

An in-vitro assessment of the genotoxic impacts of Acid Mine Drainage in the human MCF7 cell line

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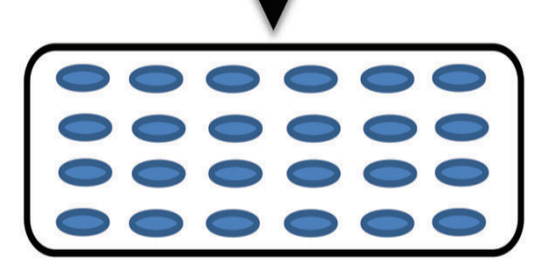
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INTRODUCTION

Chemical compounds and heavy metals can damage the DNA of living cells. If not repaired these DNA aberrations can initiate a cascade of biological consequences at the cellular, organ, whole animal; ultimately affecting the community and population level. Bio-assays evaluating short-term impacts of heavy metal contamination found in Acid Mine Drainage (AMD) have proven useful to screen the toxic potential within the environment. However, a more complete validation of the use of assays such as the Alkaline Comet Assay for the assessment of genotoxicity of AMD in humans is yet to be established. The single-cell gel electrophoresis assay (Comet Assay) was developed to measure both single and double stranded DNA breaks in mammalian cells. The advantages of this technique are the small sample size required, its rapidity and the possibility to discriminate between cell types regarding the degree of DNA damage or DNA repair level. The progression of cell death can be further elucidated with the detection of small fragmented DNA particles viewed as an apoptotic laddering pattern. Laddering of highly damaged cell types gives insight into the type of programmed cell death initiated and clearly establishes when degradation occurs. This study proposes the use of the above described tools as reliable detection mechanisms of genomic impairment and damage in human cell types exposed to AMD. Furthermore this study presents a novel opportunity as a first report in South Africa, to establish an eco-genotoxic profile of a human cell line exposed to both Raw and pH Neutralised AMD arising from gold and coal mining areas in South Africa.

METHODOLOGY

MCF7 cell line were grown and plated into 24 wells plates at a density of 3×10^5 . Cells were exposed at various time intervals and harvested for bio-assay applications



DNA isolation for Apoptotic Laddering Assay

Whole cell suspension for the Comet Assay

1. Cells are exposed to genotoxic insult

2. Cells are embedded in agarose on microscope slide and lysed in solution

3. Following electrophoresis and Fluorescent staining, damaged DNA is seen to migrate away from intact DNA

Comet "Head" comprising intact genomic DNA
Comet "Tail" composed of fragmented DNA

CONCLUSION

- Apoptotic laddering and the Comet Assay have proven to be successful tools to evaluate the genotoxic impacts of AMD from the mining areas of Mpumalanga and the Western Basin of South Africa.
- A higher rate of programmed cell death was observed in cells exposed to Kromdraai Raw and pH Neutralised AMD.
- Abrupt non-programmed cell death was shown to occur at an elevated rate in Western Basin Raw and pH Neutralised AMD, compared to that of Kromdraai Raw and pH Neutralised AMD.

