Teacher pack: Green Kid issue #2

Phytoremediation and phytomining

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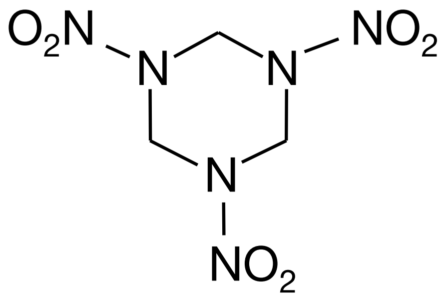
Phytoremediation

Explosive pollutant 'PARTNERS IN CRIME'

RDX

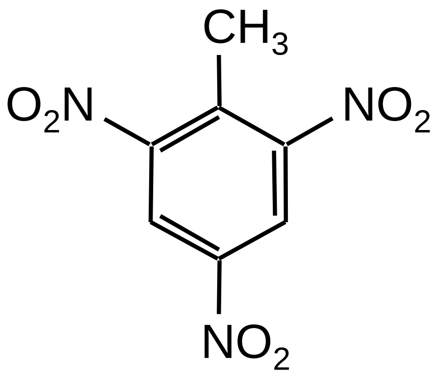
(Hexahydro-1,3,5-trinitro-1,3,5-triazine)

Royal Demolition eXplosive



TNT

2,4,6-Trinitrotoluene (TNT)

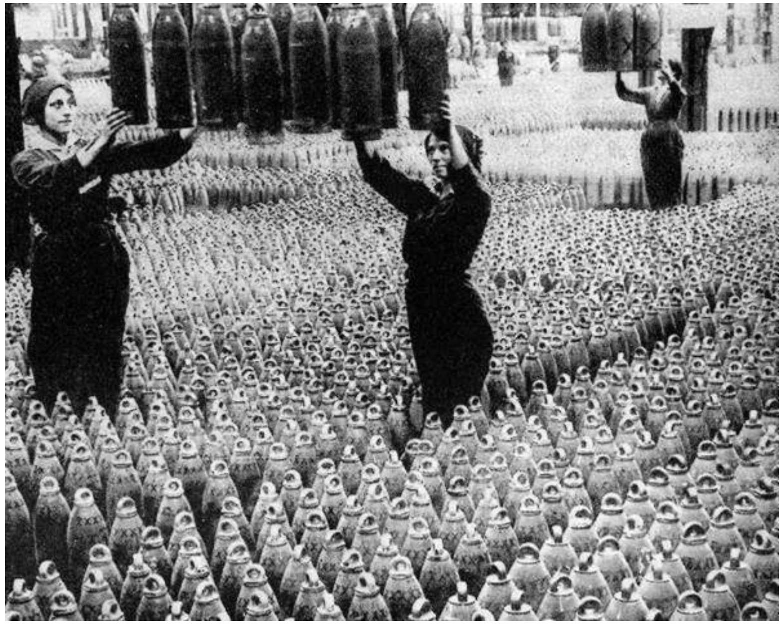


Explosives often have nitrogen groups in them because it is very energetically favourable for them to reform as N_2 (78% of air is N_2). The nitrogen really wants to form N_2 (like carbon wants to form CO_2 when we burn it, but MUCH more) so lots of energy is released really quickly when it is broken down.

Where does this pollution come from?

Manufacture

Explosives are made on big scales and any time you make a lot of something, there is the potential for escape/release. This is especially true in the past when we didn't know how polluting RDX and TNT were.



Women working in WWII munitions factory

The Kynoch Works at Witton, nr Birmingham made munitions from 1862 - 2003

Where does this pollution come from?

Use

Explosives get used in lots of things from mining and quarrying, in army training and in conflict. Not all the explosive gets used, you can see bits of yellow TNT and red RDX in these pictures.

Extent of the pollution

In the US alone:

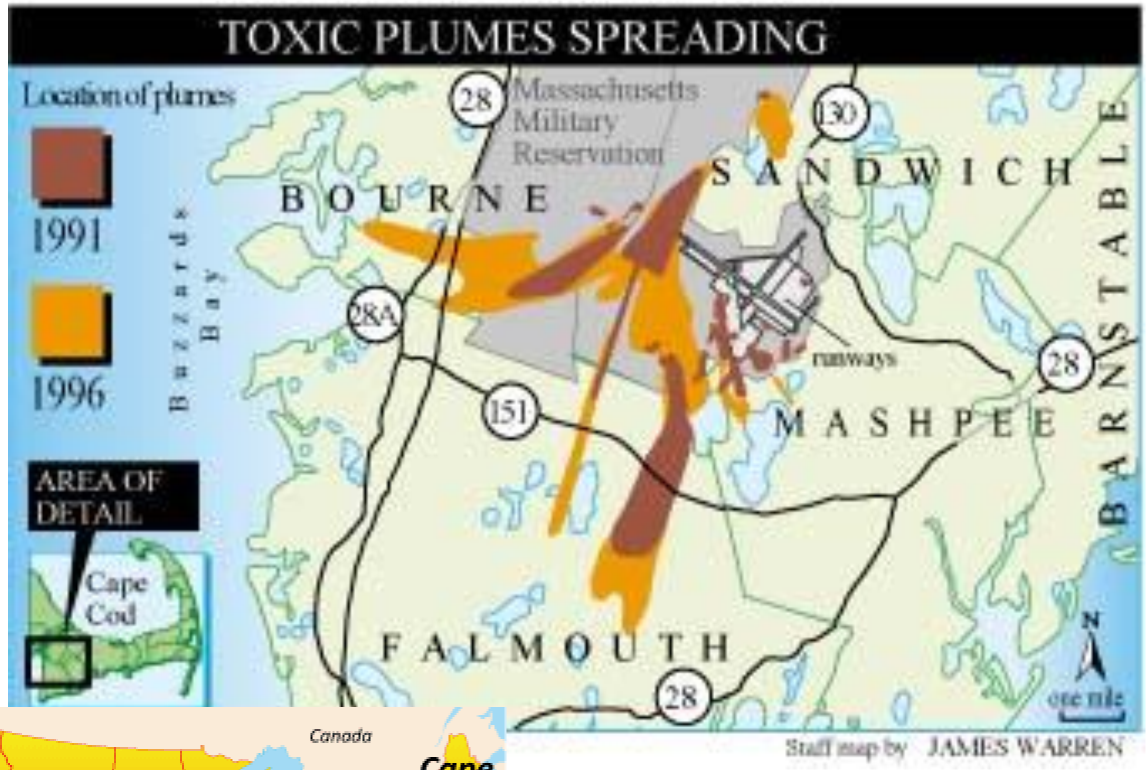
- Over 25 million acres contaminated on active ranges
- 38 billion litres of groundwater
- Treatment costs estimated at £10-100 billion

(United States General Accounting Office 2004)

Some other countries...?

The problems with RDX

- Toxic
- Little degradation in the environment in last c. 60 years
- Not found in Nature
- Readily leaches into groundwater, see the underground, toxic plumes under the Massachusetts military reserve in the US
- Threatening drinking water supplies



The problems with TNT

- Very toxic/carcinogenic (causes cancer) to all organisms tested
- Most widespread of all the explosives
- Binds tightly to the humic/organic content of soil…Like TOXIC Velcro
- Not found in Nature
- Little degradation in environ years

Main *Ex-situ* treatments – taking polluted soil away

- **Dig-and-Dump** (Landfills and Engineered Landfills; Lined, capped with leachate collection system)
  - Generation of greenhouse gases
  - Leaching of heavy metals (HM) and other wastes
  - Expensive e.g. requires long-term monitoring
  - Not really solving the problem..?

