Microbial communities in root zones of *Pennisetum setaceum* (African fountain grass) at a heavy metal contaminated site, Kabwe, Zambia

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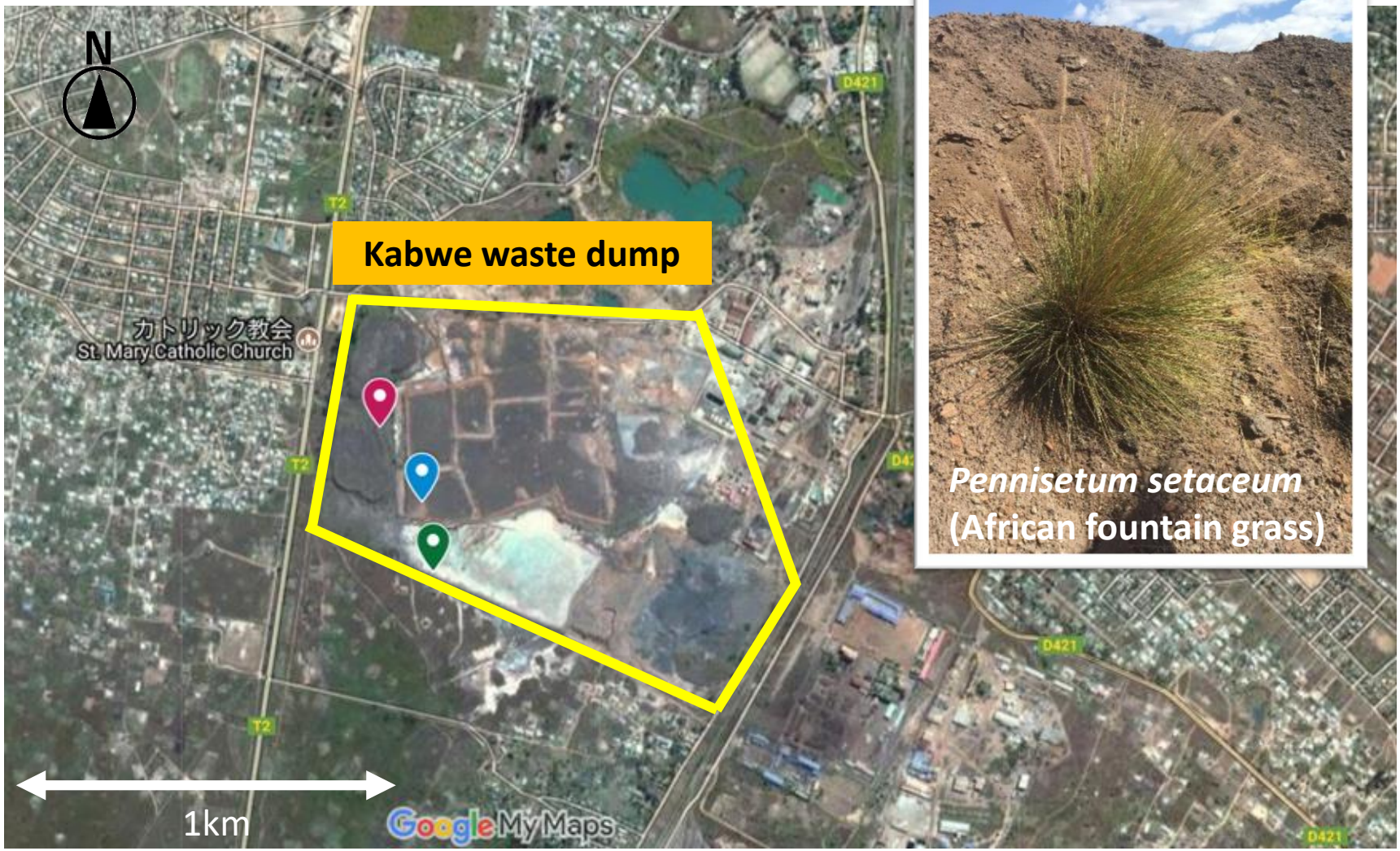
Brief view of this study