ACKNOWLEDGEMENTS

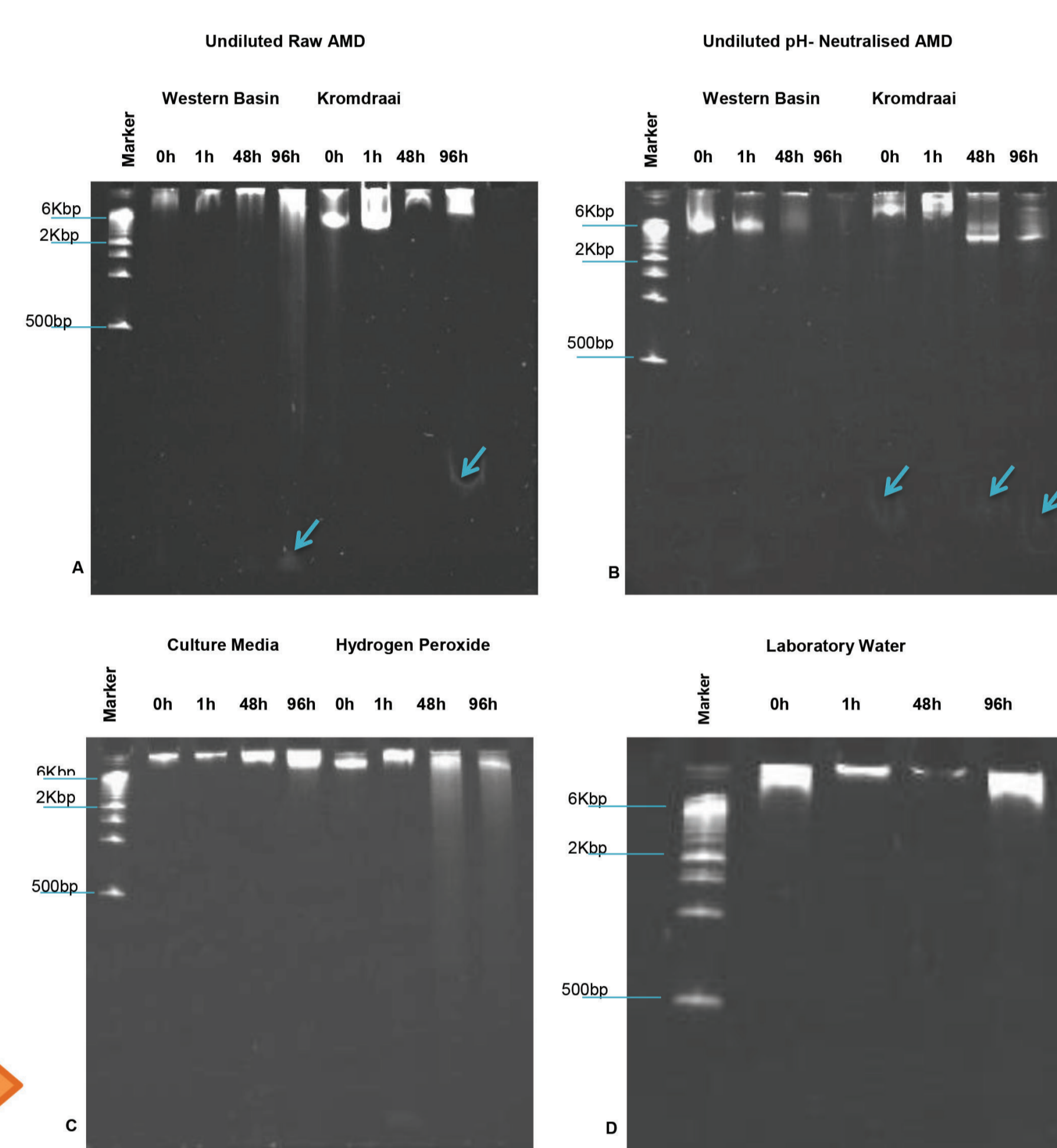
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RESULTS AND DISCUSSION

The potential of acid mine drainage to initiate internucleosomal DNA fragmentation



Cellular responses to pH Neutralised AMD from both sites may indicate the presence of regulatory mechanisms promoting the recovery of cells from DNA damage.

Figure 1: Depicts the DNA fragmented induced when MCF7 cells are exposed to harmful contaminants. DNA fragmentation is considered to be a classic hallmark of apoptosis and necrosis. Studies investigating nuclear morphology have revealed that apoptosis activates one or more caspase (cysteine-dependent aspartate-specific proteases)-activated deoxyribonuclease (CAD) enzymes which promote the breakdown of internucleosomal regions giving rise to the oligonucleosome ladder pattern (on electrophoretic analysis) of multiples of 50–200 base pair (bp) DNA fragments. In contrast, necrosis induces non-specific cleavage of DNA, producing a smear rather than a ladder when visualised under electrophoresis. Lane 1 of **A**, **B**, **C** and **D** depicts the 500 bp ladder. In **A**: lane 5 and 9 show extensive smearing until finally producing a low molecular weight band (depicted by arrow). It is speculated that these samples have undergone apoptosis. In **B**: lanes 4, 5, 8 and 9 show a decrease in DNA content at increased time of exposure. Here it is noteworthy that the pH-Neutralised AMD samples (**B**) display a significantly higher range of DNA fragmentation than the Raw AMD exposed samples (**A**) suggesting that necrosis might be involved. In **C** and **D**, all lanes depict expected intact DNA for cells exposed to Culture Media and Laboratory Water. Expected oxidative DNA damage is observed in **C**, where cells are exposed to H_2O_2 .

