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The University of Malta Research, Innovation & Development Trust

- Alfred J. Vella -
Escalation of fireworks in Malta: environmental forensic evidence from perchlorate in dust

Malta Chamber of Scientists

The Journal of the Malta Chamber of Scientists

Editor-in-Chief: Giuseppe Di Giovanni

Xjenza Online - Journal of Malta Chamber of Scientists
http://www.xjenza.org

Editorial Board

Scope of Journal

Xjenza is the Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first issue of the journal was published in 1996 and the last (No. 12) in 2007. The new editorial board has been formed with internationally recognised scientists, we are planning to restart publication of Xjenza, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide insight to a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

Instructions for Authors

Xjenza is the journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Communications
2. Research Articles
3. Research Reports
4. Reviews
5. Notes
6. News
7. Autobiography

Communications are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

Research Articles form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also apply to Research Articles.

Research Reports are extended reports describing research of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

Review Articles describe work of interest to the wide readership characteristic of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

Notes are fully referenced, peer-reviewed short articles limited to three journal pages that describe new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

News: The News section provides a space for articles up to three journal pages in length describing leading developments in any field of science and technology or for reporting items such as conference reports. The Editor reserves the right to modify or reject articles for consideration as 'news items'.

Errata: Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by ***, Xjenza, vol. *** (year). Errata should be short and consistent for clarity.

Invited Articles and Special Issues: Xjenza regularly publishes Invited Articles and Special Issues that consist of articles written on invitation by the Editor or member of the editorial board.

Submission of Manuscripts

Manuscripts should be sent according to the guidelines given hereafter to submissionxjenzaonline@gmail.com.

Referees All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with their manuscript the names and addresses of three referees, preferably from overseas. Every effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

Conflict of Interest Authors are expected to disclose any commercial or other associations that could pose a conflict of interest in connection with the submitted manuscript. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

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Preparation of Manuscripts

Xjenza accepts submissions in MS Word, Libre Office Writer and LaTeX with the latter being the preferred option. Anyone submitting in LaTeX should use the journal template, the latest version of which can be found at `http://github.com/hicklin/Xjenza-Journal-Template`. All the necessary files to run the LaTeX document should be supplied together with the rendered PDF.

If a word processor is used the styling should be kept to a minimum, only introducing bold face, italics, subscript and superscript text where the context requires it. Text should be in single-column format and the word processor options should not be used in order to justify text or hyphenate words. Together with the native format of the word processor, a pdf, generated by the word processor, must be given. Furthermore, artwork should be in accordance to the artwork guidelines give below and must be submitted separately from the word processor file. Similarly, the bibliographic data of the cited material should be submitted separately as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTEX (*.bib) file.

Article Structure

A manuscript for publication in Xjenza will ordinarily consist of the following: Title page with contact information, Abstract, Highlights, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusion, Appendices and References.

The manuscript will be divided into clearly defined numbered sections. Each numbered subsection should be given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by the section number as opposed to simply 'the text'.

Title page

- Title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide full postal address of each affiliation, including the country name and, if available, the e-mail address.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and complete postal address. Contact details must be kept up to date by the corresponding author.
- Present/permanent address. If an author has changed the address since the work described, this can be indicated as a footnote to the author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

Abbreviations Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistenly throughout the text.

Introduction State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

Materials and Methods Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results Results should be clear and concise. Numbered/tabulated information and/or figures should also be included.

Discussion This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

Conclusion The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Anyone using LATEX should use the package siunitx in all cases.

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- Save text in illustrations as 'graphics' or enclose the font.
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- Name your artwork files as 'fig$x$' or 'tab$x$' where x corresponds to the sequence number in your document.
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Formats Regardless of the application used, when your electronic artwork is finalised its file format should be one of the following (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

- PDF or SVG: Vector drawings. Embed the font or save the text as 'graphics'.
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Tables Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Large tables should be submitted in CSV format.

Citations and References Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

Citation in text References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

Kramer et al. (2010) have recently shown ...

or parenthetically

as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).

Groups of references should be listed first alphabetically, then chronologically. When writing in LaTeX use `\textcite{}` and `\parencite{}` for the respective cases mentioned.