- **Incineration** (Heat soil to 750–1200 ^{o}C)
  - Soil structure destroyed
  - Costly: build and maintenance + fuel costs
  - Release of toxins (VOCs; volatile organic compounds, and HMs)
  - Toxic ash end products

In-situ treatments – treating polluted soil where it is

- Ex-situ treatments e.g. pump and treat groundwater
  - Expensive
  - Contaminated Activated-carbon filters/foams need specialist disposal
  - Damages soil structure
  - Difficult to predict further reaches

- Monitored Natural Attenuation (leave it to fix itself and just check it isn't leaking)
  - Not fixing the problem

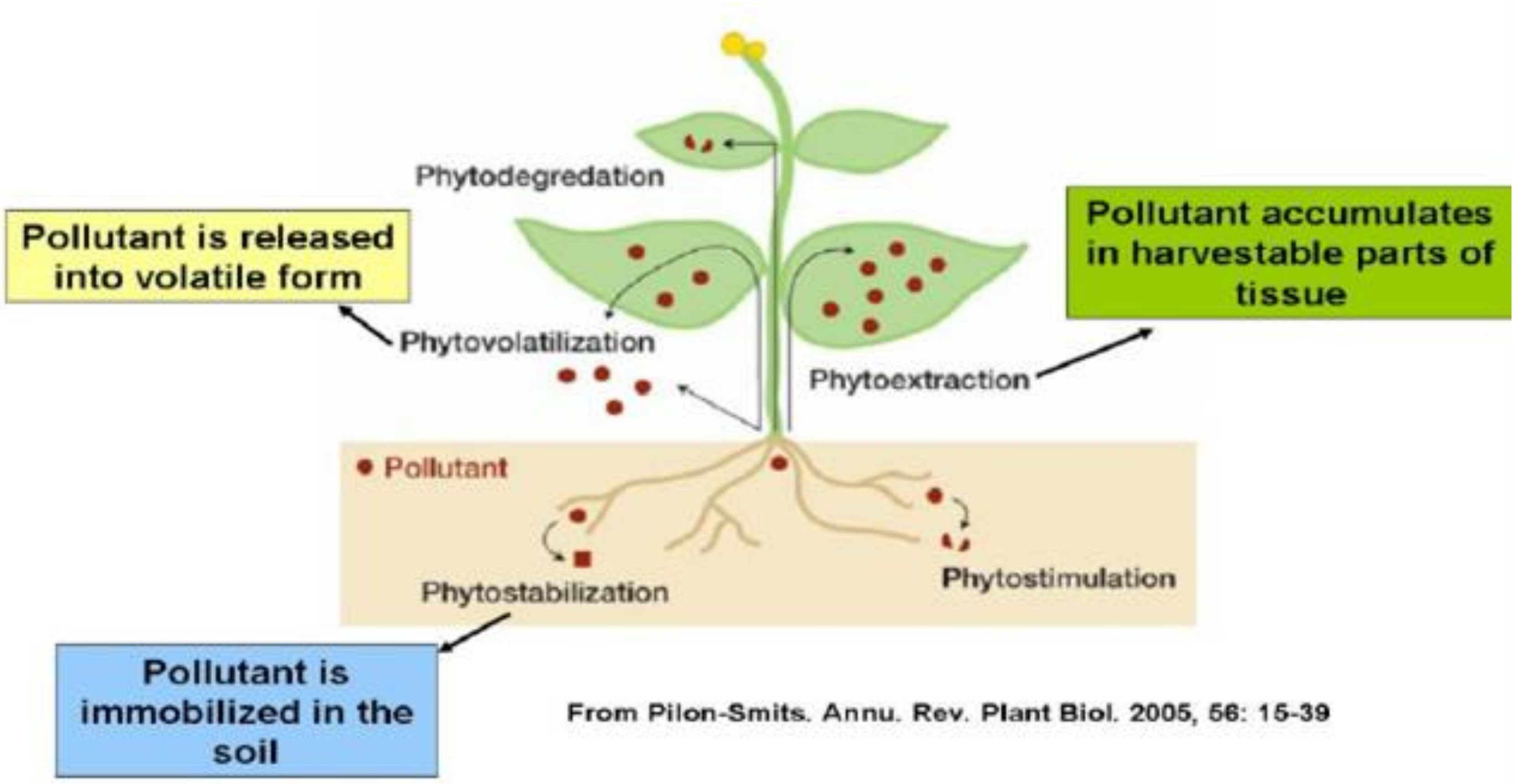
- Bioaugmentation/Bioremediation. Add organic matter and microbes
  - Microbial populations decline
  - Slow

Phytoremediation

"Use of plants to remove, contain or detoxify environmental contaminants"

- Low maintenance
- Minimally disruptive
- Can be cost-effective
- Aesthetically-pleasing
- Compatible with restoration ecology

Phytoremediation processes

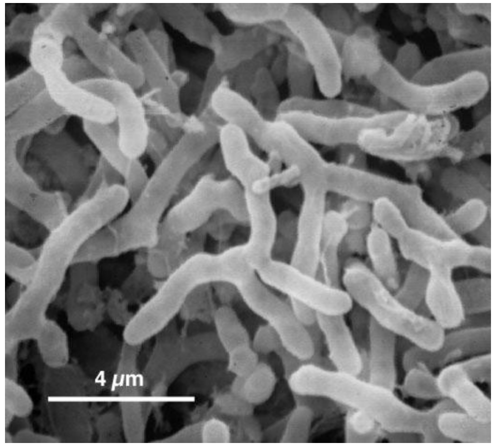


Plants have lots of ways of dealing with natural compounds they don't want and with lots of pollutants. Maybe this is a way to deal with TNT and RDX?

BUT problems:

Plants are unable to breakdown RDX…

However…some bacteria ARE able to breakdown RDX!



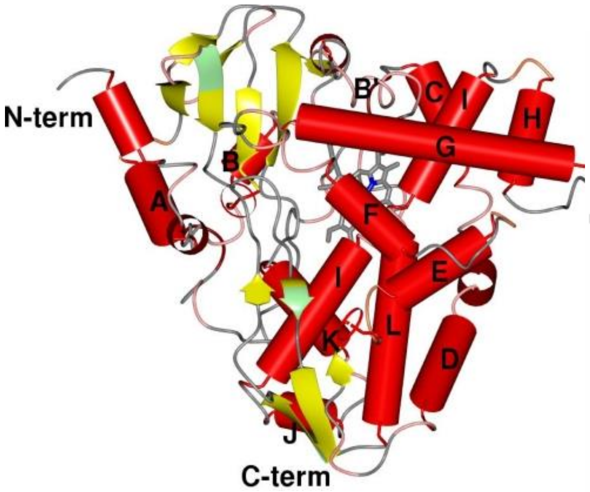
- *Rhodococcus rhodochrous* 11Y was isolated from RDX-contaminated soil
- The active enzyme: Cytochrome P450 monooxygenase XplA
- Partnering reductase: XplB

