Tracking soil contaminants using in vitro toxicity assays

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Background

•Soil contamination poses a significant and ongoing risk to both the environment and human health
•With a primarily anthropogenic origin, the effects of contamination upon human health remains poorly understood
•The quantitative measurement of known contaminants in the soil, although commonly used to determine contamination neither

1- reflects the effects of contamination on human health;
2- considers the possible additive toxic effects of contaminants in combination nor
3- identifies the presence of previously unidentified toxic chemicals

•We therefore hypothesised that soil samples could be collected and extracts prepared and tested in cell based assays in order to screen for toxic effects using a variety of viability and gene reporter assays.

Methods

• 13 soil samples were taken from around the boundary of a functioning waste site

• 3 control soil samples were taken from distant regions (2 suburban and 1 rural)

• Methanol or chloroform extraction was used to generate aqueous, alcohol and organic extracts from each sample

• The toxicity of these fractions was determined using cell viability and apoptosis assays (MTT reduction, trypan blue staining and caspase activation), a Seahorse bioanalyzer to assess mitochondrial function and using aryl hydrocarbon receptor and oestrogen receptor reporter constructs

Discussion

•MTT and caspase activity assays showed that several soil samples, and particularly phosphates 1 and 2 caused cell cycle arrest and apoptosis

•Assessment of mitochondrial function showed that phosphates 1 and 2 were also potent inhibitors with a similar effect to the known ATP synthase (complex V) inhibitor oligomycin

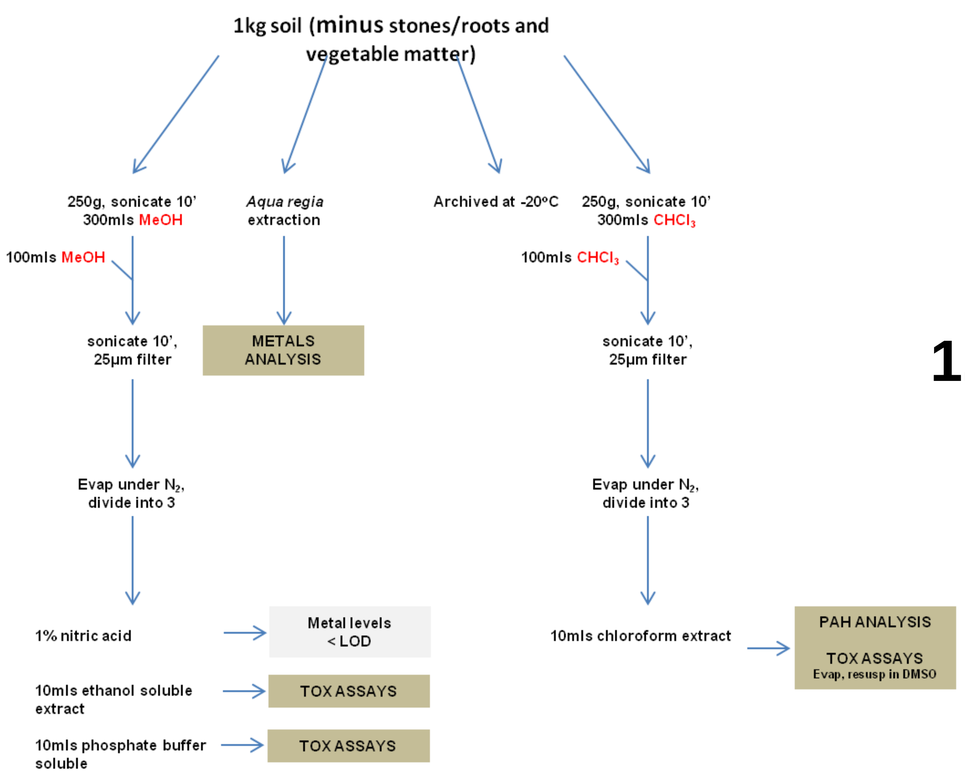
•Reporter assays showed that the organic and ethanol extracts were strong activators of the AhR and ethanol extracts of the oestrogen receptor

•These data demonstrate that extracts can be generated from soil and tested in cell-based toxicity screens and in this study this approach demonstrated that soil from around a waste site contains toxic chemicals which could pose a risk to human health