Acid mine drainage as a causative agent for elevated programmed cell death

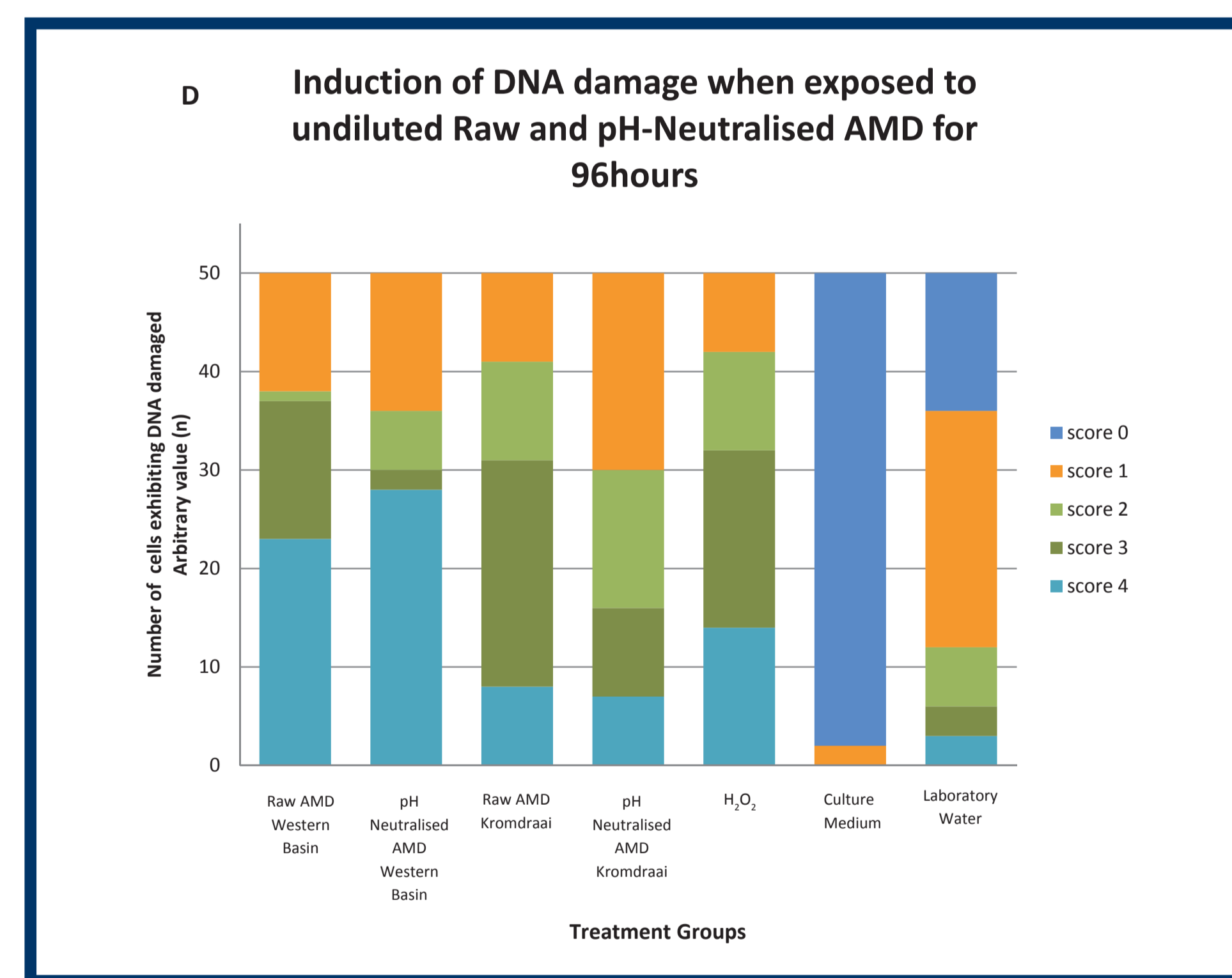
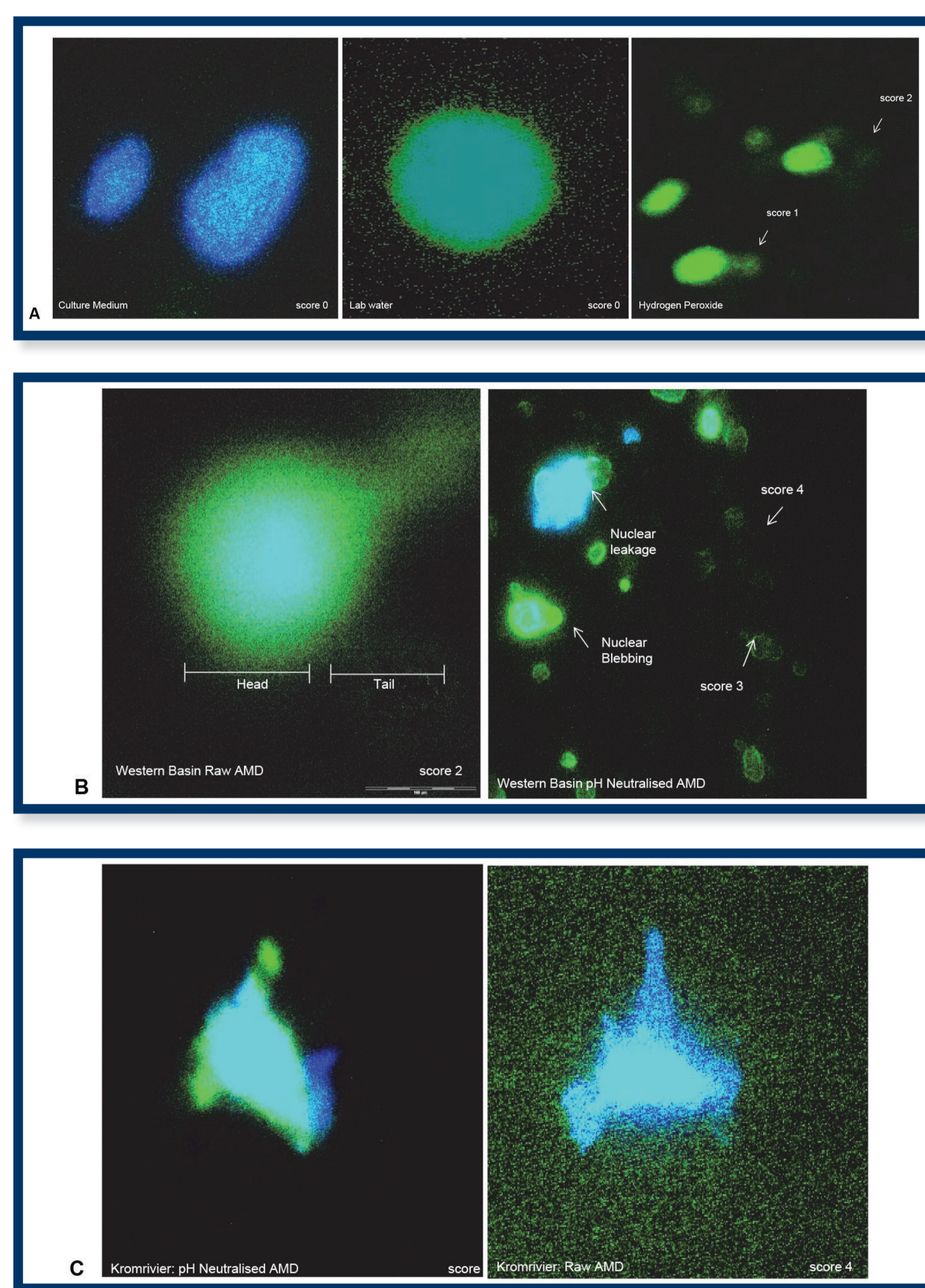