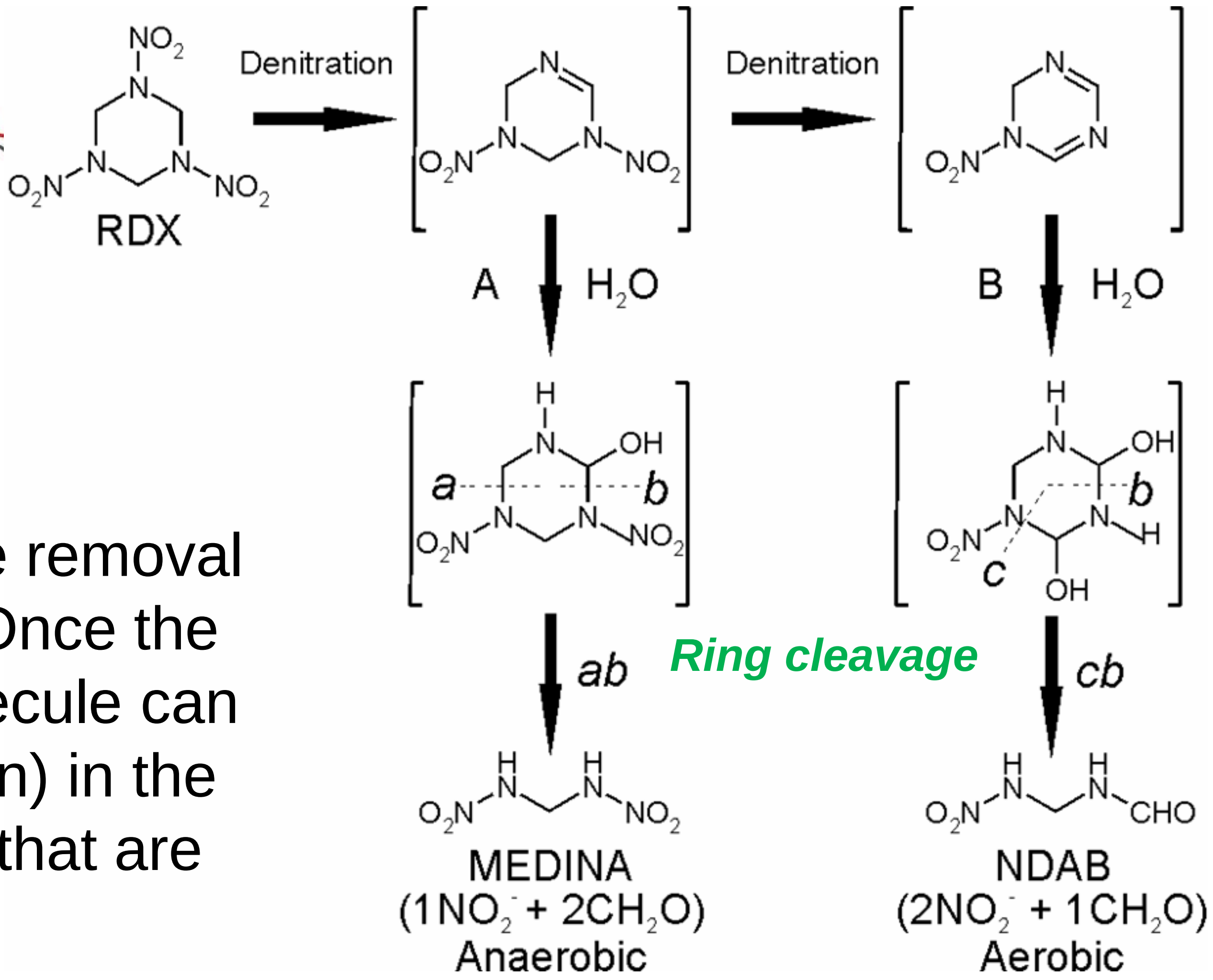
Sampling of soil on an army firing range, researchers from the University of York found bacteria that could breakdown RDX. The bacteria were not able to spread all through the soil and removed the RDX though!

Seth Smith et al. 2002 *Appl. Environ. Microbiol*.
Jackson et al. 2007. *Proc. Nat. Acad. Sci. USA.*

Rylott et al 2006 *Nature Biotech.*
Sabbadin et al. 2009 *J. Biol. Chem.*

Characterising the enzymes behind RDX degradation





XplA catalyses the reductive removal of a nitro group from RDX. Once the NO_2 group is gone, the molecule can now biodegrade (break down) in the environment to compounds that are safe.

Sabbadin et al. 2009 *J. Biol. Chem*.

Jackson et al. 2007. *Proc. Nat. Acad. Sci. USA.*

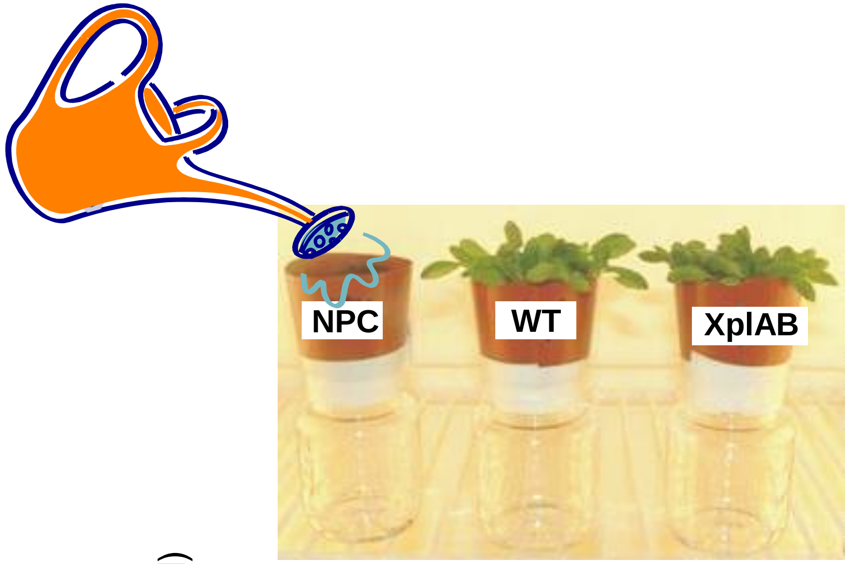
XplAB plants remove RDX from soil leachate

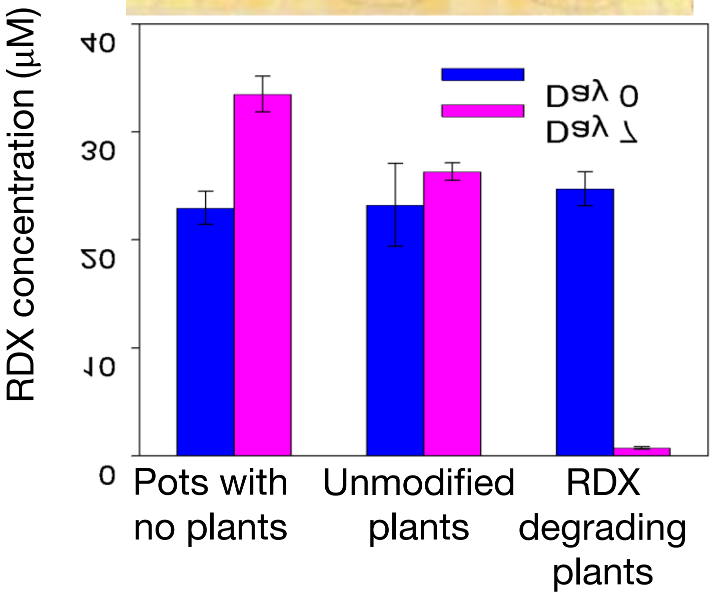
The enzyme that breaks down RDX was genetically engineered into Arabidopsis (a fast growing leafy plant that is used for lots of lab-based studies).

Water containing RDX was watered into the pots:

- A no plant control (NPC)
- Unmodified wild type Arabidopsis (WT)
- Arabidopsis containing the enzyme (XplAB).

After 7 days, the pots were flushed with water, and the XplAB-expressing plants found to have degraded all the RDX.