1. Microbes in the root zone of African fountain grass in Kabwe, Zambia

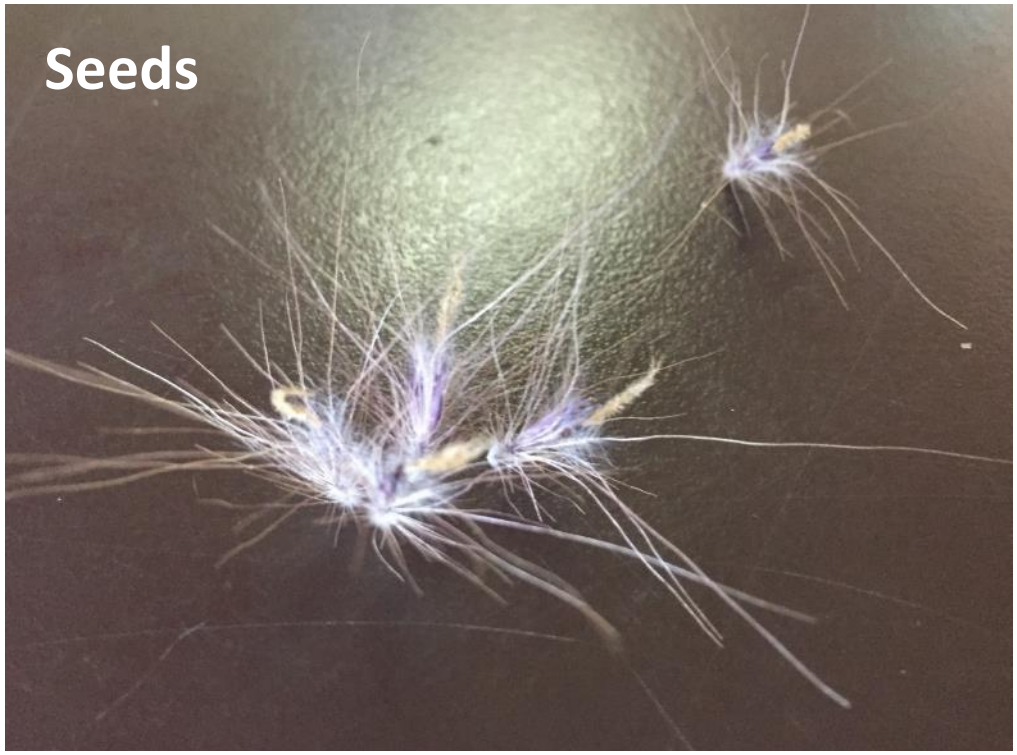
2. A new portable sequencer for microbial DNA analysis

A waste dump at the center of Kabwe city



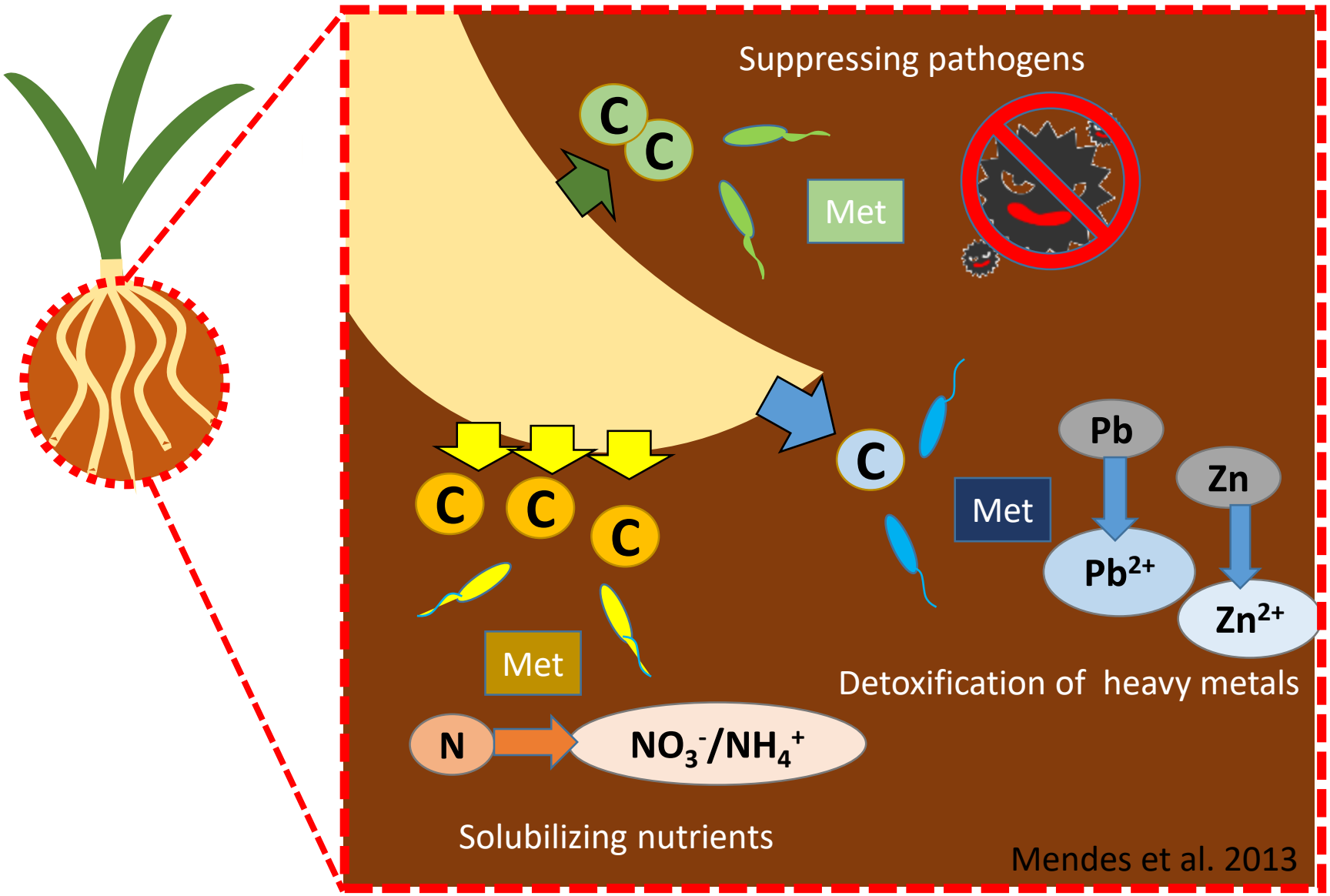
Why can they grow in such a harsh environment?

