stable thickness, consistent with approved post-mining land uses, contours and surface water drainage systems. It prevents excess compaction of the topsoil and protects topsoil from wind and water erosion.

Of greater importance than any other factor in achieving the successful reclamation of surface-mined land is the nature of the soil left at the surface after mining. The nature of this soil determines the choice of available plant species. The topsoil must be uniformly redistributed in a manner that assures placement and compaction compatible with the needs of the species that will be used to restore the distributed area to its pre-mined potential.

TOPSOIL SHELF-LIFE CONCEPT

Topsoil is essential for post-mining land reclamation. It should be noted that the period between initial removal and final replacement on the reclaimed area may be long. The topsoil will become biologically sterile if it is not preserved properly (Kundu and Ghose 1997*a*). Studies were conducted to critically examine the quantitative losses of soil quality in mine areas, to assess the impact on soil quality due to mining, and to evaluate the shelf-life of stockpiled topsoil (Ghose and Kundu 1998). By analysing the deterioration of physical, chemical and microbiological properties in soil dumps of different ages, the shelf-life can be ascertained. Shelf-life studies indicate whether biological reclamation is required for the stockpiled topsoil. Biological reclamation must be adopted to preserve the topsoil if the storage period exceeds the shelf-life period. If the shelf-life of topsoil in a particular area is ascertained, then the mining authority can decide whether it is essential to choose biological reclamation for the preservation of topsoil or whether they can preserve the soil by technical reclamation alone (Kundu and Ghose 1998). A prior knowledge of topsoil shelf-life would enable mine planners to draw up an appropriate strategy for topsoil excavation *vis-à-vis* mine scheduling. An appropriate concurrent and post-mining reclama-

tion strategy can also be determined. This will not only save time but also money.

Topsoil preservation

It is advisable to avoid topsoil storage, especially long-term. However, if storage is unavoidable, upon completion of the surface of the heap, the following steps are to be followed to keep the soil in good condition:

- (a) the surface should be thoroughly ripped with suitable sub-soiling machinery for the purpose of: (i) relieving surface compaction caused by the passage of scrapers and other machines; (ii) aeration of the soil; (iii) encouragement of deep rooting plants by introduction of vegetation;
- (b) following ripping, the heap should immediately be cultivated with suitable low-maintenance species, such as dwarf grasses, to prevent erosion and gully formation;
- (c) the surface vegetation should be actively maintained with seeding and weed control operations.

SOIL MICROORGANISMS

Symbiotic microorganisms

Plants form beneficial symbiotic associations with a number of soil microorganisms, including fungi, bacteria and actinomycetes (single-celled plants usually found in soil). Mycorrhizae are a natural component of the ecosystem in most Indian soils. They are very important in India, as they are necessary to ensure the establishment of some plant species. The majority of native plant species used in restoration probably form associations with vesicular arbuscular mycorrhizae (VAM) and ectomycorrhizal fungi. These fungi have been shown to be effective in increasing the uptake of phosphorus by plants growing in phosphorus-deficient soils. Some species of orchids only become established in the presence of particular mycorrhizal fungi. The ability of VAM fungi to associate with plants is rapidly depleted by topsoil disturbance and stockpiling. This often results in low levels of infection in the early years of restoration (Miller and Cameron 1976). Similarly, only limited numbers of ectomycorrhizal fungi species have been seen in recent restoration. As a result, some species may not recolonise restored areas until specific mycorrhizae have recolonised. To conserve mycorrhizal inocula, topsoil should be:

- directly returned wherever possible; and
- when stockpiling is unavoidable, the piles should be low and revegetated as soon as possible.

Nitrogen fixation by legumes relies on a symbiotic association between the plant and the bacteria known generally as rhizobia. Rhizobia appear to be more tolerant to disturbance and stockpiling than mycorrhizal fungi. An actinomycete of the genus *Frankia* forms nodules with the capacity to fix nitrogen on the roots of species from the genera *Casuarina* and *Allocasuarina*. These genera are often included in revegetation programmes. Little is known of the tolerance of *Frankia* to soil disturbance and stockpiling.