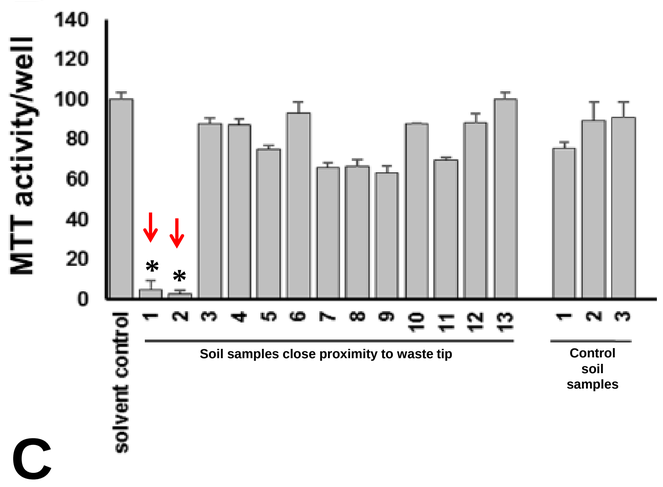
•Use of this approach at contaminated locations would enhance our understanding of the potential effects of contamination upon disease

Results

Figure 1. A



1. B



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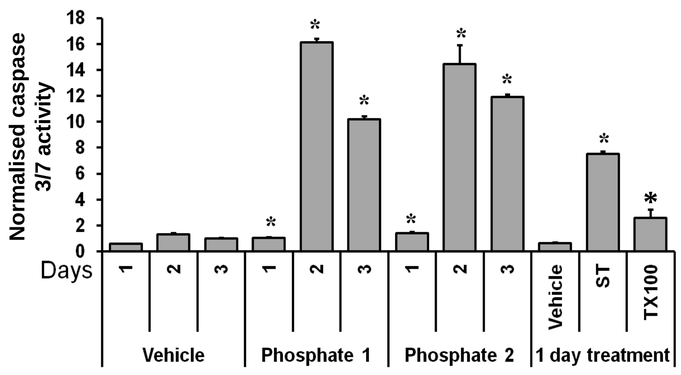
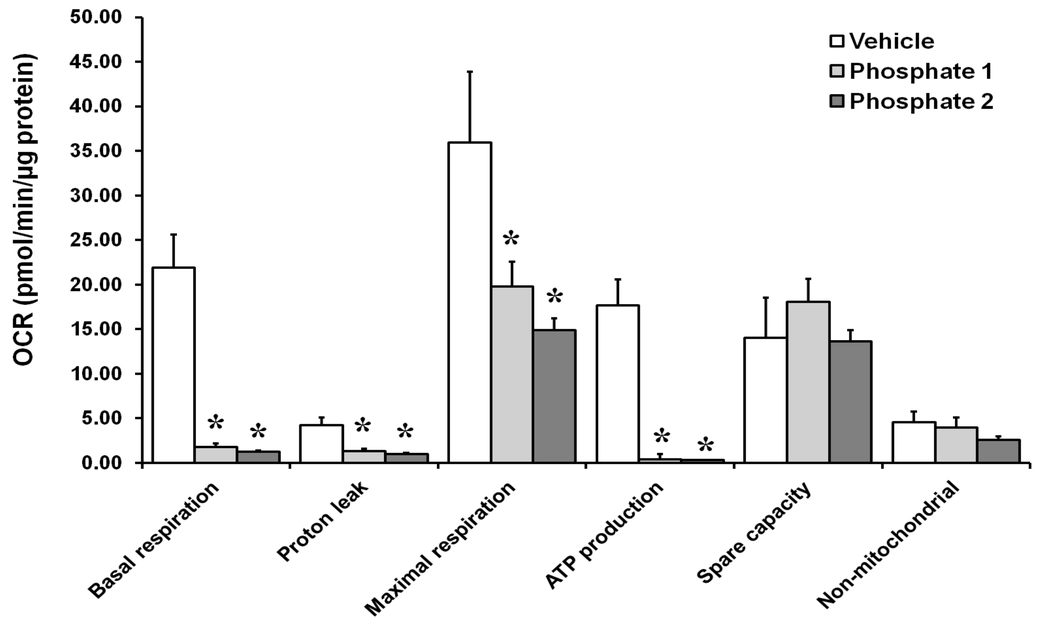


Figure 1: Phosphates 1 and 2 significantly reduce proliferation and induce apoptosis. A. Sampling methodology. **B.** MTT activity in AR42J-B-13 (B-13) cells treated with 1% (v/v) waste site and control phosphates over 24 hours. **C.** Caspase 3/7 activity in B-13 cells treated as indicated. Abbreviations: ST- 1µM staurosporine. ;TX100- 0.1% (v/v) triton X-100. For **B** and **C**, bars indicate mean and SD. * indicates significant difference compared to respective control at p<0.05.