(1) Morphology



The unique characteristics protect African fountain grass from several stresses (e.g. heat and drought).

(2) Microbes in a root zone



Previous technique

- Isolation of single microbial species
- 1% of soil microbes can be isolated

New technique

- Multiple microbial species can be identified at the same time
- Non-culturable microbes are included

DNA level studies for microbial community

DNA samples
from any environments

Iontrrent
PGM

- High running cost
- Clean and well-equipped environments

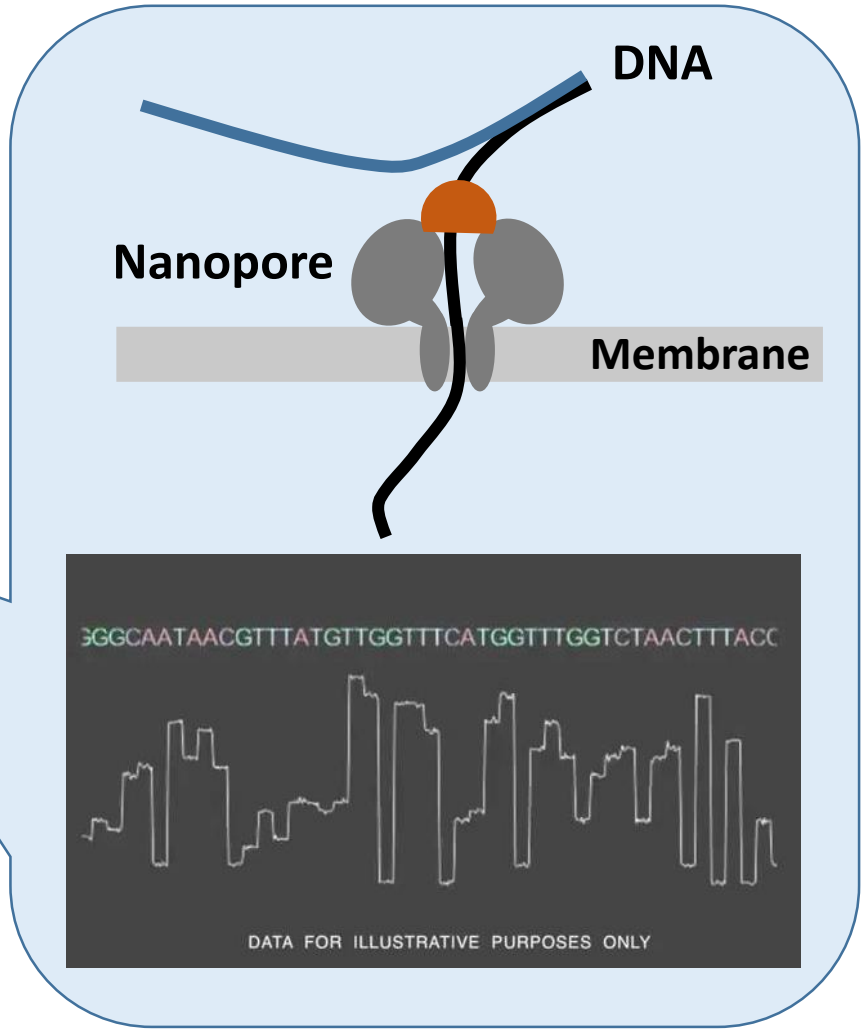
Reference database

Esherichia coli
GGTCATTAAATACTA

Obtained sequence data

GGTCATTAAATACTA
AGCCTTCCCATATAG
CGTTTCCTTCCATAA
ACTTCATATAGTTGA
GGCCTTATAGCCCAG

New sequencing device “MinION” (Oxford Nanopore)



- USB-like device
- Reusable

We have to evaluate the usability of MinION sequencer for analyzing microbial community in Zambia

