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Evaluation of the NTM Elite Agar for cultivation of non-tuberculous mycobacteria from different types of environmental samples

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A commercially available medium, NTM Elite Agar (bioMérieux, Poland), is highly recommended for cultivation of rapidly growing mycobacteria (RGM) from clinical samples [1][2]. However, to date its applicability for environmental samples was tested only on water samples [3]. The aim of this study was to evaluate the performance of the NTM Elite agar for NTM isolation from four different types of environmental samples i.e. natural and potable water, air and soil.

MATERIALS AND METHODS

This study included **63 environmental samples (12 natural water, 14 potable water, 31 air, 6 soil)** collected from April to July 2023 in two major cities in Poland, i.e. Warsaw and Cracow. All samples were cultured on two types of media, i.e. (i) **NTM ELite Agar (RGM)** and (ii) modified **Middlebrook 7H11 (MG25)** agar, parafilm-sealed, and incubated at 30°C for 6 weeks, weekly checked for mycobacterial growth. Detailed **methodology of processing water** (**Fig. 1**), **soil** (**Fig. 2**) and **air** (**Fig. 3**) **samples** is depicted below.

WATER SAMPLES

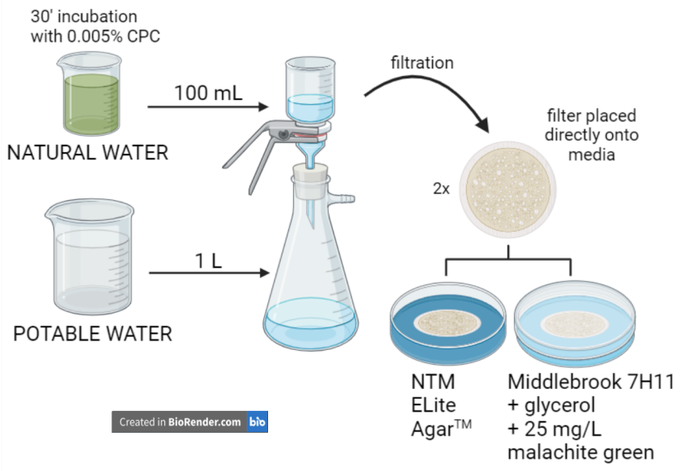

Fig. 1. Prior filtering, 100 mL of natural and 1 L of potable water samples were decontaminated using 0.005% CPC. Filter membranes were placed directly onto agar media plates.

SOIL SAMPLES

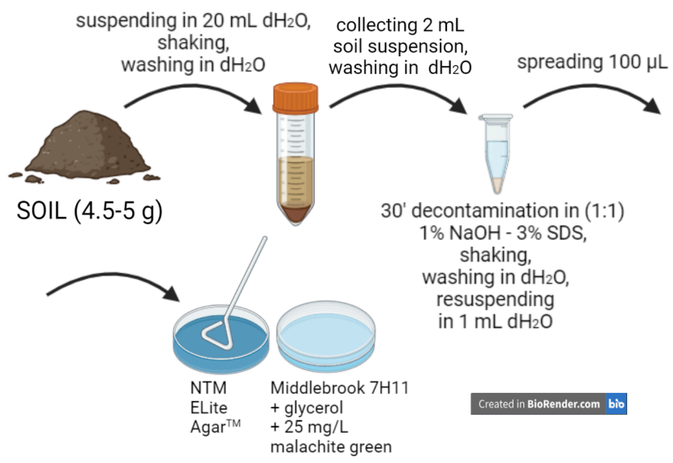

Fig. 2. Soil samples (4.5-5 g) were decontaminated for 30 min. with 1% NaOH-3% SDS solution, centrifuged, washed twice, and then spread (100-uL suspension) onto plates.