Plant pathogens

If the soil is infected with plant pathogens, for example *Phytophthora* or *Armillaria*, then specific procedures may be necessary to limit their spread and the intensification of their impact on plant communities. This will generally involve:

- strict separation of infected and uninfected soils;
- control of the movement of infected soil into uninfected areas;
- the use of procedures which avoid creating conditions which are conducive to the spread and intensification of the disease; and, possibly,
- the use of control measures such as fungicides.

ALTERNATIVES TO TOPSOIL

If topsoil is unavailable, the cost of transportation is prohibitive, or the topsoil contains such high levels of weed seed or plant pathogens that it is unsuitable for restoration, then subsoil, overburden, waste rock or similar materials must be used as a substitute for revegetation (Riley 1978). These materials will generally require techniques to increase their organic matter content and nutrient status. Their physical characteristics may require amelioration and their pH may need to be adjusted. The physical and chemical properties of the proposed substrates should be thoroughly investigated prior to their use in restoration.

The following are techniques which can improve the ability to support plant growth in the long term.

- application of organic matter such as animal manures, sewage sludge or other wastes;
- chemical amendments such as:
 - gypsum to improve the structure and reduce the pH of highly alkaline substrates;
 - lime to raise the pH of acid substrates; and
 - inorganic fertilisers;
- soil conditioners. Many proprietary soil conditioners, such as polyvinyl alcohol polymers, are available which may be useful in certain situations. Trial

areas should be treated to assess their value before large areas are treated;

- growing green manure crops which can be incorporated into the substrate;
- establishing nitrogen-fixing species such as legumes to increase the organic matter and nitrogen content of the substrate; and
- applying mulch.

Seeding rates will probably have to be increased compared to those for topsoil, in order to establish a satisfactory cover of plants on these alternative substrates.

RESTORATION EARTHWORKS

Land form design and reconstruction

The re-shaping and grading of a site is an essential aspect of restoration. The need for extensive reshaping of spoil piles can sometimes be minimised by good mine planning and management. The final landform must be hydrologically compatible with the surrounding area. Slopes must be stable and will be less obtrusive if they have a similar gradient to natural slopes in the area, although this is not possible in many cases. The following factors should be considered with respect to landform design:

- *Stability*
The maximum angle and length of slope that will be stable at a specific site depends on site-specific variables such as spoil and topsoil characteristics, and rainfall intensity. The erosion potential of the different materials on site needs to be assessed and a geo-technical investigation may be required. Terraced landforms with short, steep (angle of repose) slopes and gently sloping terraces (<5%) may be as stable and have a higher land capability than a conventional landform of around 15–18% slope.
- *Drainage density*
The drainage density of surrounding areas will provide a guide. An increase in drainage density may be required if there has been an increase in the gradient of slopes or changes in the nature of the surface materials.

Erosion control

Control of erosion is important both during mining and in the restoration programme. A major objective of restoration should be to establish an adequate cover of vegetation to stabilise the site and prevent or control erosion (Ghose 2002). Until an adequate cover of vegetation has been established, it is imperative that provision be made to control erosion from disturbed areas.

Soil particles can be lost in three ways; they can be blown away, washed away, or the whole surface may slip away or slump. Soils containing more than 60% unaggregated sand grains and individual granules in the size range 0.1–0.5 mm are very susceptible to wind erosion; those with less than 40% are not usually susceptible.

Before a vegetation cover is established, wind erosion can be controlled by three basic methods:

- protecting the soil surface with a mulch of natural or manufactured materials;
- maintaining the soil in an erosion-resistant condition (i.e. moist, or with a compacted surface crust or a cloddy structure); and
- reducing wind velocity across the disturbed areas by establishing wind breaks.

Erosion by water involves two stages. First, large soil aggregates are broken up into finer particles and, second, these fine particles are transported downslope. The loss of soil through water erosion is a function of the erosivity or intensity of the rainfall, the erodibility of the soil, the area of catchment, the length and gradient of the slope, the amount of vegetation cover and the erosion control measures undertaken. Soil erodibility depends on soil texture, structure and the degree to which soil particles disperse when in contact with water. Soils with high levels of exchangeable sodium tend to disperse readily.