Figure 2. A



2. B

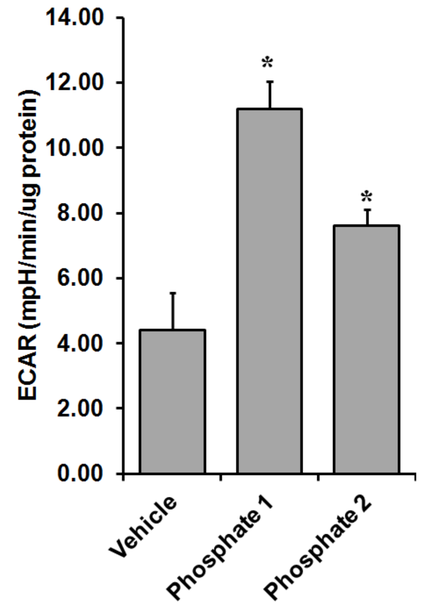
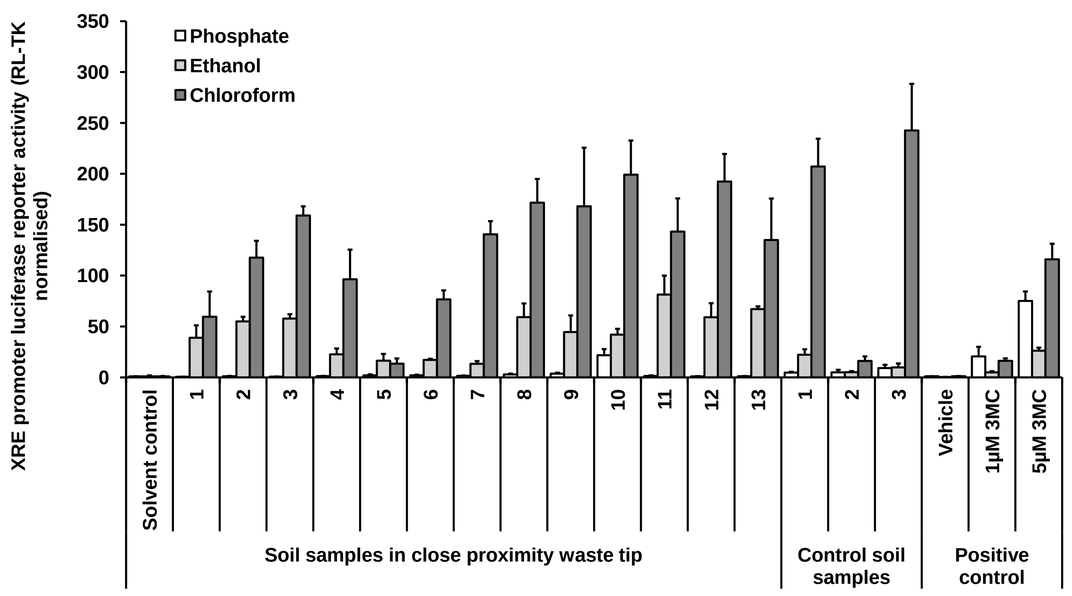


Figure 2: **Phosphates 1 and 2 impair mitochondrial function. A.** Mitochondrial parameters in response to phosphates 1 and 2 (1% v/v) in B-13 cells after 1 hour. Determined using a Seahorse bioanalyzer in combination with the Mito Stress Test Kit and expressed as normalised oxygen consumption rate (OCR). **B**. Extracellular acidification rate (representative of glycolysis) in B-13 cells treated as A. Bars are the mean and SD. * indicates significant difference compared to respective control at p<0.05.

Figure 3. A



3. B

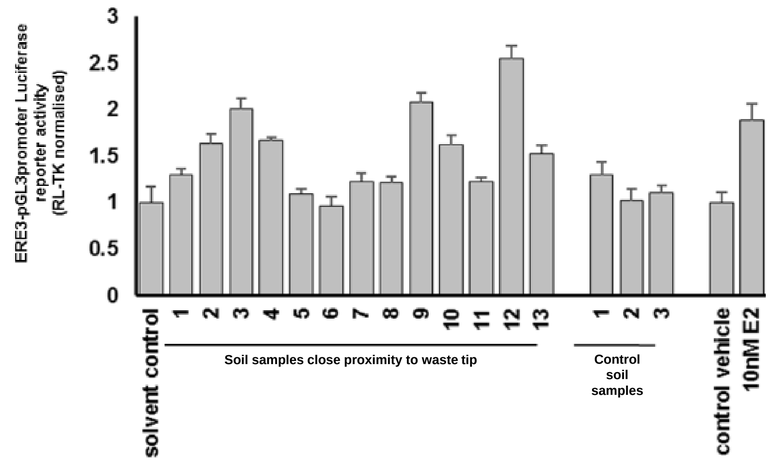


Figure 3: **Soil samples activate the aryl hydrocarbon receptor (AhR) and oestrogen receptor (ER)**. **A**. XRE-LUC reporter activity in HepG2 cells treated for 24 hours with soil samples at 0.1% (v/v). **B.** ERE-LUC activity in MCF7 cells treated with ethanol soil extracts at 0.1% (v/v) for 24 hours.

Acknowledgements

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