The reference section Every reference cited in the text should also be present in the reference list (and vice versa). The reference list should also be supplied as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. Consult the APA v6 guidelines for multiple authors. Below are some examples of referencing different bibliographic material.

Reference to a Journal Publication:

Agree, E. M. and Freedman, V. A. (2011). A Quality-of-Life Scale for Assistive Technology: Results of a Pilot Study of Aging and Technology. *Phys. Ther.*, 91(12):1780–1788.

McCreadie, C. and Tinker, A. (2005). The acceptability of assistive technology to older people. *Ageing Soc.*, 25(1):91–110.

Reference to a Book:

Brownsell, B. (2003). *Assistive Technology and Telecare: Forging Solutions for Independent Living.* Policy Press, Bristol.

Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

Reference to a Chapter in an Edited Book:

Brownsell, S. and Bradley, D. (2003). New Generations of Telecare Equipment. In *Assist. Technol. Telecare Forg. Solut. Indep. Living*, pages 39–50.

Web references The full URL should be given together with the date the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately or can be included in the reference list.

References in a Special Issue Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

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-List of title word abbreviations: `http://www.issn.org/2-22661-LTWA-online.php`;

-CAS (Chemical Abstracts Service): `http://www.cas.org/sent.html`.

Video data Xjenza accepts video material and animation sequences to support and enhance the presentation of the scientific research. Authors who have video or animation files that they wish to submit with their article should send them as a separate file. Reference to the video material should be clearly made in text. This will the modified into a linked to the paper's supplementary information page. All submitted files should be properly labelled so that they directly relate to the video files content. This should be within a maximum size of 50 MB.

Submission check list

The following list will be useful during the final checking of a manuscript prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

- One author has been designated as the corresponding author with contact details:
  - E-mail address.
  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations
  - Abstract does not exceed about 250 words.
  - Manuscript has been 'spell-checked' and 'grammar-checked'.
  - References are in the required format.
  - All references mentioned in the reference list are cited in the text, and vice versa.
  - Bibliographic data for all cited material has been given.
  - Permission has been obtained for use of copyrighted material from other sources (including the Web).

– A PDF document generated from the word processor used is given.

After Acceptance

Use of the Digital Object Identifier The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

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Happy Birthday Xjenza

As Foundation President and on behalf of the newly elected council of the Malta Chamber of Scientists, we are happy to celebrate with the editor and his editorial board the 20 year of our journal, now known as, XJENZA_ONLINE.

XJENZA_ONLINE reflects the history of our Chamber. Together, we have come a long way from the birthplace on campus in 1906.

Since then, the editors, Angela Xuereb, Joseph Grima and Giuseppe Di Giovanni have striven to ensure the highest standards of peer review and gave the journal a good reputation in Malta and elsewhere. Thanks to their efforts, undoubtedly, the quality of the content has been high. They have provided exposure for that part of the scientific research experience that has been mainly directed in the local context. Local interest science is extremely important underlying policy that impacts a variety of sectors in the economy, ecology, and in society.

Secondly, with the editor, the Chamber is working to develop XJENZA into a regional English language platform for training of young researchers in the writing of scientific research manuscripts, and science communication, in general.

We hope that with your support and the international partners that we are seeking, XJENZA has a long life of success in modern Science Communications.

I take this opportunity to wish Giuseppe and his board the best of success for many years to come.

Happy Birthday XJENZA.

Alex Felice
Foundation President

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Special Editorial

20th Anniversary of Xjenza (July 1996–July 2016), the official Journal of the Malta Chamber of Scientists

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It was twenty years ago today that the Malta Chamber of Scientists published the first issue of Xjenza. At one of its meetings, some sixteen months prior to the first issue in July 1996, a member of the Chamber of Scientists put forward the idea of starting a scientific journal. After much discussion, the idea was approved by a majority. It was not a unanimous decision, however. Some members even felt that Xjenza would cease to publish after the first few issues. How wrong they were! Twenty years later and Xjenza is still going strong, thanks to the hard work of its current Editor-in-Chief Professor Giuseppe Di Giovanni, and other Editors Prof. Richard Muscat and Prof. Joseph N. Grima who kept the journal going between 2002 and 2007.