Measures to protect the soil from water erosion should be carried out on a catchment basis. Drainage from external catchments must be controlled by diversion channels or holding structures such as banks, drains or dams. Water leaving the site or diverted around the site must also be controlled. It is necessary to discharge this water so that it does not cause erosion or carry sediment downstream. Sediment dams are the most common means of controlling sediment levels in run-off.

On disturbed areas, control of water erosion is achieved by:

- slowing the water flow across the soil surface;
- reducing the impact of raindrops on the soil surface; and
- maintaining the soil in an erosion-resistant condition.

Encouraging infiltration and building drainage control structures to channel water off the site can reduce water flow across the soil surface. Infiltration can be encouraged by ripping and cultivating on contours and constructing contour banks. Water can be channelled

off-site by drains, graded banks and stabilised waterways.

Ripping encourages infiltration, relieves soil compaction, increases the volume of soil readily accessible to plant roots and binds the topsoil to the subsoil. Ripping should always be along the contour. Areas can be ripped after the topsoil has been returned or before topsoiling, in which case the area will nominally need to be cultivated before revegetating. When ripping after topsoil return, care must be taken to avoid burying significant quantities of the topsoil and therefore losing its benefits. The design of the ripping type and the way that it is adjusted can be important in reducing topsoil burial. Ripping should normally be as deep as possible. The rip lines should be spaced at approximately the ripping depth apart. Ripping should not continue when conditions are too wet to allow the soil to shatter or when it brings large amounts of rock to the surface. A 'winged' ripping type can improve soil shattering, especially in moist soil conditions. Mulches can be used to protect the soil from raindrop impact. Examples of suitable materials are brush matting, stubble mulch, hay mulch, sawmill wastes, bitumen and other chemical stabilisers. These materials may also aid, or in some cases inhibit, germination of seeds in the revegetation program. It is recognised that mulches and additives are not cost-effective on large areas, but could be useful for small problem areas. Soils are most resistant to erosion when they are maintained in a cloddy condition. This is difficult to achieve in sandy soils.

REVEGETATION STRATEGY

When attempting to restore a native ecosystem, the initial revegetation effort is unlikely to produce a vegetation identical to the original (Donahue *et al.* 1990). This does not mean that the final canopy species cannot be established in the first instance: merely that other species may dominate the vegetation in the early stages of restoration. The initial revegetation effort must establish the building blocks for a self-sustaining system, so that successional processes lead to the desired vegetation complex (Foy 1974). The best time to establish vegetation is determined by the seasonal distribution and reliability of rainfall. All the preparatory works must be completed before the time when seeds are most likely to experience the conditions that they need to germinate and survive, i.e. reliable rainfall and suitable temperatures.

Species selection

The species selected for establishment will depend on the future land use of the area, soil conditions and cli-

mate. If the objective is to restore the native vegetation and fauna, then the species are pre-determined. Some indigenous species may not thrive in areas where soil conditions are substantially different after mining. If this is the case, and the objective is to re-establish vegetation which fulfils the functions of the original native vegetation, then some species from outside the mining area will have to be introduced. Species which have similar growth forms to the original vegetation, and thrive in areas with comparable soil types, drainage status, aspect and climate to the restored area, are the most appropriate. One of the major approaches, at least in terms of establishment of the native ecosystem, should be to search the local area for natural analogues of the post-mining landscape and mine soils, and use these as models for the proposed post-mining ecosystem. Care must be taken to avoid introducing a species which could become an unacceptable fire hazard, invade surrounding areas of native vegetation, or become a weed for the local agricultural industry.

Where the future land use is agriculture, then the species selected should be those which are commonly used for pastures on soils of similar texture, drainage status, pH and fertility. Suitable legumes should always be considered for their ability to improve soil fertility. Cover crops can be considered where a quick cover for erosion control is required. When establishing native plants with a cover crop, a compromise must be reached between the density of the cover crop needed to provide rapid erosion control, and the tendency of the cover crop to suppress or smother the native species. Dense cover crops may also pose a fire hazard. Hybrids such as sudax (a commercial fodder species) can be useful cover crops, as they do not set viable seed and so will not persist on the site.

