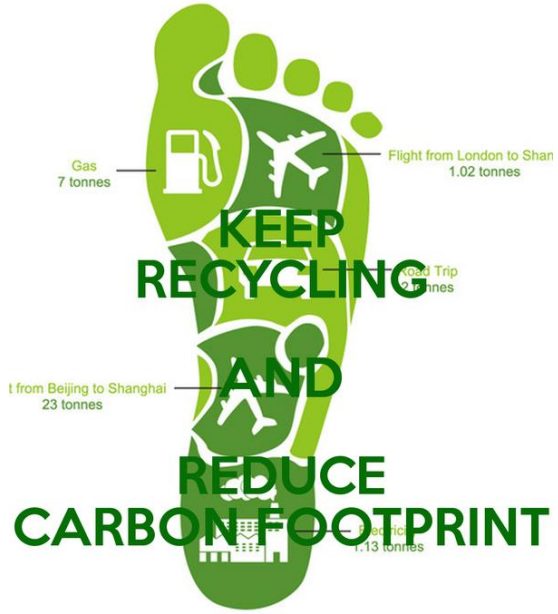
Transnational Meeting and Staff Training

6-9 October, Inail Piazzale Pastore, 6 - Rome

Boccia Priscilla

Italian Workers' Compensation Authority (INAIL), Department of Technological Innovation and Safety of Plants, Product and Anthropic Settlements (DIT) Rome

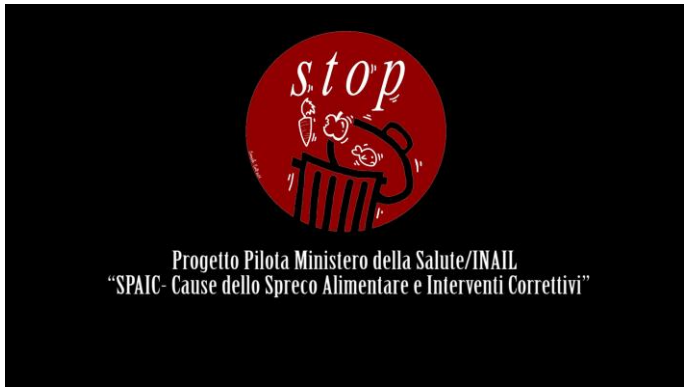
Department of technological innovations and safety of plants, products and anthropic settlements



- ✓ Biotechnological research to environmental health protection in the living and working environment with a particular attention to genotoxic effects due to anthropic activities or xenobiotic substances.

- ✓ Food waste: bringing research in to the school, showing specific aspects of the waste footprint and on sustainability. The students represented the active part, creating themselves their own message with video products and apps.

THE SOLUTION



Scientific collaboration in the field of validation of cytogenetic methods: a pre-validation study of comet assay on plants, recognized as an excellent indicators of cytogenetic and mutagenic effects caused by environmental contaminants in order to study the inter-laboratory variation in DNA strand breaks in plants and identify key factors affecting comet assay performance through a pre-validation study.

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Abstracts of the 12th International Comet Assay Workshop held at the University of Navarra, Pamplona, Spain, 29–31 August 2017 (https://icaw.vito.be/)

hCOMET: a COST Action dedicated to the comet assay in human biomonitoring

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The COST Action hCOMET has members from 23 countries, with a common interest in using the comet assay to measure DNA damage and repair in humans. The purpose of this Action is two-fold: first, to collect as much human comet assay data as possible into a single database so as to allow a pooled analysis; and second, to improve the inter-laboratory reproducibility of the assay. In the first year we have succeeded in creating the database, with DNA damage estimates for around 20,000 human samples; analysis is now proceeding, to determine which factors (smoking, age, nutrition, sex, occupational exposure etc.) affect DNA damage and repair, and to what extent.

To achieve the second aim, we need first to understand better the technical factors that affect assay performance, in measurement of both DNA damage and DNA repair, and working groups are actively engaged in these topics. Standardised methods will be tested in a ring study; and the findings of this will be incorporated into standard operating procedures that, we hope, will be adopted as best practice in future biomonitoring studies. Another working group is studying the applicability of the comet assay to different cell types, for example cells from normal and tumour tissue, isolated peripheral blood mononuclear cells (the most commonly used cells) compared with whole

found that consistency of removal is important, whereas lung and lung lavage less so.

Five cohorts of 10 animals in each (A, B, C, D and E) were tested in five independent experiments. In each cohort, five animals were dosed with the vehicle, 0.9% saline and five were dosed with the positive control EMS at 200 mg/kg b.w. Animals were dosed 3–4 hours prior to euthanasia. All animals were euthanized by CO_2 inhalation, nasal tissues (turbinate and septum lining) were collected. Tissues were minced with scissors and single cells were prepared and processed for the comet assay. EMS was dosed at 200 mg/kg b. w. The vehicle control was 0.9% saline (10 mL/kg). The positive control, EMS, was dissolved in 0.9% saline and was prepared fresh just prior to dosing. Vehicle control dosed male rats had % tail DNA values in all 5 cohorts ranging from 0.07 to 0.89 with a mean value of 0.30 ± 0.22 in nasal tissue.

Positive control EMS dosed male rats had % tail DNA values in all five cohorts ranging from 7.24 to 29.02 with a mean value of 15.73 ± 5.18 in nasal tissue. These values were statistically significant compared to the concurrent vehicle control values.