Rylott et al. 2006 *Nat. Biotechnol.*
Jackson et al. 2007. *Proc. Nat. Acad. Sci. USA.*

BUT Problems:

- We have a plant that breaks down RDX!
- But, plants do not like TNT

TNT is toxic to plants:

Five-week-old Arabidopsis growing in soil + TNT

0 25 50 100 250 500

mg TNT/ kg soil

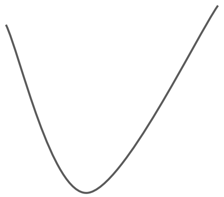
If there is too much TNT, the plant won't grow, it is too toxic, the TNT sticks to the roots and kills the plants.

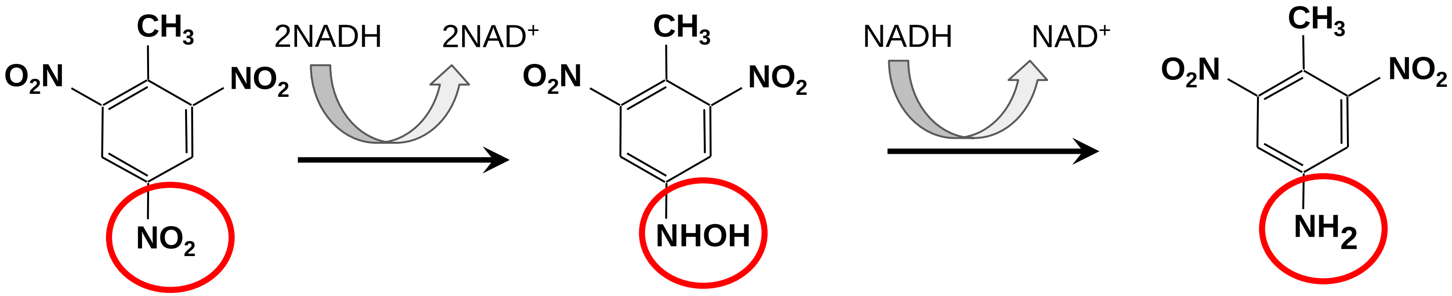
Toxic to plants but again bacteria have evolved resistance

Going back to army firing ranges, a bacteria that could deal with TNT was found!

- Bacteria isolated from TNT-contaminated soil
- Genes encoding explosives detoxification identified
- A nitroreductase isolated from *Enterobacter cloacae*

A bacterial nitroreductases detoxifies TNT





2,4,6-trinitrotoluene (TNT) | 4-hydroxylamino-2,6-dinitrotoluene (4-HADNT) | 4-amino-2,6-dinitrotoluene (4-ADNT)

Nitroreductase

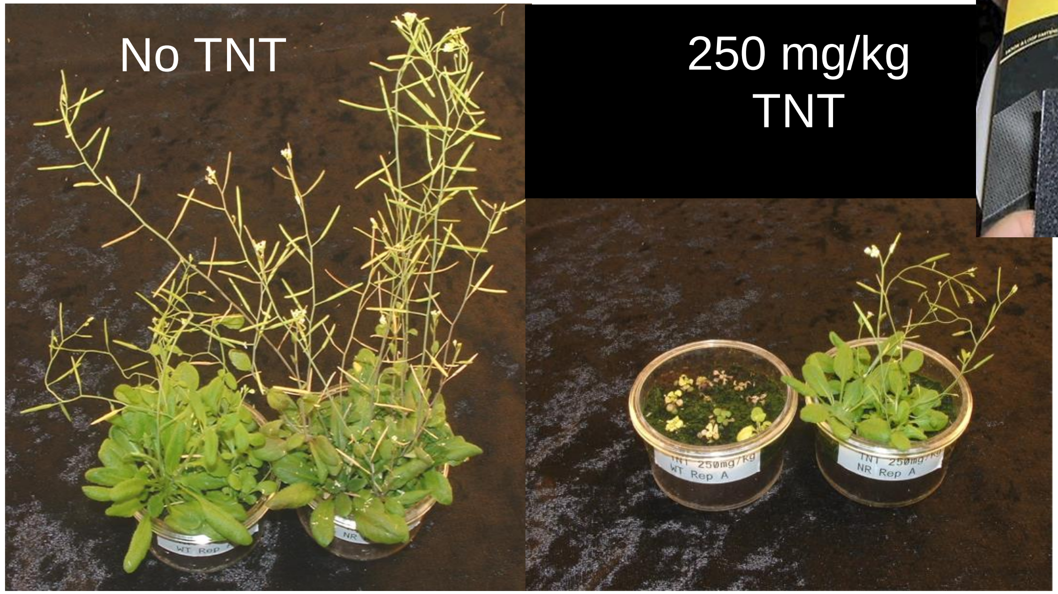
Less toxic
(and less ‘sticky’!)
compounds

Changing an NO_2 to an NH_2 doesn’t make TNT biodegrade, but it does make it safer.

Hannink et al. 2001 *Nature Biotech*.
Rylott et al. 2011 *New Phytologist*

Nitroreductase confers TNT resistance to plants

Arabidopsis plants engineered with the bacterial nitroreductase (NR) have enhanced tolerance to TNT



Unmodified +NR Unmodified +NR

Putting the enzyme into Arabidopsis (+NR) means that the plant can now grow on contaminated soil

Hannink et al. 2001 *Nature Biotech.*
Rylott et al. 2011 *New Phytologist*

Combining NR and XplAB in Arabidopsis

Unmodified plants

TNT detoxifying (NR)

RDX degrading (XplAB)

TNT detoxifying and RDX degrading (XplAB-NR)

Uncontaminated soil

250 mg/kg TNT & RDX

Combining both enzymes in one plant means it can deal with both TNT and RDX and grow vigorously even on contaminated soil.

Rylott *et al.* 2011 *New Phytologist*

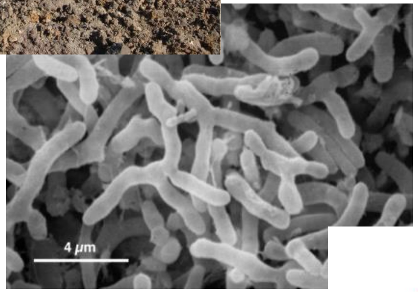
But can you drive a tank over Arabidopsis..?

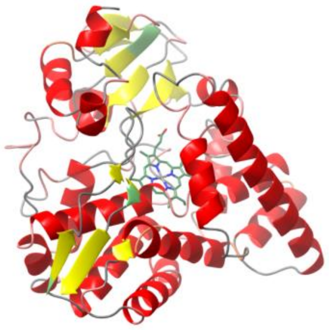
Transferring technology to US-native range grasses