Provenance selection

Provenance is used here to help in differentiating between examples of a native species. For restoration programmes directed towards re-establishing native species, a decision must be made as to whether only species of local provenance should be used for restoration, or whether to use a wider range of provenances. The argument for using only local provenances is the preservation of the genetic integrity of the area, and the premise that local provenances are best adapted to the local soils, climatic conditions and ecological processes. The argument for using a wide range of provenances is that conditions at a restored mine will always be different, to a greater or lesser extent, to the original conditions at the site, and that the local species will not necessarily be the best-adapted to these altered conditions. Selection of species will be influenced by the pri-

orities for restoration and the circumstances of each case.

Establishment

Plant species can be established on restored areas from:

- propagates (seeds, lignotubers, corms, bulbs, rhizomes and roots) stored in the topsoil;
- sowing seed;
- spreading harvested plants with bradysporous seed (seed retained on the plant in persistent woody capsules) on to areas being restored;
- planting nursery-raised seedlings;
- transplants of individuals from natural areas;
- habitat transfer – the transfer of substantial amounts (around 1 m² or more in area and 200–300 mm depth) of relatively undisturbed soil with its vegetation intact from natural areas;
- invasion from surrounding areas through vectors including birds, animals and wind.

Topsoil

The topsoil has an important role, particularly in the establishment of native species. Maximising the return of species from the seed resource in the topsoil is one of the most important aspects of restoring the full suite of pre-mining species to restored areas. The numbers and diversity of seeds in the topsoil could not be duplicated economically by collecting and sowing seed.

Seeding

Sowing seed is an economical and reliable method for establishing some species. Seeding results in a more random distribution of plants than planting seedlings, and leads to more natural-looking vegetation. The species best established from sown seed are those which produce large numbers of easily collected, viable seeds, which have high germination and survival rates in the field. These often include colonising species such as many of the Indian acacias. These species are easy to establish and quickly provide cover, erosion control and other benefits. However, they can also have a significant and long-lasting effect on the numbers and cover of small shrub and herb species. Care must be taken, when re-establishing the original land use in areas of native vegetation, that these easy-to-establish species do not dominate the restoration at the expense of botanical diversity. Seeding techniques, seed collection, seed treatments and seedbed preparation are discussed below.

Bradysporous species

Bradysporous seed (seed retained on the plant in persistent woody capsules) can be important in some eco-

systems. Where species with bradysporous seeds are an important part of the ecosystem, the vegetation can often be harvested using a forage harvester before clearing, and can then be spread on areas being restored. The bradysporous seed is shed from the capsules on to the soil surface as the harvested vegetation dries out.

Recalcitrant species

Some plants fail to re-establish on restored sites where the aim is to restore the native flora, despite the application of seed and the use of fresh topsoil. These are often species which typically respond to disturbance by epicormic buds under the bark or from various underground storage organs (e.g. lignotubers, corms, bulbs, rhizomes and roots). These can sometimes be introduced by transplanting or habitat transfer (see below).

Planted seedlings

It is usually more economical to establish plants by direct seeding than by planting seedlings. Planting nursery-raised seedlings is most appropriate when the particular species cannot be established in suitable numbers through seeding or topsoil return. It may be possible to propagate these species from seed, cuttings, divisions or tissue culture, grow them on in containers in a nursery and then plant them as part of the restoration process. Planting seedlings may also be appropriate where the restoration objectives require a systematic layout of plants, as in the case of establishing a plantation. Planting seedlings on a regular basis requires a reliable supplier of consistent quality seedlings or an on-site nursery. Seedlings should always be sun 'hardened' before being planted in the field.

Transplanting and habitat transfer

Direct transplanting of species (which cannot be established by other means) is possible by transferring slices or front-end loader buckets of soil with the vegetation intact. However, this is an expensive option. Successful transplanting is influenced by the climatic conditions. In general, transplanting is best carried out in cool, wet conditions. For this operation, the top 200–250 mm of the soil profile was removed in slices using a bulldozer. The soil and plants were retained in large lumps and stored to one side of the mine path. The 100–150 mm of soil below this top layer of soil was stripped and stored separately. The topsoil layers were replaced in the correct sequence on top of the tailings immediately the tailings were returned (after one to three months).