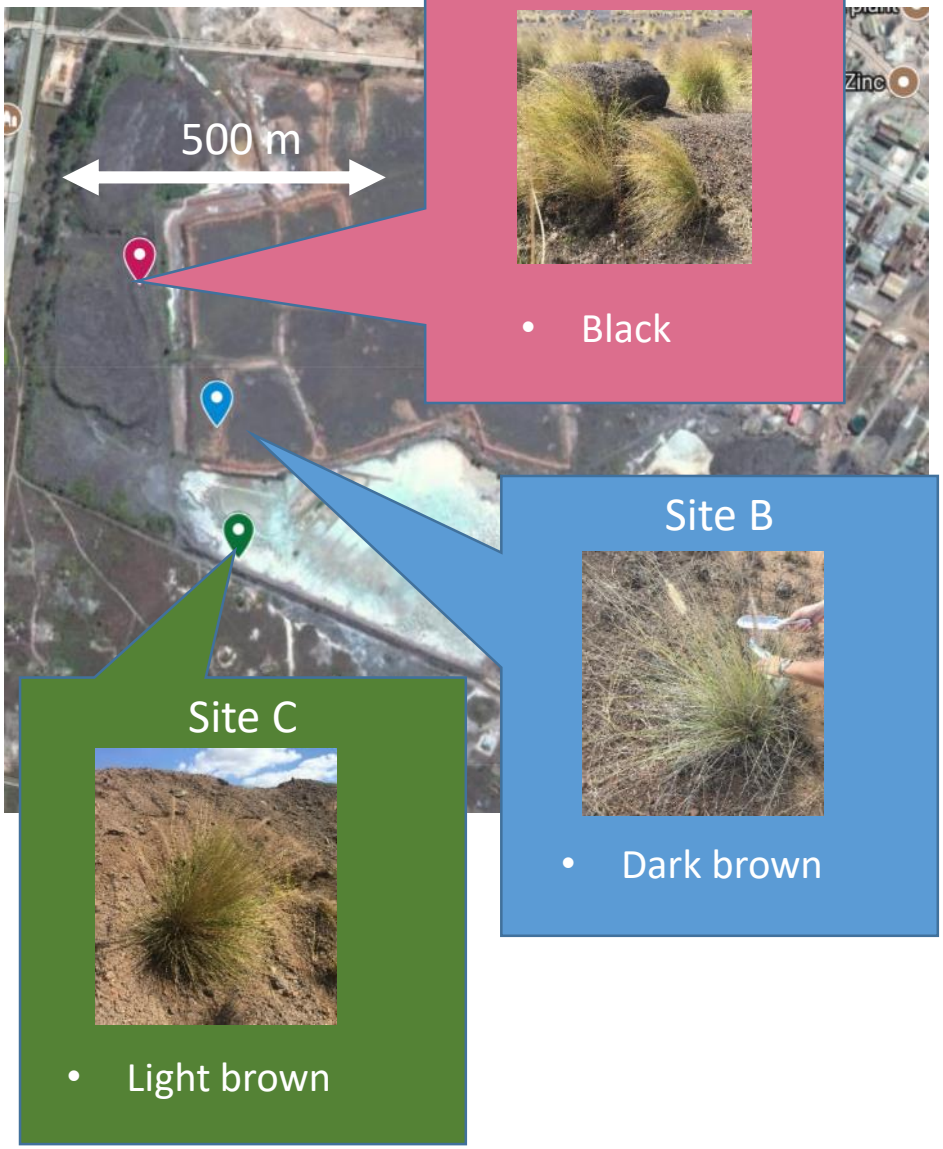
The purposes of the study

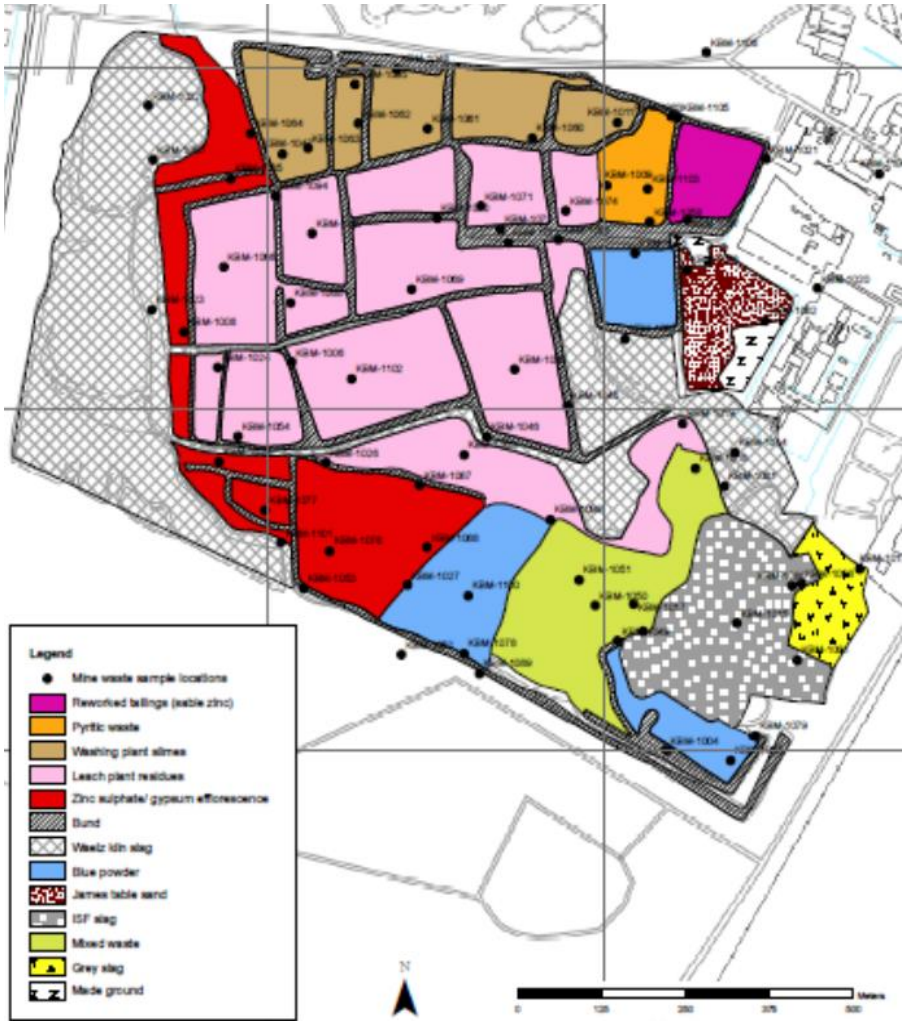
1. **To identify microbes in the root zone of African fountain grass in Kabwe, Zambia**