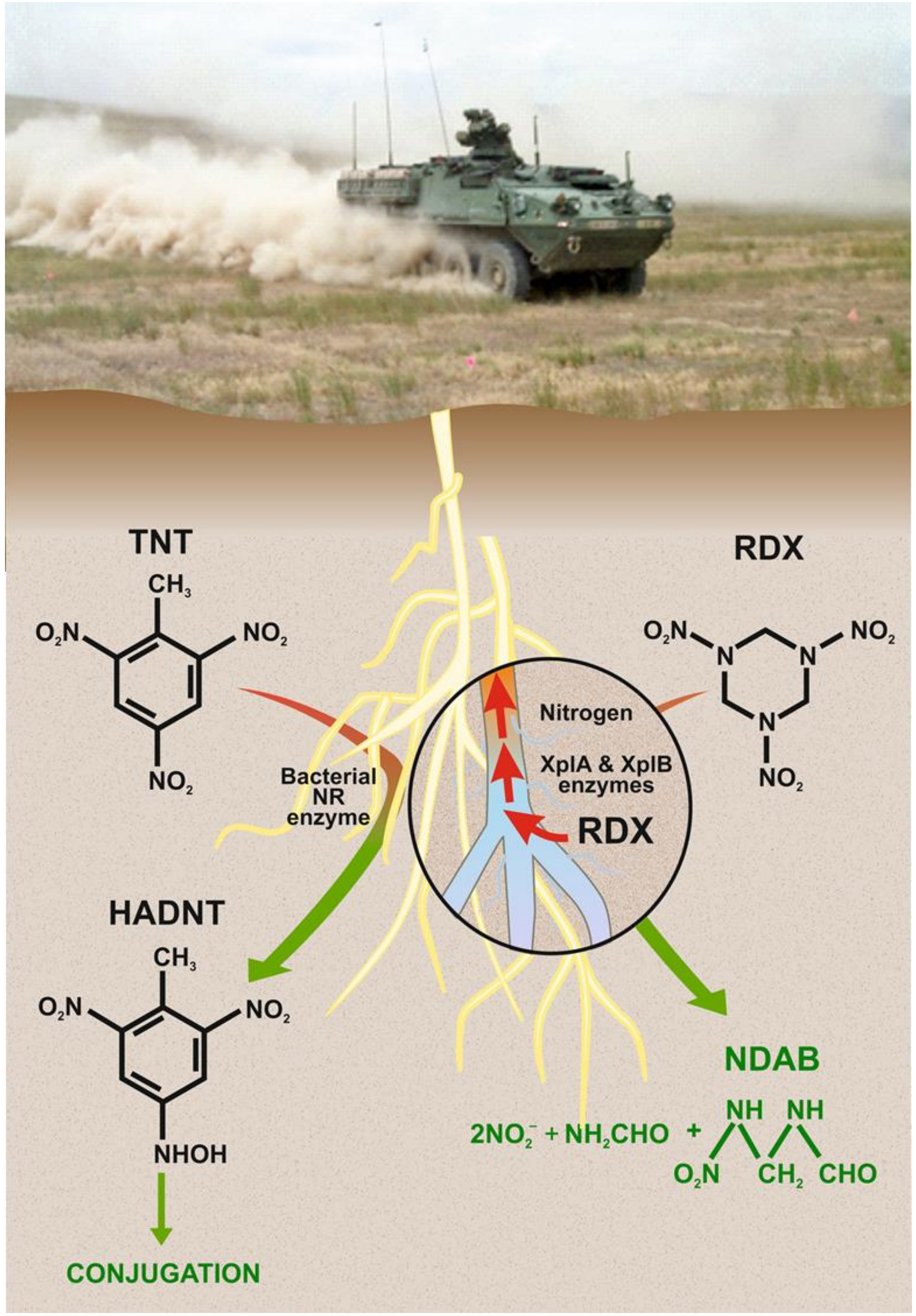
Switchgrass
(*Panicum virgatum*)

- Provide year round cover and all season growing
- Excellent adaptability for different environments (can be grown on marginal lands)
- Good erosion control (decreased windflow and evaporations, less surface run-off)
- Aesthetically enhancing
- High density and deep rooting systems: pollutant accessibility
- Excellent nesting and invertebrate habitat
- Fast growth rate
- Can be blown from the face of the earth…

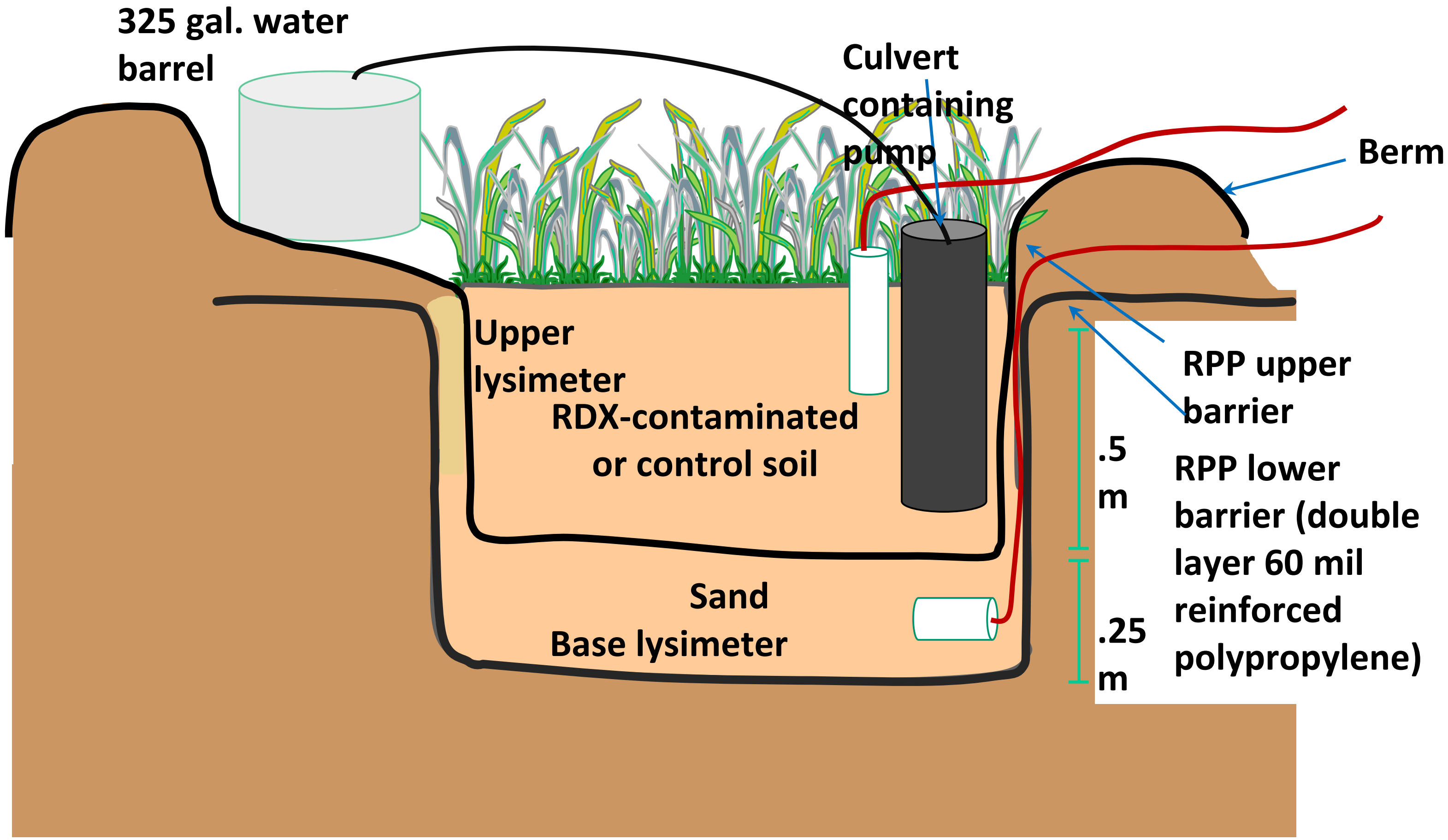
Summary: Put bacterial genes into plants
Remediate and contain explosive contamination