Seed collection and purchase

A consistent supply of quality seed is essential for the success of revegetation. There are many difficulties

inherent in collecting native seeds. In many years, seed set may be poor due to climatic conditions, insect pests, grazing or fire. Some of the points to be considered when collecting seed are as follows:

- consider establishing a seed orchard of species which are rare, produce limited seed, or have seed which is difficult to collect;
- identify collection areas before the seed matures;
- collect seed only when it is mature. Seed of some annual species of the jarrah forest can be shed within a day of ripening, which means that very close observation of seed-bearing plants is required. Differential ripening within one species or a single plant may necessitate several visits;
- avoid seed or fruits that have been attacked by insects or show signs of fungal infestation;
- some seed can be collected by mechanical means, including vacuum methods;
- when operating in forest areas, seed can often be collected from trees that have been felled for saw milling.

Some of the points which should be considered when purchasing seed are:

- purchase from reputable seed merchants. This should avoid problems of incorrect identification of species, contamination with weed species and non-viable seed;
- consider setting up long-term contracts with seed merchants for particular species. With the security of contracts, merchants can concentrate on improving their knowledge of the seed biology and location of the species, and build up inventories knowing that the seed will be purchased;
- seed should be from known areas;
- date of collection and conditions of storage should be known. Ideally, the germination rate of the seed should be given. The State Department of Agriculture or Primary Industry should certify seed of agricultural species.

Seed processing and storage

Seed should be cleaned before storage, to remove as much debris and chaff as possible. Seed can be extracted from pods, capsules or other fruits by a variety of means. Processing methods are often species specific. Techniques include: drying the capsules or pods in the sun, or in an oven; threshing, using commercially available threshers; burning seed cones to release the seed; soaking fleshy fruits in water before recovering the seed, etc. Clean seed should be stored in dry insect- and vermin-proof containers. The containers should be clearly labelled with details of the spe-

cies, date collected and collection location. Seed will often need to be treated with insecticide and fungicide before storage, to prevent insect and fungal attack. Exposure to carbon dioxide for one or two days before storage has been shown to be an effective treatment against insect attack for many tropical native species. Storage areas should be fumigated regularly. Loss of seed viability during storage is common. Seed must be stored at low humidity (10% relative humidity) and low temperature. Temperature requirements may vary for temperate and tropical species.

Seed treatments

Seed of some species requires pre-sowing treatments. Germination of most native legumes and a number of other species is enhanced by heat treatment. These species are commonly immersed in boiling water for 30 seconds to five minutes before sowing. There is evidence that the germination of some species, which normally have low germination rates, is enhanced by treatment with smoke from burning plant litter. These species can either be treated directly with smoke or with water, which contains the soluble components of smoke. Agricultural legume seed should be inoculated with the appropriate rhizobia. This is usually done in conjunction with lime pelleting, which is a simple procedure easily carried out on-site. Rhizobia inocula and details of the pelleting procedure are readily available from seed merchants. Rhizobia, which form associations with native legumes, seem tolerant of soil disturbance and storage, and will often readily infect native species after restoration. However, inoculation may be necessary if infection does not readily occur after restoration, or when establishing native legumes in overburden, tailings, or subsoil. This will involve isolating native rhizobia, growing them on suitable media, and attaching them to the seeds. While this is a relatively simple procedure, specialist guidance will probably be needed. Suitable techniques for inoculating large quantities of seed with mycorrhizal fungi are not yet available.

Seedbed preparation

The preparation of a suitable seedbed is an important factor in the successful establishment of plants from seed. The objective in creating a seedbed is to place the seed in a suitable place for germination. The seed must be in good contact with the soil to ensure that it can take up water easily, and the soil must be well aerated. The soil around the seed must be loose enough for the seedling to grow up through the soil and allow root growth. The seedbed should be free of weeds. Care should be taken not to over-prepare the soil, as a rough surface provides more niches for the seeds and encourages

infiltration of rain. A variety of conventional agricultural equipment can be used to prepare seedbeds. Soils should be cultivated when moisture levels are adequate, to avoid powdering, but not so wet that compaction and loss of structure becomes a problem. If seed is to be broadcast, it should be done before the seedbed has the chance to consolidate and form a surface crust. Seeding machines generally cultivate as well as sow. Where the topsoil contains significant quantities of seed of desirable species, care must be taken not to disturb the soil after these seeds have started to germinate, as this will cause a substantial reduction in plant establishment. Mulches can improve the microenvironment for seed germination and establishment.