2. **To evaluate the usability of MinION sequencer for analyzing microbial community**

Sampling in June, 2017

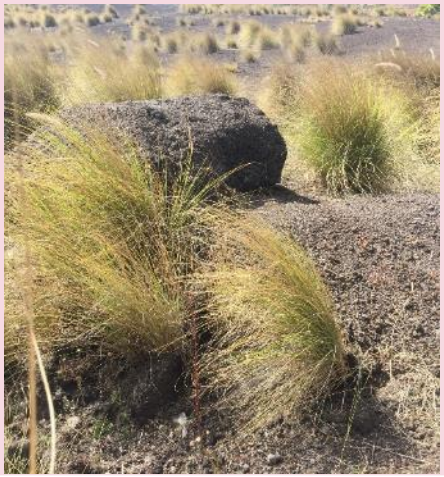
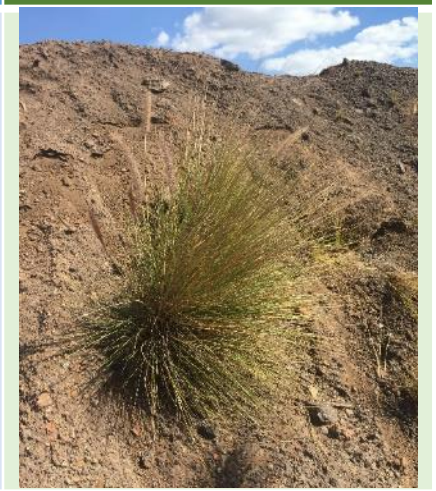
- African fountain grass
- Different colored soils





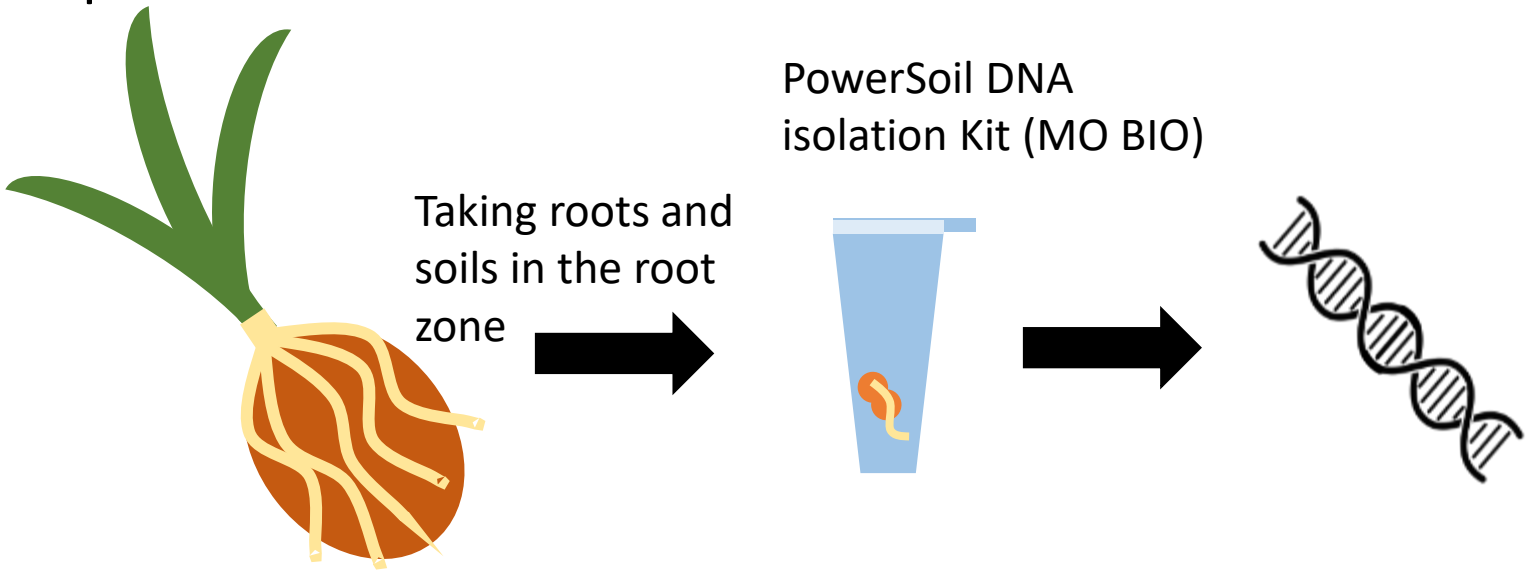
Reference:Kabwe scoping and design study phase 1 complement report by Water Management Consultants Ltd, UK (2006)