With much encouragement, I opted to serve as Editor-in-Chief, with four Associate Editors to assist me in what seemed a mammoth task at the time, namely: Dr. Martin Ebejer, Professor Richard Muscat, Professor Christian Scerri and Professor Emmanuel Sinagra.

The Editorial Policy established the setting up of high standards of peer review, which would have automatically led to equally high standards of scientific research. However, the editorial policy at the time was not to solely publish research work by established scientists but also to encourage young scientists, be they undergraduate or postgraduate students, with promising new areas of research to write papers and submit them for publication. Xjenza was to provide a suitable training ground for these young scientists seeking their first publication.

The Editorial Board was keen to publish original research articles. Review articles on topical issues were to be encouraged. It was also the intention of the Editorial Board to establish visible research profiles to increase awareness among members of the Chamber and the general public of the scientific research being conducted in Malta at the time in the various disciplines.

Angela A. Xuereb Anastasi, Editor of Xjenza, 1996–2002

Xjenza was meant to be a record of Maltese scientific development and achievement, and also a forum for the initiation and development of debate on the major specific scientific issues of the day.

Importantly, we also envisaged Xjenza as a means by which links could be forged with local scientists and Maltese scientists working abroad. The idea was for a register to be compiled with a view to familiarizing local colleagues with their work and encouraging cross-border Maltese collaboration.

Twenty years ago, University science in Malta was in need of much nurturing, still in its infancy. Most of us had passed long periods of time abroad working towards a doctoral degree or on postdoctoral fellowships conducting research at various universities and research institutes mostly in the UK and USA. There were some

members of the Chamber of Scientists putting in a lot of energy to improve the visibility of science in Malta. There was a vibrant atmosphere, with most trying to set up research laboratories and to publish in international peer-reviewed journals to put the University of Malta on the map. And that is what spurred us on to start Xjenza.

Angela Xuereb (angela.a.xuereb@um.edu.mt)

The journal's production was interrupted between 2007–2012 and the Chamber, revitalised by a new spring, wanted to publish it again and fill the gap left in the scientific Maltese community, this time as on online only journal. I was excited to take the challenge of leading Xjenza and very grateful for the confidence expressed in me by the ChoSci council in recognition of my extensive international editorial experience.

Generally, I have continued the vision for the journal as established by the founding Editor Prof Xuereb and pursued by her successors, but I aimed higher by taking Xjenza to an international level. I have strived to publish national and international scholarly research, which focuses on important issues from different branches of science, technology and the humanities, of general interest, and understandable to as many scholars as possible.

More specifically, together with my new appointed editorial team I feel confident to affirm that we have pursued four major goals regarding the new Xjenza Online, to:

1. increase the diversity and quality of submissions and open the journal to international authors;
2. encourage and publish student papers (both reviews and research articles);
3. advertise local and international opportunities for research collaborations, grants, meetings and successful stories (news articles);
4. improve the production process and decrease the time required for reviewing manuscripts.

We have broadened the appeal of Xjenza by engaging well recognized scientists. Xjenza Online has taken back its role of forum for academic discussion. At the same time we have started the internationalisation of this journal, opening it up to researchers worldwide. Our goal is to create a place for Xjenza among the other international journals. We have now enough issues (7), high-quality papers and a strong editorial board to apply to have Xjenza indexed in the major databases, such as MEDLINE, THOMSON REUTERS SCIENTIFIC.

We have continued the old and important aim of Xjenza Online, besides highlighting the exciting research being done nationally and internationally, to offer training in the art of scientific publishing in a peer-reviewed environment for graduate students and young researchers. Indeed, publication of research work is essential in order to advance science. It is also essential for people pursuing a scientific career as their recognition as researchers depends on their publications and contributions to scientific progress. Scientists live in a culture of "publish or perish". Xjenza Online, therefore, has become an essential tool in the scientific development of early-stage researchers, and represents a learning platform where they can learn not only how to write a scientific paper, but also how to get it published.