AIR SAMPLES

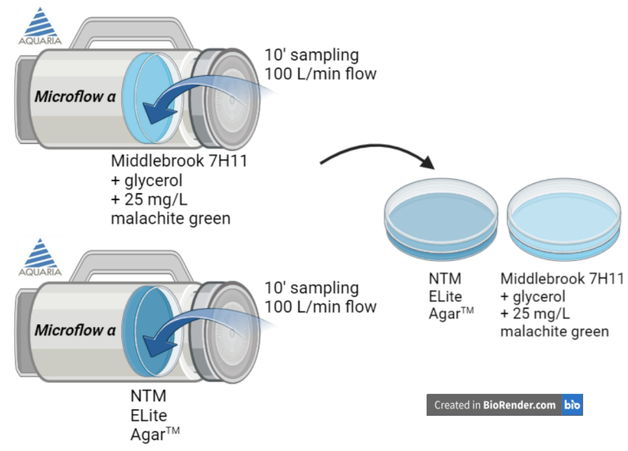

Fig. 3. Air was filtered directly onto plates using *Microflow α* (Aquaria, Italy) sampler.
Each sampling too 10 min. (100 L/min. flow; 1 m^3 filtered).

RESULTS

Of all **126 cultures, 62 (49.2%) overgrew with moulds** over 6-week incubation. Each media manifested moulds growth on approximately half **(31/63 each; 49.2%)** of the plates. However, the **commercial medium was almost twice more effective in recovering NTM** from environmental samples. Out of **184** colonies suspected to be NTM, ca. **2/3 (121; 65.8%)** were retrieved from RGM, while only **1/3 (63; 34.2%)** from MG25. Exeamplary plates are presented on **Fig. 4**.

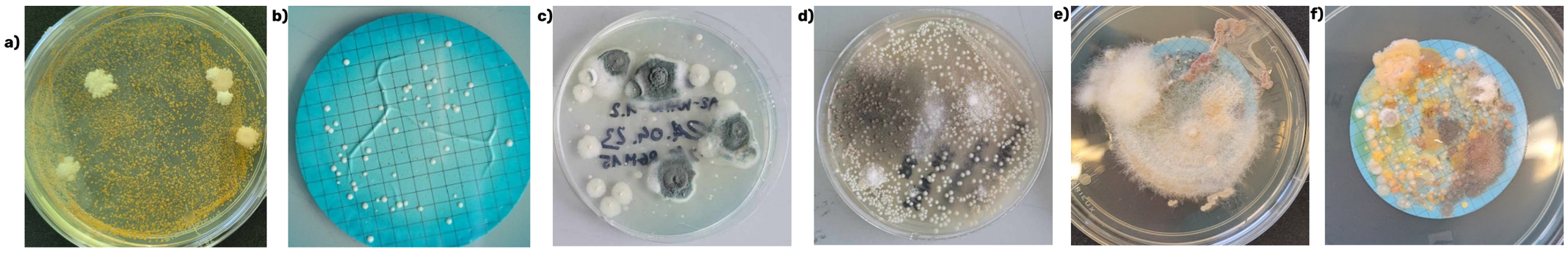

Fig. 4. No moulds growth on **a)** RGM (soil sample), **b)** MG25 filter (water sample); **moulds overgrowth** on **c)** RGM (air sample), **d)** MG25 (soil sample), **e)** RGM (water sample) **f)** MG25 (water sample).

CONCLUSIONS

- For environmental samples, **different decontamination methods** should be applied for each sample type.
- There was **no difference** between NTM Elite and modified Middlebrook 7H11 agar media in preventing mould overgrowth when culturing environmental samples.
- NTM Elite Agar provided a **higher mycobacterial recovery** than Middlebrook 7H11 agar.

Related Literature

[1] Broncano-Lavado et al. J Clin Microbiol. 2023 Apr 20;61(4):e0003623. **doi: 10.1128/jcm.00036-23**.

[2] Ditommaso et al. Int J Environ Res Public Health. 2022 Aug 26;19(17):10645. **doi: 10.3390/ijerph191710645**.

[3] Alexander et al. PLoS One. 2021 Mar 3;16(3):e0247166. **doi: 10.1371/journal.pone.0247166**.

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