Soil properties

	Site A	Site B	Site C
			
pH(H_2O)	8.73	6.39	7.22
Total C [g kg dry soil]	148	50.8	9.43
Total N [g kg dry soil]	5.30	3.20	0.38
Total Pb [mg kg dry soil]	3732	11573	19319

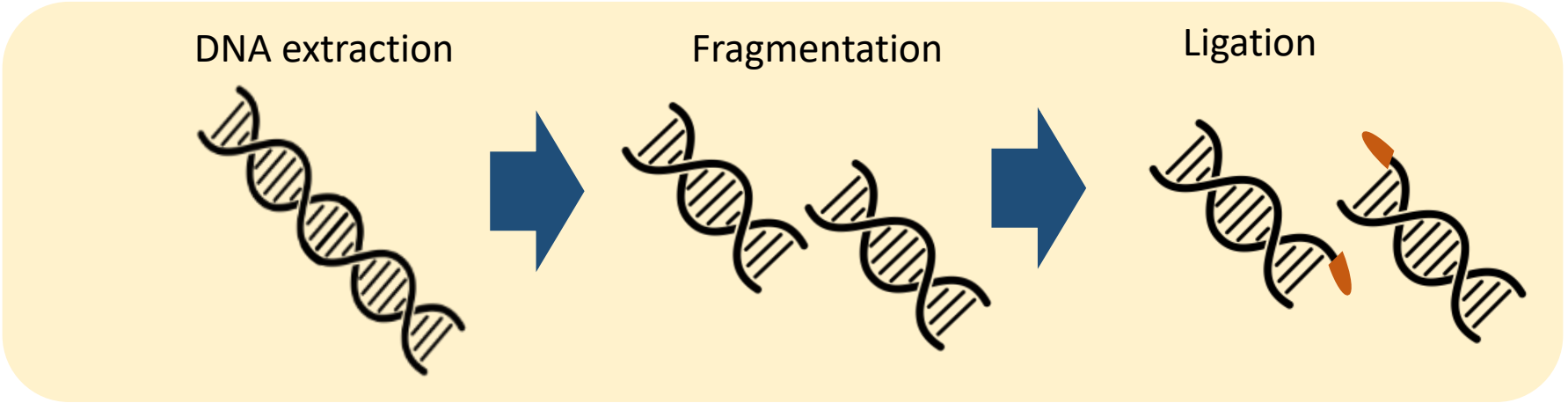
Microbial analysis

- Step 1: DNA extraction

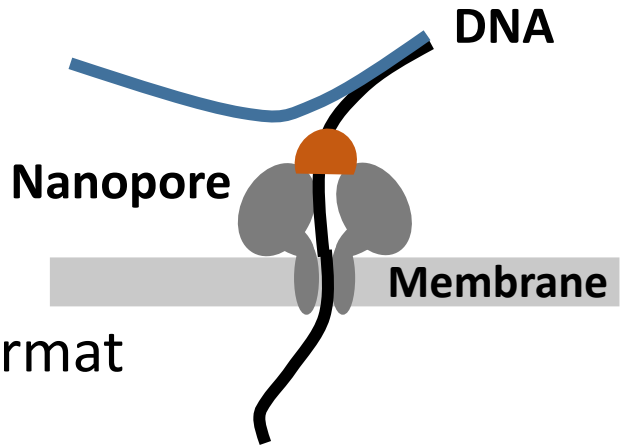


- Step 2: PCR-free preparation

  Rapid Barcoding Kit (Oxford Nanopore)