As mentioned in diverse parts of this Editorial, our work as Editors would not be possible without the tireless support and contributions from our Associate Editors, Members of the Editorial Board, and our production staff. Special thanks go to our Associate Editors who are instrumental in helping us assess submissions, and communicate often several times per week with the Editors and Managing Editor. They are as much a part of the Xjenza team as the Editor-in-Chief. The Xjenza's team is made up of eleven editors, Ian Thornton (Cognitive and Social Sciences), Ian Cassar (Economics) Sebastiano D'Amico (Geoscieces), David Mifsud (Life Science), David Magri (Physics and Chemical Science), Philip Farrugia (Engineering Science), Liberato Camilleri (Mathematical and Statistical Science), Nicholas Sammut (Information and Communication Technologies), Carmel Cefai (Psychological Science), Godfrey Baldacchino (Social Sciences), Joseph Galea (Medical Sciences). In addition there is the Project Editor Jackson Said, Copy Editor Gabriel Farrugia and the Editorial Assistant Caitlin Davies.

In addition, I have included an Advisory Board made of international scholars, such as Prof. David Eisner, Manchester University, UK, Prof. Vincenzo Crunelli, Cardiff University, UK; Prof. Angela A. Xuereb Anastasi, University of Malta; Prof. Frank Vella, University of Saskatchewan, Canada; Prof. Giacomo Rizzolati, University of Parma, Italy. Moreover, we have also add some Editorial Board Members (Dr Maurizio Casarrubea from the University of Palermo, Italy, Dr Katya De Giovanni from the University of Malta, Malta, Prof. Maria Attard from the University of Malta, Malta, Prof. Mauro Pessia from the University of Malta, Malta and Dr Sandro Lanfranco from the University of Malta, Malta who served as an AE from 2013–2015).

Xjenza Online New Look

A principle well known in the world of communication is that for material to be seen as trustworthy and fresh, the manner in which a topic is presented is as important as the content itself. Therefore, it is important to assess regularly the "look" and image of the journal with the goal of improving our ability to communicate with our

readers. An important example of this improvement is seen in this new edition of ***Xjenza Online***, where we introduce several new features. The most visible is our front cover, where we have a new logo and a new design We hope our readers find this new look fresh and exciting, and that it helps brand ***Xjenza Online*** as the Maltese leader in science publishing.

As we look toward the future, Xjenza Online will continue to strive to improve content, expand distribution and access, and improve functionality for our readers and all others interested in the recent development in the different branches of science in Malta and abroad.

In the coming months, we would like to start a new annual Xjenza prize for the best research paper published in the previous calendar year. We will also be looking at ways to improve the synergy between, and the ChoSCi website, with an eye toward creating a strong portfolio of content for the Xjenza international community. As we identify ways to improve the Journal, if you have comments or suggestions, we as Editors would like to hear from you. Thank you for our 20 years, and we look forward to year 30 and beyond.

Giuseppe Di Giovanni
(giuseppe.digiovanni@um.edu.mt)

Giuseppe Di Giovanni, Editor of Xjenza, 2013–Present

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Research Article

Escalation of Fireworks in Malta: Environmental Forensic Evidence from Perchlorate in Dust Fall

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Abstract. Summer in Malta is accompanied by fireworks as part of the numerous (about 85) religious festivals (*festa*) that occur throughout the period. We attempted to establish whether firework activity is truly following official trade statistics, which imply a decreasing trend, or otherwise. Firework manufacture critically depends on the availability of oxidising agents, two of which (KNO_3, $KClO_3$) being controlled by permits but not potassium perchlorate ($KClO_4$) which is freely available. Recent changes in legislation have slightly decreased the quantity of $KClO_3$ and increased that of KNO_3 and according to official trade statistics, consumption of $KClO_4$ has decreased from 17 t/a in 2010 to $\approx$ 2.5 t/a during 2011–2014. However, from levels of perchlorate in dust fall and rate of total deposition we calculated the quantity of $KClO_4$ used during 2012 and obtained $\geq$ 90 t/a, 36 times the official figure. This situation is serious since the danger of accidental explosion during manufacture escalates as production intensifies. Also, contamination of the environment by firework-waste appears destined to remain high with possible consequent effects on human health.