Figure 2: Detection of DNA damage in the form of apoptosis and necrosis induced by undiluted Raw and pH Neutralised AMD exposed for 96 hours. Cells were stained with DAPI and Vista green specific for nuclei and fragmented DNA, respectively. **A**, **B** and **C** depict comet cells with given scores, specifying the extent of DNA damage. Percent DNA in Tail together with visual scoring is used to categorise morphologies observed within various comet cell types. Percent DNA in Tail is a representation of DNA intensity within the tail region and gives insight into the kinetics of cells when exposed to harmful substances. Hence the higher the score the more advanced the DNA damage.

In **A**, culture medium and Lab water show undamaged cells with % DNA in Tail of less than 0.02%, denoted score zero. In the third panel of **A**, Hydrogen peroxide display comet cells of two categories. Score one denotes % DNA in Tail occurring between 10–30%. Score two denotes % DNA in Tail occurring between 30–60%. In **B**, undiluted Western Basin Raw AMD depicts a score two comet cell with typical features of a comet cell. The comet cell is divided into a head and tail region where the head represents intact DNA and tail an extent of damaged DNA. In **B**, panel two, cells exposed to undiluted Western Basin pH Neutralised AMD depicts a variety of morphological parameters when exposed for 96 hours. This population show comet cells from scores 1–4, displaying an initiation of early to late stage apoptotic and necrotic cells. **C**, displays cells undergoing extensive nuclear leakage leading to necrosis when exposed to undiluted Kromrivier Raw and pH Neutralised AMD. Scores three and four denotes % DNA in tail occurring between 30–60% and 60–100%, respectively.

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In **D**, non-DNA damaging properties are displayed in cells exposed to culture medium (score 0). When cells are exposed to a non-nutrient environment (laboratory water), a notable population of undamaged and early apoptotic (score 1) cells occur. A standard response to oxidative stress is observed when cells are exposed to H_2O_2 . Cells exhibiting late stage apoptotic cell formation (score 3) are highly distributed in Western Basin Raw AMD and Kromdraai Raw AMD populations. This response denotes a propensity towards extensive DNA mutations and cell death. Although abrupt cellular necrosis (score 4) is exceedingly distributed in Western Basin pH Neutralised AMD, this population, together with Kromdraai pH Neutralised AMD exhibits a notable minimised effect with the development of early stage apoptotic cells (score 1). Thus cellular responses to pH Neutralised AMD from both sites may indicate regulatory mechanisms present promoting the recovery of cells from DNA damage.