Seeding methods

Seed can either be broadcast on to the surface of the soil or drilled into the soil using some form of mechanical seeder. Target densities for each of the seeded species, and an estimate of how much seed is required to obtain these densities, need to be established. The seeding technique chosen will depend on local factors, such as topography, the size of the area being restored and the type of seed. Agricultural species can usually be sown with conventional agricultural seeders. Various machines have been developed for sowing native species, but in many cases broadcasting by hand is still the best option. Mechanically sowing native seeds is difficult because of the large range in seed size, which makes an even coverage of seed hard to obtain. In addition, many native seeds have awns or other appendages, which create problems for mechanical seeding. Broadcast seed can be buried after spreading, by harrowing or cultivating with a range of equipment, or it can be left on the soil surface. Care must be taken not to bury the seed too deep for successful establishment when cultivating after seeding. Seed can also be applied by hydro seeder, hydro mulching or by helicopter or fixed-wing aircraft. Hydro seeders are a convenient way of seeding steep areas. Aerial seeding is used to seed large areas, especially when the soil is too wet or the slope too steep to use tractor-mounted seeders. Timing of seeding can also be important for successful revegetation. In most cases, seed should be sown immediately prior to the expected onset of reliable rains, or after the start of the rainy season. Native seeds may require specific moisture and temperature conditions to germinate, so that they establish at the optimum time of the year for survival. This need for multiple cues may allow seed to be sown well before it would normally be expected to germinate. Seed theft by ants, birds and small mammals is a potential problem when seed is sown early, although less likely than in areas of

native vegetation, because of the impact of disturbance on fauna populations.

Weed control

Controlling the introduction and spread of weeds is an important consideration in restoration. Weed infestations on restored areas can be very difficult to control and so the emphasis should be on prevention rather than cure. Weeds in areas adjacent to the disturbed areas should be controlled to reduce the potential seed load. Care must be taken that weeds are not introduced to the area in manures or as contaminants in seed of the desirable species. Fertilisers and manures should always be used carefully to stimulate weed growth, seed set and spread. A vigorous cover of the desirable plant species is often an effective impediment to invasion by weed species. Cultivation, hand weeding, burning and herbicides can all be used in attempts to control weed infestations. However, control can be difficult where there are plants that need to be retained growing amongst the weeds. Selective grass herbicides can be used for grass weeds in areas revegetated with non-grass species. Hand weeding is expensive, but can be effective for smaller areas. Herbicides can be applied selectively using wick applicators in some cases, for example when the weeds are much taller than the desirable species.

FERTILISERS AND SOIL AMENDMENTS

Most restoration programmes will include an application of fertiliser in the establishment phase. Areas restored to agriculture or other intensive use will also normally require a maintenance fertiliser programme (Reeder and Berg 1977). Initial applications of fertiliser have been shown to increase species numbers, plant co-density and growth rates in a number of areas in India where the objective is to restore the native vegetation. The type of fertiliser and the application rate will vary according to the site, soil type and post-mining land use (Keeny and Bremner 1966). Application rates of up to 80 kg/ha of nitrogen, 5–80 kg/ha of phosphorus and varying rates of potassium and micronutrients have been used. Care needs to be taken when preparing fertiliser prescriptions and applying fertilisers on restored areas. The roots of seedlings can be damaged if fertiliser is placed too close to the plant. Fertilisers, particularly nitrogen fertilisers, may stimulate the growth of weed species. These weeds can jeopardise the success of restoration by outcompeting the more desirable species or by becoming a fire hazard. A wildfire may then kill the more beneficial species and succession will proceed in an undesirable direction.