Keywords: perchlorate, environmental forensics, Malta, fireworks, dust fall

1 Introduction

Fireworks in Malta are synonymous with summer since the three month long period between June and September is the time of celebration of scores of religious feasts (*festa*), each one of which is accompanied by the burning of abundant fireworks over several days per week. In addition, other events of importance and even an annual competitive fireworks festival are adding to the local firework activity. It is a well-established fact that the burning of fireworks lowers air quality and introduces into the air and other environmental phases compounds of toxic metals such as copper, barium, lead and antimony and other chemicals which are harmful to health. Thus, it is of interest to know whether local firework activity is not just 'traditionally intense' but actually rising, as the anecdotal evidence would suggest despite contrary indication from trade statistics.

Manufacture of fireworks is practically controlled by placing limits on the acquisition of oxidising agents, which are key to pyrotechnic compositions. The principal oxidising agents employed in Malta are potassium nitrate (KNO_3), potassium chlorate ($KClO_3$) and potassium perchlorate ($KClO_4$). Local legislation dating back to the early part of the twentieth century has imposed limits on the use of KNO_3 and $KClO_3$. Recently (2014), the limits have been modified by a slight lowering to 1000 kg per year for $KClO_3$ and an increase to 3200 kg per year for KNO_3. There are about 30 firework factories currently active in Malta. Potassium nitrate and chlorate can only be obtained from importers if the proper police permits are presented. On the other hand, perchlorate, imported as the potassium salt ($KClO_4$), has only become popular relatively recently and its acquisition is not limited. According to the National Statistics Office (NSO), imports of $KClO_4$ increased from 2300 kg in 2002 to 17 000 kg in 2010 and then suddenly and inexplicably fell and stabilized at about 2500 kg thereafter. Meanwhile, anecdotal observation of firework activity suggests that these displays have not decreased in number during the last five years or so. It is not known whether the chemical is being (legally) introduced directly into Malta from EU producers whose imports are not reported to the NSO.

The objective of this paper is to establish whether

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firework activity (manufacture and burning) is declining substantially, as the NSO data suggest, or whether the information from trade statistics is incorrect and pyrotechnic activity is actually escalating. Imported fireworks may also be contributing to this activity. This matter is of interest not only because firework burning exerts a negative impact on the quality of the environment (Shi, Zhang, Gao, Li & Cai, 2011; Munster, Hanson, Jackson & Rajagopalan, 2009; Shi et al., 2011; Camilleri & Vella, 2010), especially if it is intensive and prolonged in time, but also because it exposes the general public to greater personal risk associated with illegal manufacture, which takes place covertly in private residences and the legal and dangerous transportation through inhabited areas of significant loads of pyrotechnic articles on their way from factory to burning fields (Vella, Axiak, Delicata & Theuma, 2011).

We have recently shown (Vella, Chircop, Micallef & Pace, 2015) that perchlorate (ClO_4^-) is present in indoor dust in Malta where it appears to derive from the only source of contamination that is significant locally, namely, the burning of fireworks. Twenty eight of 37 indoor dust samples (76%) collected during the period 2011–2013 contained perchlorate at concentrations which ranged from 0.79 ppm to 53 ppm (median value 7.8 ppm). In a study by Wan et al. (2015) on perchlorate in indoor dust from twelve countries, including Greece, USA, Japan and China, it was found that perchlorate levels were typically less than 0.5 ppm and for China, the value was highest at 4.25 ppm: thus, Malta's indoor dust contains almost double the perchlorate in China's dust, making the local situation the worst case of contamination by this chemical reported so far. Perchlorate is also found in local atmospheric dust fall with concentrations that range from 0.52 ppm to 561 ppm (median concentration 6.2 ppm) (Vella et al., 2015). Most significantly, these levels peak during the summer and decrease again during the rest of the year, only to rise again during the next summer period. This temporal variation, which closely follows the firework activity on the island, leaves no doubt about the source of this otherwise rather exotic chemical. Worldwide, the principle use of perchlorate, mainly as the ammonium and potassium salts, is in the manufacture of solid rocket propellant and military explosives although it has other uses, e.g. in air bags, highway flares, tanning and some other manufacturing applications (Urbansky, 1998, 2002; Gu & Coates, 2006). In Malta, potassium perchlorate is imported exclusively for use as an oxidising agent for the manufacture of pyrotechnic compositions. Compositions made from mixtures of potassium perchlorate and aluminium or other metals (magnesium, titanium, magal) are far more stable than those containing potassium chlorate (locally known as "putassa") and for this reason, the latter compositions have recently (2014) been declared illegal in Malta.