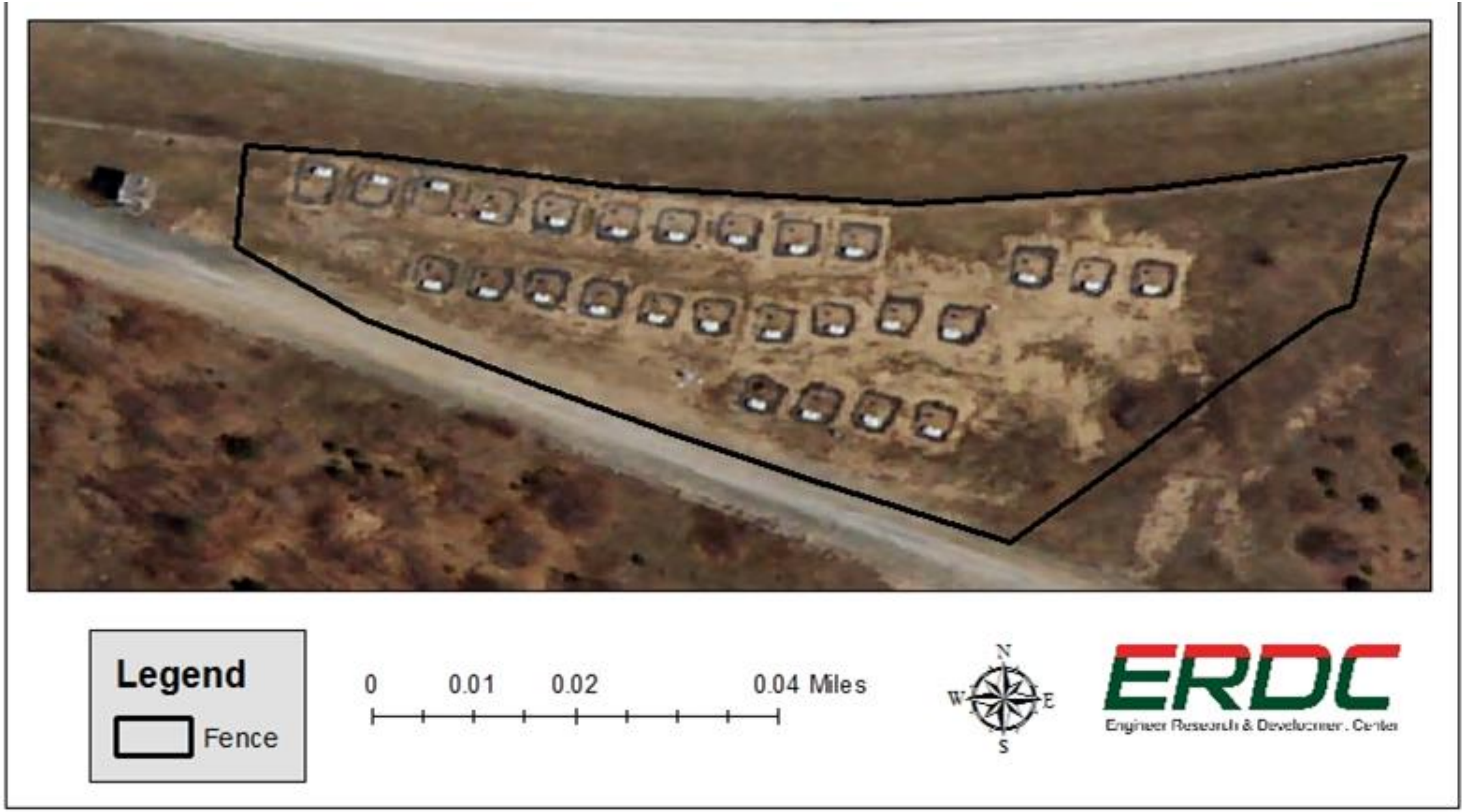


Go-ahead for a field trial



Plots were grown on a US military base but had to be contained so that no TNT or RDX was released.

Aerial view of field trial plot



Growth of switchgrass on the plots

1 June 2016

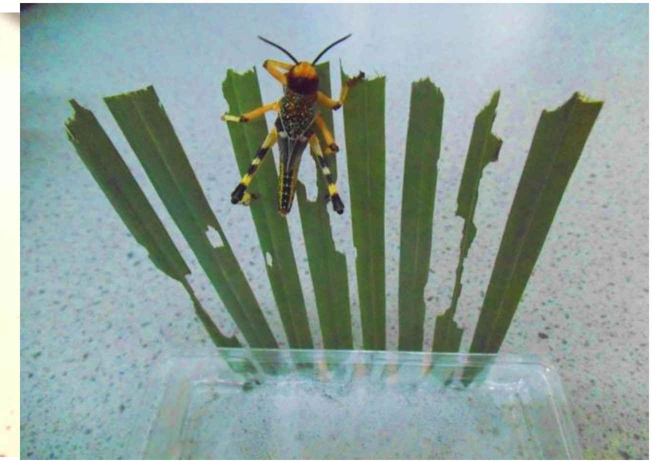
15 June 2016

12 July 2016

9 August 2016

RDX-degrading, TNT-detoxifying plants

- Remove RDX from soil leachate
- Remove RDX from soil leachate in presence of TNT
- Plants have up to 30-fold higher biomass than unmodified plants
- Plants contain 30 to 100-fold less RDX than unmodified plants



The genetically engineered switchgrass grew more than wild type plants **AND** had much less toxic RDX in them so would be safer for insects and animals to eat

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DoD EPA DOE SERDP Strategic Environmental Research and Development Program

BBSRC bioscience for the future

[dstl]® Defence Science and Technology Laboratory

How to distribute the grasses?

- Ranges contain unexploded bombs…
- How to sow the plants without getting injured?

The genetically engineered switchgrass seeds have been made into seed balls which can be dropped from a height and start growing. The plan is to to use helicopters to plant the grass over firing ranges in the USA.