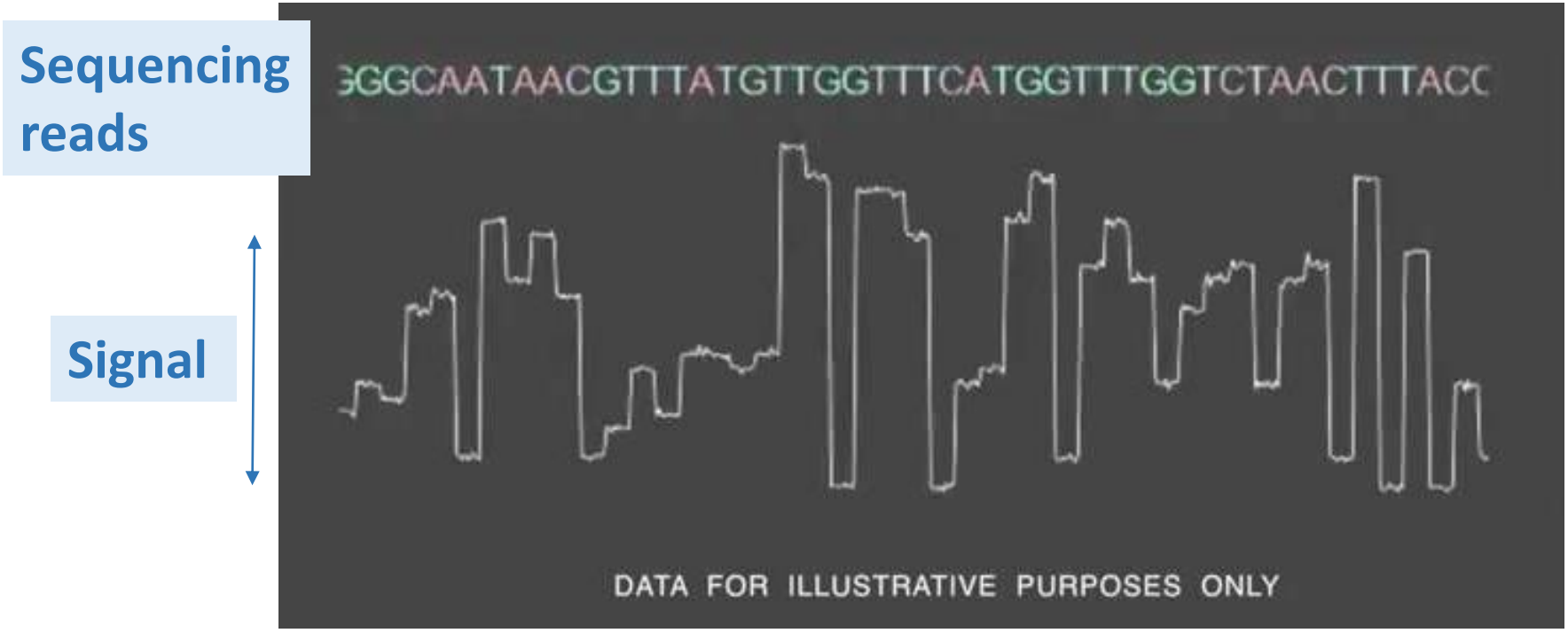


Step 3: Sequencing



- MinKNOW software
  - To collect sequencing dataset in Fast5 format
    - Signal data
    - Sequencing reads (e.g. TAACG…)
    - Quality scores (e.g. 19,20,22,18,20)

*It shows reliability of the sequencing read data.



Step 4: Sequence classification

- What's in My Pot (WIMP) workflow

1) Filtering

To remove sequences with low quality (QS)
[Averaged QS < 10]