The shift from the use of chlorate-metal mixtures towards perchlorate containing systems is expected to reduce the toll exacted by accidents during the manufacture of fireworks which claimed an average of 2.2 lives per annum in the period 1980–2010 (Axiak, Delicata, Theuma & Vella, 2012). On the other hand, the shift will have an impact on the quality of the environment, since firework displays release into the atmosphere unburnt solid residues that precipitate as dusts thus contaminating homes and soils. Dust is a significant source of human exposure to chemicals: the fine particulates present an inhalation risk while coarser material is a risk for ingestion (Christoforidis & Stamatis, 2009; Shi et al., 2011), especially for infants and children who are more prone to transfer matter from hand to mouth (Glorennec, Lucas, Mandin & Le Bot, 2012). Contaminated dust fall also has potential to affect water quality and that of locally grown agricultural produce (Smith, Yu, McMurry & Anderson, 2004; Shi et al., 2007), which become potential sources for consumers of these foods (Lee, Oh & Oh, 2012; Asami, Yoshida, Kosaka, Ohno & Matsui, 2013).

The concern over perchlorate relates to the chemical's known interference with thyroid function: perchlorate blocks the uptake of iodine into the thyroid thus reducing production of thyroxine and triiodothyronine (Wolff, 1998; Greer, Goodman, Pleus & Greer, 2002). Females appear to be more at risk than males, especially if their dietary iodine intake is limited (CDC (Centers for Disease Control and Prevention), 2006). A recent first study of its kind by Taylor et al. (2014), which considered the effects of perchlorate on pregnant women with hypothyroidism and iodine deficiency and the neurodevelopment of their offspring, has provided evidence to show that the chemical affects cognitive development so that their children had lower IQs when measured at three years of age.

In this paper, we attempt to use perchlorate in dust fall as a proxy for the determination of the quantity of fireworks burned in Malta using the rate of total dust fall and the concentration of perchlorate in this dust as the environmental forensic evidence.

2 Materials and Methods

2.1 Collection of field samples

Dust fall was collected from a total of 43 sites from both the main island of Malta and the smaller island of Gozo (Fig. 1), 38 sites of which were sampled during September and October 2011 and 17 sites during March, May, July, August, October and December 2012 and February 2013. Dust fall at each site was collected in four Beatson jars of 10 cm diameter which were exposed for