Some species, particularly from the family Protease, are reported to be adversely affected by application of phosphorus fertiliser. These adverse effects are likely to be seen principally on sandy soils, and are less likely to occur on finer soils with a greater capacity to adsorb phosphorus. Relatively high rates of phosphorus and low rates of nitrogen fertiliser will favour the growth of legumes. The pH of acid soils can be increased by the application of lime (calcium carbonate). Low pH (below about 5.5 when measured in water) can cause aluminium or manganese toxicity and reduce the availability of some nutrients. Application rates of lime are usually in the range of 2–5 tonnes/ha, but will vary according to soil type, initial pH and the source of the lime. Gypsum can be used to improve the structure of poorly structured sodic soils. An exchangeable sodium proportion of greater than 6% can indicate an unstable soil structure. Gypsum is normally incorporated into the soil at around 5–10 tonnes/ha. The application of gypsum results in the replacement of sodium with calcium on the soil exchange surfaces, which can improve soil structure, reduce surface crusting and increase water infiltration. It may also reduce the pH of sodic soils (soils with pH >8.5). Various organic wastes (e.g. animal manures, sewage sludge, and blood and bone) can have value as both fertilisers and soil amendments. However, supply may be unreliable, and they are often too expensive, variable in composition, and too hard to spread, to be used for large-scale restoration.

NUTRIENT ACCUMULATION AND CYCLING

In a self-sustaining ecosystem the nutrient demands of the plants must be met principally by nutrient cycling, supplemented by inputs in rainfall, dry deposition, rock weathering and, in the case of nitrogen, by fixation of atmospheric nitrogen. When the objective of restoration is agriculture or another more intensive use, these processes can be augmented by fertiliser addition. Maintaining or increasing the ability of the soil to supply nutrients, to store and supply water, and support root growth, should be a major concern in developing a sustainable ecosystem. Improving water infiltration, relieving soil compaction and increasing the volume of soil accessible to plant roots is a major aim of the deep ripping normally carried out as part of the erosion control measures in restoration. The re-establishment of nutrient cycles is essential to the sustainability of restoration.

Mining removes the vegetation and inevitably leads to the loss of some plant nutrients from the site. This is particularly important where the proportion of the total nutrients in the ecosystem, which are contained within

the vegetation, and the plant litter on the soil surface, is high, as is the case for many Indian ecosystems. In these cases, there must be an input of nutrients to the systems if they are going to reach a productivity level equivalent to the pre-mining ecosystem, and be self-sustaining in the long term (Lyle 1987). The nutrients which are usually most limiting to plant growth in Indian ecosystems are nitrogen and phosphorus. Most of the nitrogen held in the soil is in the organic matter, and is released for uptake through mineralisation. As mineralisation rates are generally low, a large capital of nitrogen must be accumulated. The most practical way to increase the nitrogen capital of ecosystems is to establish nitrogen-fixing plants, usually legumes, which can quickly increase the nitrogen levels in the system. The accompanying increase in soil organic matter is important in improving soil structure, and the ability of the soil to hold and supply water (Keese 1968). Most soils, except some sandy soils, have a considerable ability to retain phosphorus fertiliser. Some of this phosphorus can remain available to plants, and an initial application of phosphorus fertiliser may supply sufficient phosphorus for plant growth until nutrient cycles become established. Symbiotic association with mycorrhizal fungi can increase the growth of many Australian native species in phosphorus-deficient soils (Anon. 1995). Research into methods of inoculation with mycorrhizal fungi may be advantageous, especially in areas where the application of high levels of phosphorus fertiliser is considered undesirable, for example when restoring heath vegetation on coarse sands.