Sample 1

G	C	C	C	A	T	T	…
15,	13,	16,	7,	10,	13,	10…	

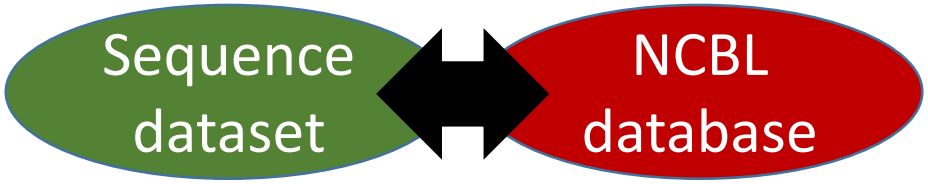
Further analyzed

Sample 2

G	C	C	C	A	T	T	…
5,	9,	10,	7,	10,	9,	10 …	

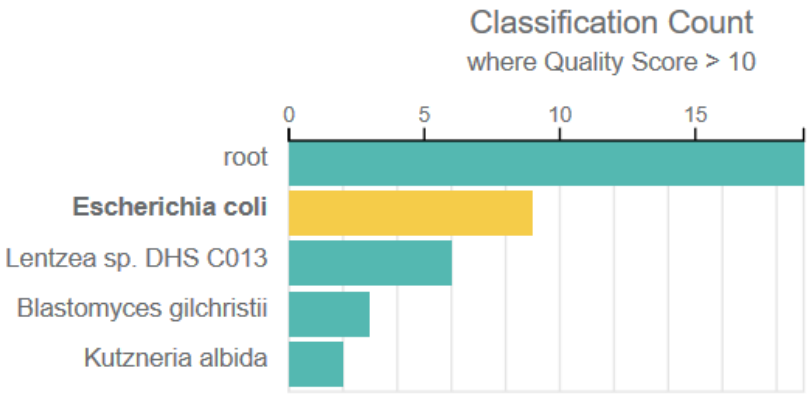
Removed

2) Classification



Sample 1
GGTCATTAAATA…

Escherichia coli
GGTCATTAAATA…



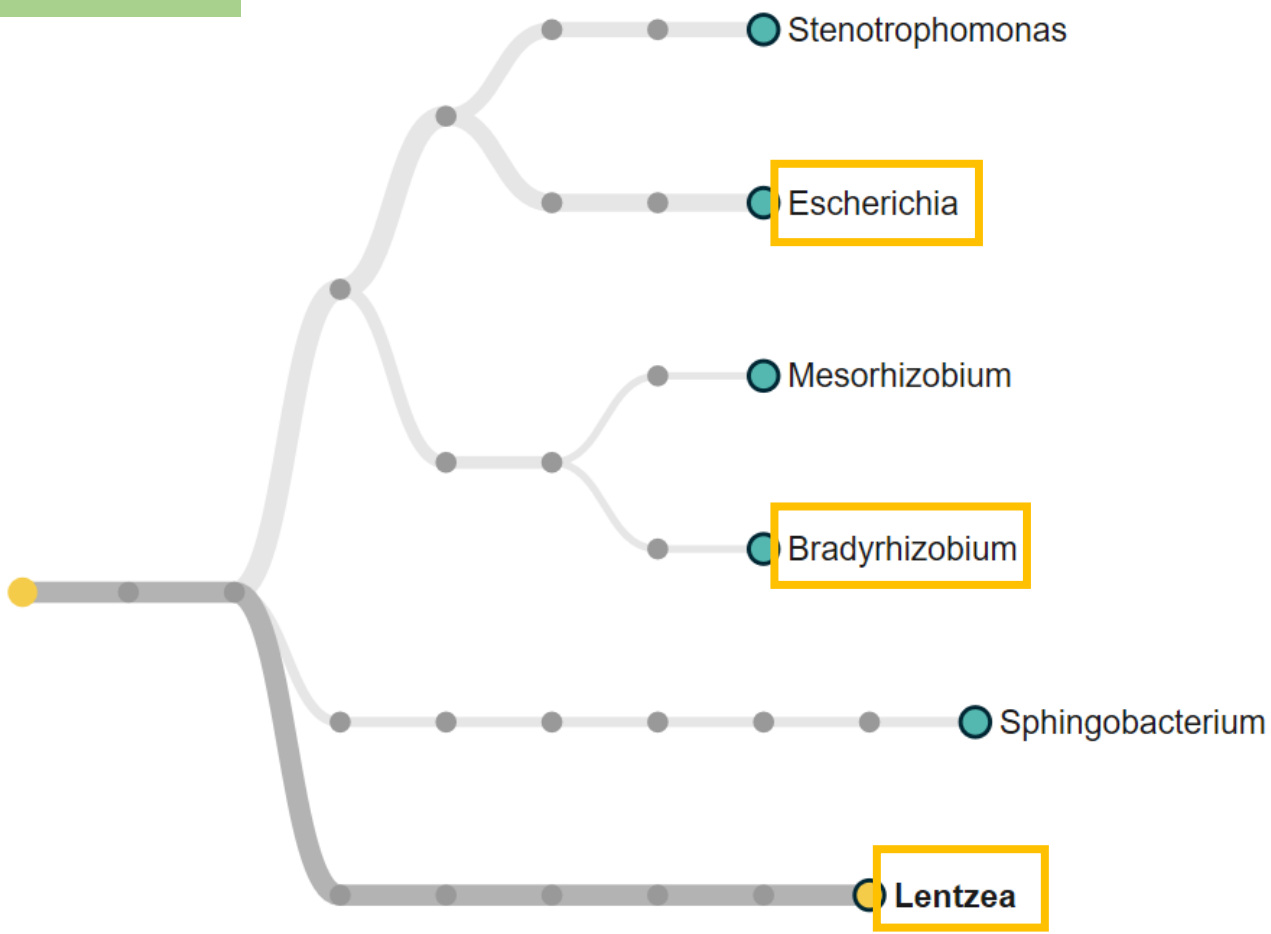
Result and discussion: Classification

	A	B	C
No. of reads	897	220	618
No. of classified reads	106	32	86
No. of identified species	6	8	11
Taxa (QS>10)	**Root**	**Root**	*Lentzea*
	Escherichia	*Escherichia*	**Root**
	Lentzea	*Bradyrhizobium*	*Bradyrhizobium*
	Bradyrhizobium	*Mesorhizobium*	*Escerichia*

- The reads with low quality were removed during the filtering step for the classified reads (Average quality scores 7.15).
- Long reads with higher quality are more likely to be classified (min 5 bases, max 220,480 bases).

Result and discussion: Phylogenetic tree

Site C



Result and discussion: Common bacteria among the different sites

Escherichia spp.

- Ubiquitous soil bacteria

Lentzea spp.

- Capable of degrading high molecular weight ester [3]

Bradyrhizobium spp.

- Symbiotic bacteria of legume plants
- N_2-fixation [4]
- Common in South Africa and Western Australia soil [5]

Photo from "*A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships*"

[3] Jarerat et al. 2002, [4] Zahran et al. 1999, [5] Stępkowski et al., 2005

Conclusions

1. Despite the low quality scores, the MinION data showed the key bacteria in the root zones.

2. *Bradyrhizobium.* spp and *Lentzea.* spp were common bacteria among the three sites.

Future research

- The number of classified sequences should be improved by modifying the protocols.
- Can *Bradyrhizobium.* spp and *Lentzea.* spp detoxify heavy metals?

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