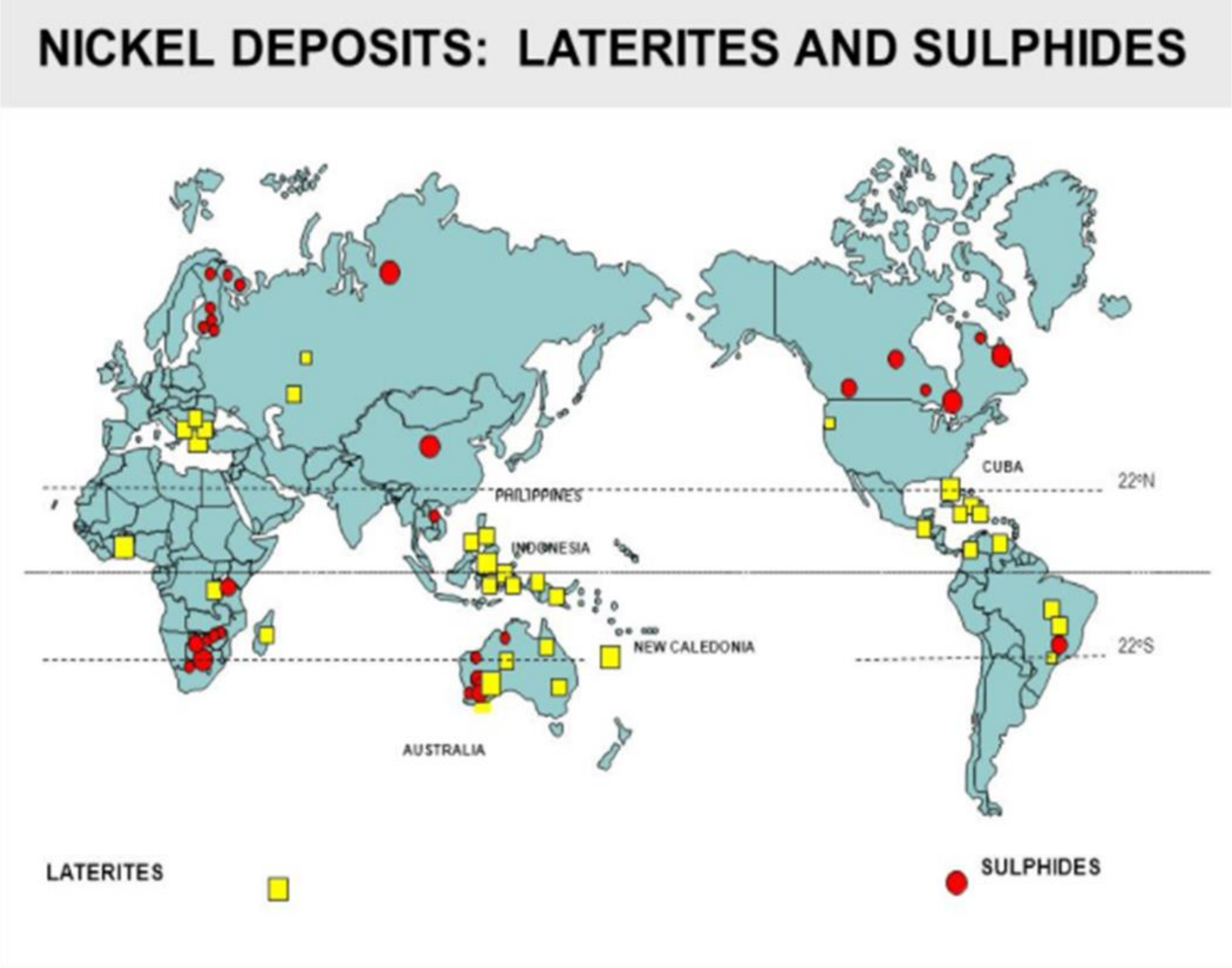
Phytomining

Case Study #2 - Nickel Phyto/Agromining

Element	'Normal' plant conc. (mg/kg)	Hyperaccumulator (mg/kg)
K	5600	7500
Ca	9600	9500
Mg	1600	7100
Fe	240	220
Co	<2	25
Cr	4	20
Mn	330	226
Ni	**27**	**12,400**

This example is of *Alyssum bertolonii*, an Italian plant that will take up lots (hyperaccumulate) of nickel.
Element concentration is mg metal / kg plant (ppm)

Data from Reeves, 1992



Map courtesy of Dr. Charles Butt

- Ultramafic soil is not suited to food crop growth
- Chemistry is wrong (low Ca, high Mg, high Ni)

- Land is essentially degraded

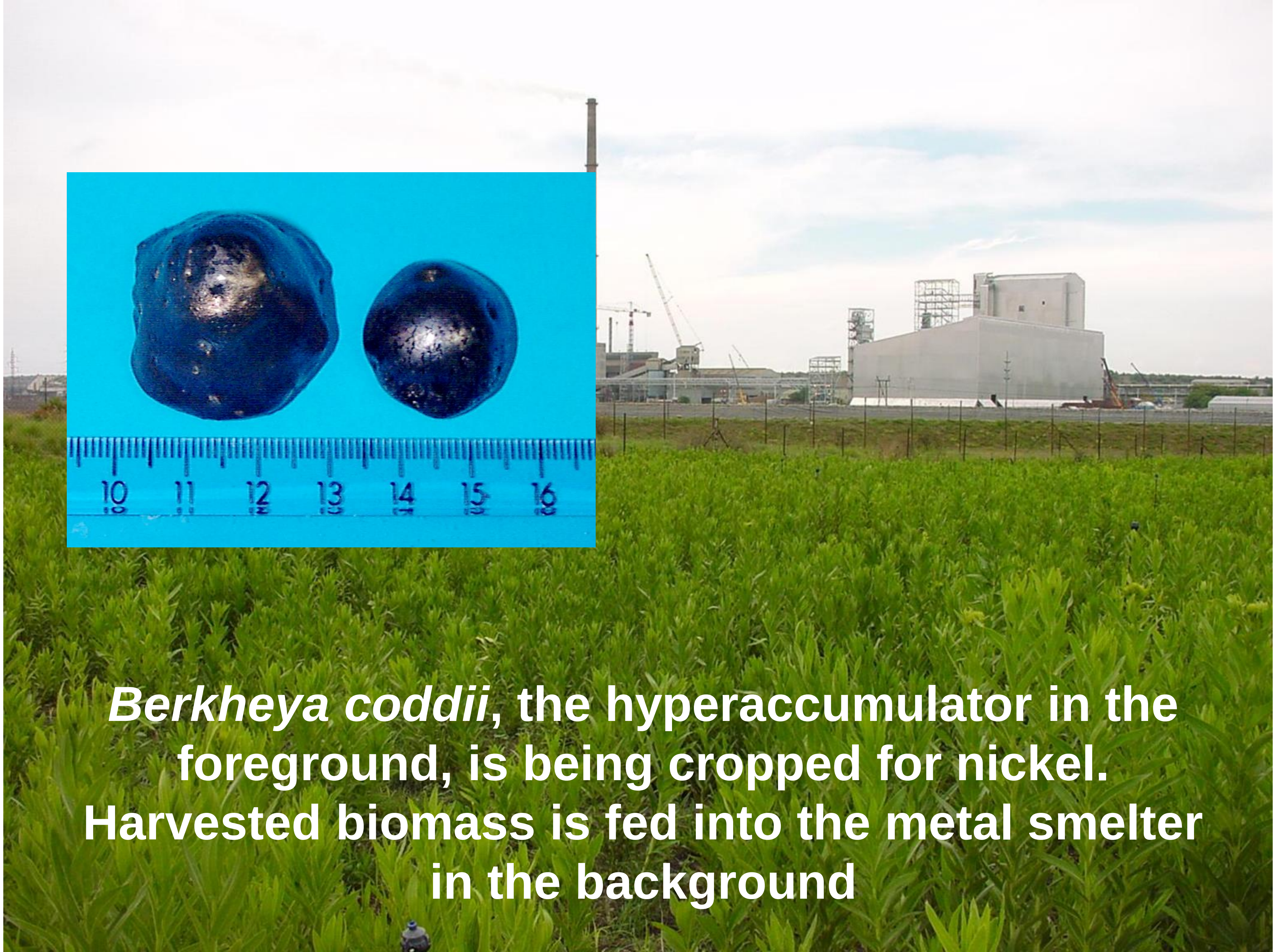
- Ultramafic ‘laterites’ exploited for Ni and vast areas have been cleared of topsoil
- ‘Agromining’ is being pushed as a viable land use in these areas

On the island of New Caladonia, there are trees where their sap is blue/green due to the very high amounts of nickel they have taken up and stored from the soil. The trees don’t need the nickel, it is their way of dealing with it.