FAUNA

Encouraging the native fauna to return to areas cleared for mining is a fundamental part of any restoration programme that aims to restore a natural ecosystem (Schafer and Nielson 1970). Some invertebrate species will be introduced if fresh topsoil is placed on the area, but most fauna species will need to recolonise from surrounding areas. A range of factors, including the size of the restored area, the fauna populations in surrounding areas and the success of the revegetation programme influence the rate of recolonisation by fauna. Many fauna groups will quickly colonise any areas which contain the resources that they require, such as food, shelter and breeding sites. In many cases the main aim in a fauna return strategy should be the re-establishment of the native vegetation. If this is successful, then the fauna should colonise from surrounding areas. Difficulties in re-establishment will occur when fauna population levels are low in surrounding areas, or a

species is locally extinct (Wali and Freeman 1973). In these cases, a captive breeding and release programme, or re-introduction programme from another area, may be appropriate. However, the reasons why the populations are low need to be understood and controlled, so that the introduced animals do not succumb to the same pressures which reduced the original populations. Fauna may also be slow to return where species require resources, which are not available in young restoration. For example, some jarrah forest species live in logs or stumps on the forest floor and others require tree hollows to breed. These may not be available for a very long time after restoration. The return of these species can be expedited by creating fauna habitats and corridors during restoration, using logs, stumps and other natural materials. Fauna corridors running from the surrounding areas to the centre of the restored areas encourage smaller species of mammals and reptiles, which are reluctant to traverse large distances of open ground, to colonise. Tree hollows can be substituted for by providing nest boxes of appropriate size for the target species in developing restored areas. Animals, particularly invertebrate species, are important in many ecological processes, such as nutrient cycling, litter decomposition, soil aeration, seed dispersal, seed predation and plants' ability to survive fire or set seed, so that they can become re-established after fire. Fire control strategies can include free breaks, hazard reduction burns in adjacent areas, prescribed cool burns in restored areas, and weed control.

SUCCESS CRITERIA AND MONITORING

It is essential to monitor the success of any restoration programme and to be prepared to rework any areas of restoration not developing adequately. Success criteria should be defined and agreed to by all interested parties. In India there are no recognised criteria for determining when restoration is complete. The debate on whether an area has been completely restored cannot be resolved until there is widespread agreement on issues such as: what constitutes a viable ecosystem, what is an acceptable level of species diversity, how indistinguishable should a restored area be from neighbouring untouched areas, etc. These are issues of continuing debate and research. However, the industry and regulatory agencies have had to address this issue on numerous occasions. Handing back responsibility for managing the land to the relevant landowner or government agency will often be possible when some mutually satisfactory endpoint has been reached. If the aim of restoration is to develop a new higher-value land use for the area, then the land manager may accept respon-

sibility for the increased level of management that will be required before the mining company's responsibility is relinquished. Restoration can be considered to be successful when the site can be managed for its designated land use without any greater management inputs than other land in the area being used for a similar purpose. Restored native ecosystems may be different in structure to the surrounding native ecosystems, but there should be confidence that they will change with time along with or towards the make-up of the surrounding area. The restored land should be capable of withstanding normal disturbances such as fire or flood.

The success criteria could include:

- physical (stability, resistance to erosion, re-establishment of drainage);
- biological (species richness, plant density, canopy cover, seed production, fauna return, weed control, productivity, establishment of nutrient cycles);
- water quality standards for drainage water; and
- public safety issues.

Monitoring techniques must be designed to provide statistically valid results with the desired order of accuracy. The sampling intensity will usually have to be a compromise between the level of precision of the data collected and the cost of collecting the data. There will always be a compromise between the number of samples collected and the number of decimal points that their analysis is expressed in.

CONCLUSION

Restoration is an essential part of developing mineral resources in accordance with the principles of ecologically sustainable development. Restoration is not an operation which should be considered only at, or just before, mine closure. Rather, restoration should be part of an integrated programme of effective environmental management through all phases of resource development, from exploration to construction, operation and closure.

Ecosystem restoration is relatively new science, even though humans have been disturbing the land for many centuries. Mining organisations are developing the expertise to re-assemble species into communities that have a chance to grow, develop and rebuild local biodiversity. They are achieving this through careful attention to all aspects of restoration and revegetation: from initial planning, clearing, soil removal, storage and replacement, through species selection and re-establishment of vegetation with its associated organisms, to maintenance of areas into the future. To achieve such recognition requires commitment from all

the personnel associated with mining operations. The strategies of restoration outlined in this paper should help mine managers to develop a restoration plan which, with the support of a committed management and workforce, will help to achieve a high standard of restoration of mine-degraded land, for a sustainable mine closure.

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