3D Skin comet assay: Genotoxicity assessment addressing the dermal route of exposure

Kerstin Reisinger[1], Joep Brinkmann[3], Tom Down[4], Anja Fischer[1], Andrea Haase[3], Frank Henkler[3], Sebastian Hoffmann[2], Manfred Liebsch[3], Andreas Luch[3], Claudia Petrick[1], Ralph Pirow[3], Astrid Reus[5], Andre Said[3,6], Monika Schäfer-Korting[6], Markus Schulz[7], Stefan Pfuhler[4]

O25 - A PRE-VALIDATION STUDY OF COMET ASSAY ON PLANTS

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In the 1990s the comet assay began to be used in plant models, with several limitations in the protocol as a result of the differences in the structure of the plant cell as compared to the animal cell. Because of the conserved structure of plant genetic material, numerous varieties of species can be used in plant genotoxicity testing. In the case of plant cells, several discrepancies and dissimilarities in the protocol exist across labs, despite the fact that an optimized protocol was recently released [1]. In addition, several labs continue to adopt conventional methods into their protocol, which are both time-consuming and redundant, reducing the reliability of the assay.

The aim of this work was to initiate a collaboration between two research institutes in order to study the inter-laboratory variation in DNA strand breaks in plants and identify key factors affecting comet assay performance through a pre-validation study. Two model plants were selected; *Vicia faba* (broad bean) and *Trifolium repens* (white clover). Several optimizations in Pourrut's protocol were evaluated at different steps such as chopping, unwinding, and electrophoresis time [1]. Preliminary significant results have been obtained, identifying the extraction as the main critical step. The chopping method resulted in good efficiency of nuclei isolation in terms of integrity and yield obtained for *Trifolium repens* while not for *Vicia faba* variety used because of its cellular structure, as its larger nuclei, thus a different method of isolating nuclei from *Vicia faba* has been required.

Further research is required in order to optimize the same protocols using different sensitive plant species to ensure reliability as well as an extrapolation of results to provide a guideline for plant comet assay.

[1] Pourrut, B., Pinelli, E., Mendiola, V. C., Silvestre, J., Douay, F. (2015). Recommendations for increasing alkaline comet assay reliability in plants. Mutagenesis, 30, 37–43.

Proceedings of the 3rd World Congress on New Technologies (NewTech'17)
Rome, Italy – June 6 – 8, 2017
Paper No. ICEPR 123
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Use of Comet Assay as an Efficient Biomarker for Plant Biomonitoring and Phytomanagement of Contaminated Sites

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Extended Abstract

During the last decade plants have been increasingly used in ecotoxicological studies and environmental biomonitoring. In order to evaluate the impact of stress (biotic or abiotic) on plants, it is important to evaluate their health. This can be realized at the macroscopic scale (growth, dry or fresh weight…) or at the molecular scale, using biomarkers. During the same period, the application of the comet assay has been established as one of the most interesting techniques in eco-genotoxicology. It is a rapid, versatile, sensitive and relatively inexpensive method for measuring DNA damages and repairs in individual cells. The aim of this work was to evaluate the interest of the comet assay to monitor pollutant impacts on higher plants growing on contaminated sites and to select plant species to remediate contaminated areas.

In a first study, we investigated the potential impacts of contaminants from a hazardous waste site, in a controlled environment, on *Vicia faba*, as a bioindicator plant. Soil samples were collected from a former industrial area in Italy and their phytotoxicity and genotoxicity were investigated. In the case of the controlled environment we evaluated the environmental damage after a simulated accidental release of toxic substances in soil. In this case we evaluated the contamination effects on soil-plant system and detected DNA damages by short-term genotoxicity tests (comet assay and micronuclei tests) performed on polluted soils and on gravitational water. Our studies demonstrated that the comet assay is a sensitive, rapid and cost-effective technique for the detection of DNA damage, which is ideally suited as a biomarker of

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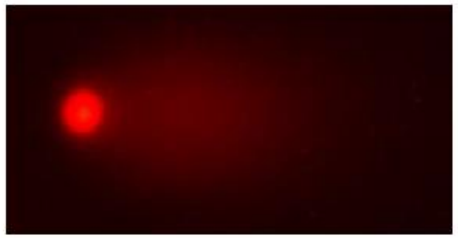
Intermediary Research Report

Optimization of the Comet Assay in Plant Cells



In preparation for getting a master's in sustainable management of pollution

For validation of M1

Internship supervisor, Università di Roma La Sapienza: Dr. Elena Sturchio

Internship supervisor, ISA: Dr. Bertrand Pourrut

VIJAYARAJ Vinita

2015-2016

September 2016

Erasmus+

Higher Education: Traineeship Certificate

Bertrand Pourrut ISA ENSAT

Inter-laboratory study → INAIL /DIT

The idea was to continue the study by the implementation of interlaboratory tests that hopefully will involve a European network of laboratories

Stephen Sterling,"Sustainable education«: a whole institutional change as to re-orient higher education to address the extra-ordinary global challenges that face societies and which will affect young people's lives in this century .

An education and a vision that enhances the individual and nature in their complexity and interdependence.

It is a vision of education and of ecological and rational learning that leads to a change in the educational culture in order to reach a sustainable future