Rinorea bengalensis

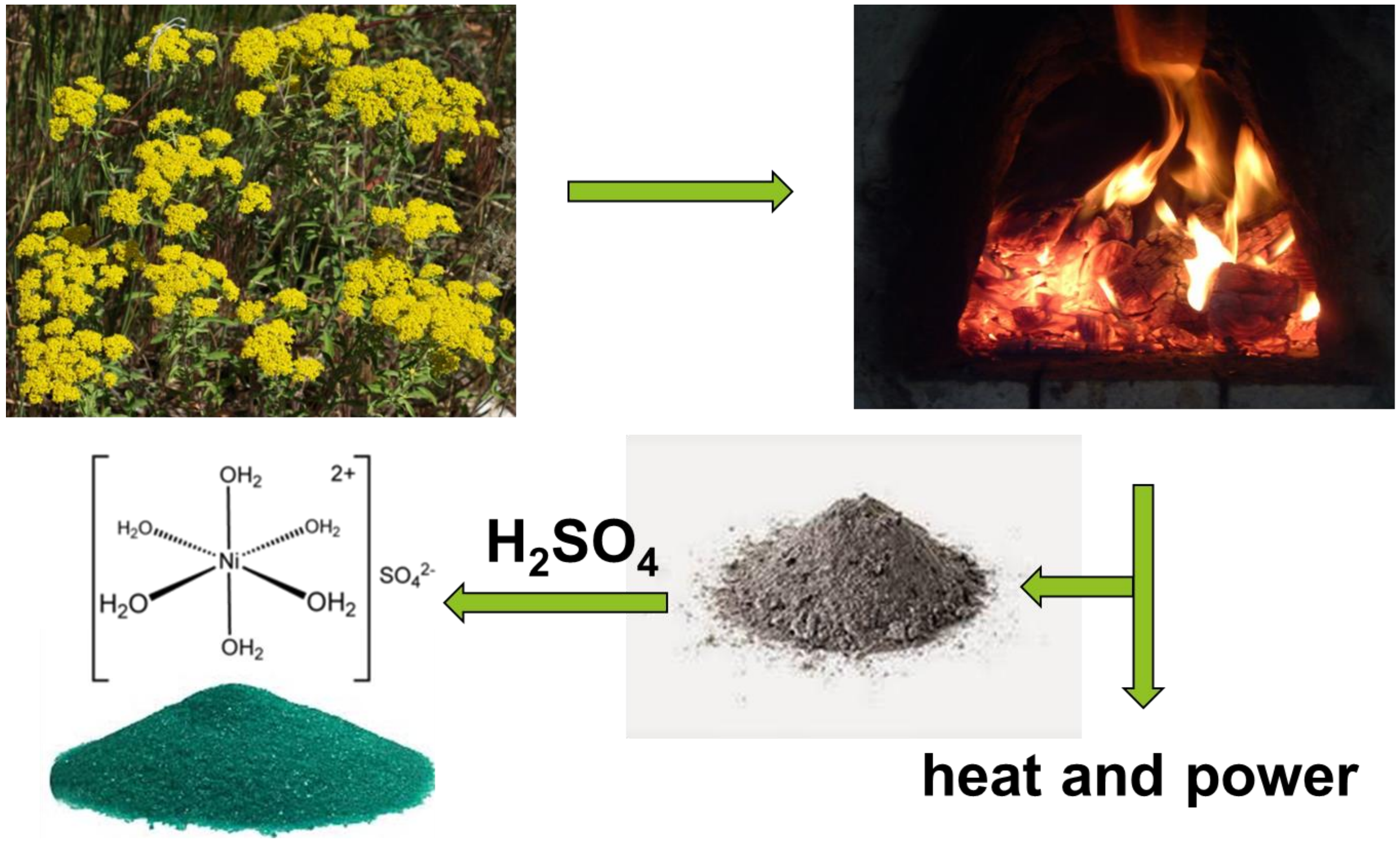
Phyllanthus securinegiodes

Photo courtesy Antony van der Ent



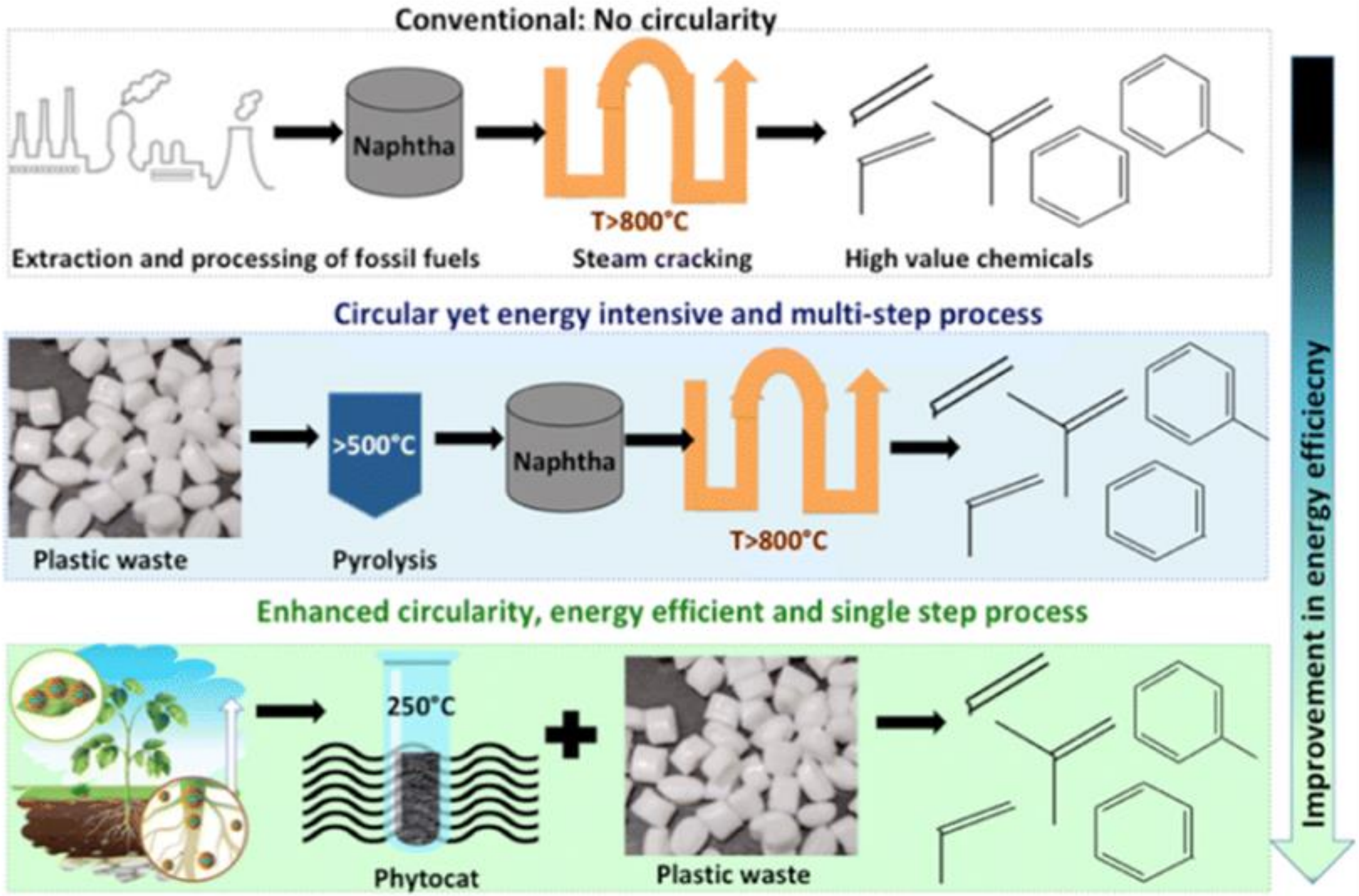
Berkheya coddii, the hyperaccumulator in the foreground, is being cropped for nickel. Harvested biomass is fed into the metal smelter in the background

Agromining



Large trials in Albania led by University of Lorraine

Alyssum bertolonii is grown on soil not fit for farming (ultramafic soil). It accumulates lots of nickel. The plant is harvest and burnt. Nickel salts are collected from the ash using an acid. This process is much more energy efficient than traditional mining, but it won’t replace mining.



Researchers at the University of York have been able to use plants containing nickel to turn very hard to recycle plastic waste (mixed plastics and very highly dyed/coloured plastics) into chemicals that can be used to make new things.

Johar et al. 2022 *RSC Sustainability.*
Johar et al. 2021 *Green Chemistry.*

The Team

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