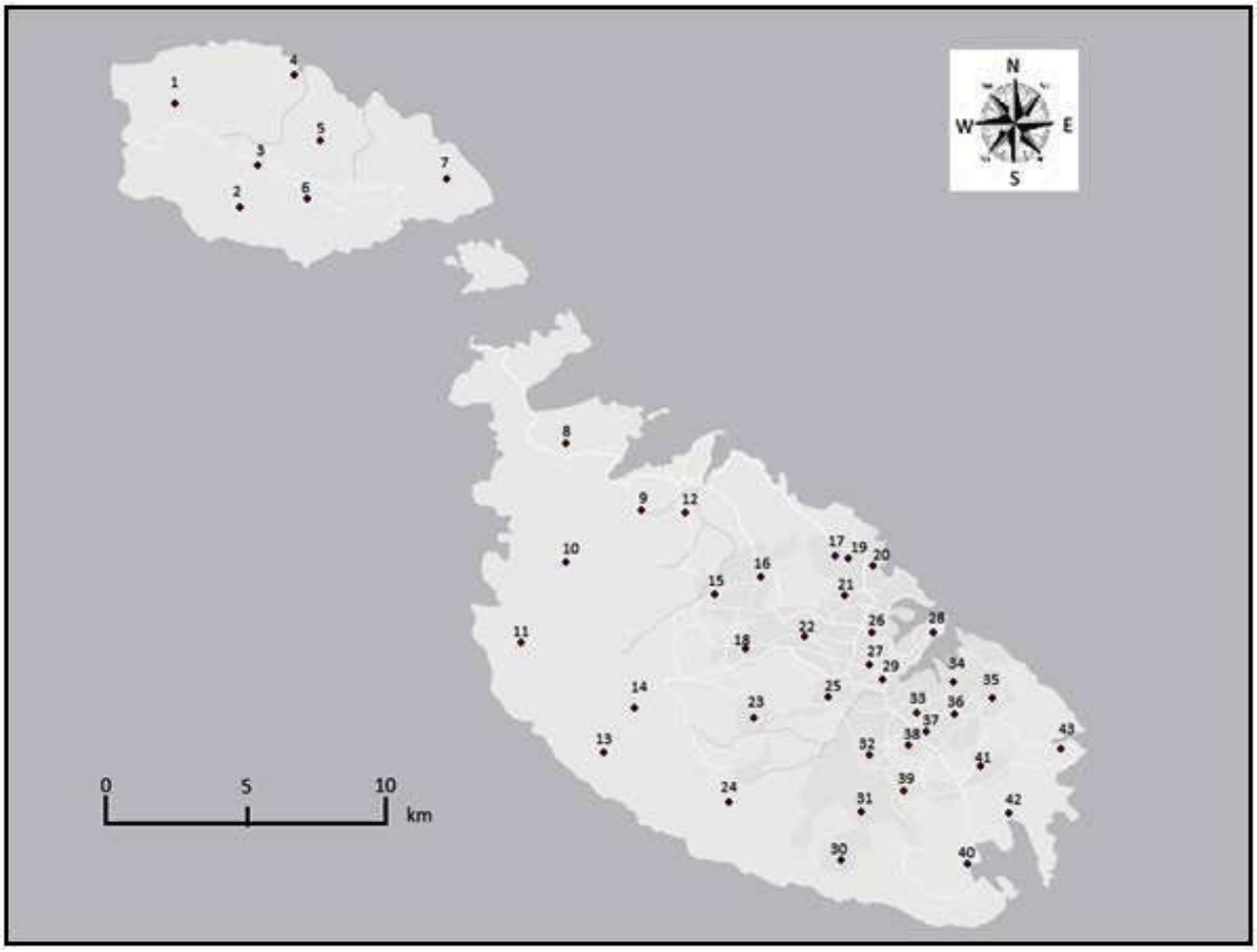

Figure 1: Map of the Maltese Islands showing localities from which dust fall was collected during September 2011 to February 2013.

about 30 days on rooftops of houses, away from walls. After collection, the jars were capped and stored at room temperature pending analysis.

The quantity of dust in each jar was determined by quantitative transfer into tared vials using deionised water which was then evaporated at 100 °C to constant mass. This allowed the rate of dust fall to be measured.

2.2 Measurement of perchlorate in dust fall

The method of analysis of perchlorate in the dust is described in detail elsewhere (Vella et al., 2015) and was based on extraction of the perchlorate in dust using deionised water and analysis of the extract by ion chromatography.

3 Results and Discussion

Fig. 2 shows the rate of dust fall in $\mathrm{mg\,m^{-2}\,d^{-1}}$ calculated as monthly means for the period September 2011 to February 2013. It is clear that, for most of the time, Malta experiences considerable dust fall which actually well exceeds $200\,\mathrm{mg\,m^{-2}\,d^{-1}}$, this value being the "limit of nuisance" suggested for the UK (Quality of Urban Air Research Group, 1996).

Several sources likely contribute to this coarse atmospheric dust and the major elements present in it (Ca, Si, Fe, Al) as reported by Vella et al. (2015) suggest that it is, in part, transboundary in nature and in part consists of re-suspended settled dust derived from soils and the local limestone terrain by attrition. Another strong local contributor is the quarrying and building industry (Vella & Camilleri, 2005).

The quantity of dust fall is much dependent on wind direction and rainfall: thus, for example, strong western and southern winds transport to Malta dust from the Sahara while rainfall effectively scavenges particulate matter and brings it to earth thus increasing the rate of deposition. For these reasons, the dry summer months July–August 2012 (precipitation in mm was 0.2 and 0.8 respectively) produced very low dust deposition during the period of measurement, although values were still above the mentioned nuisance value. The extremely high values registered during March 2012 and February 2013 were due to dust intrusions from the Sahara desert

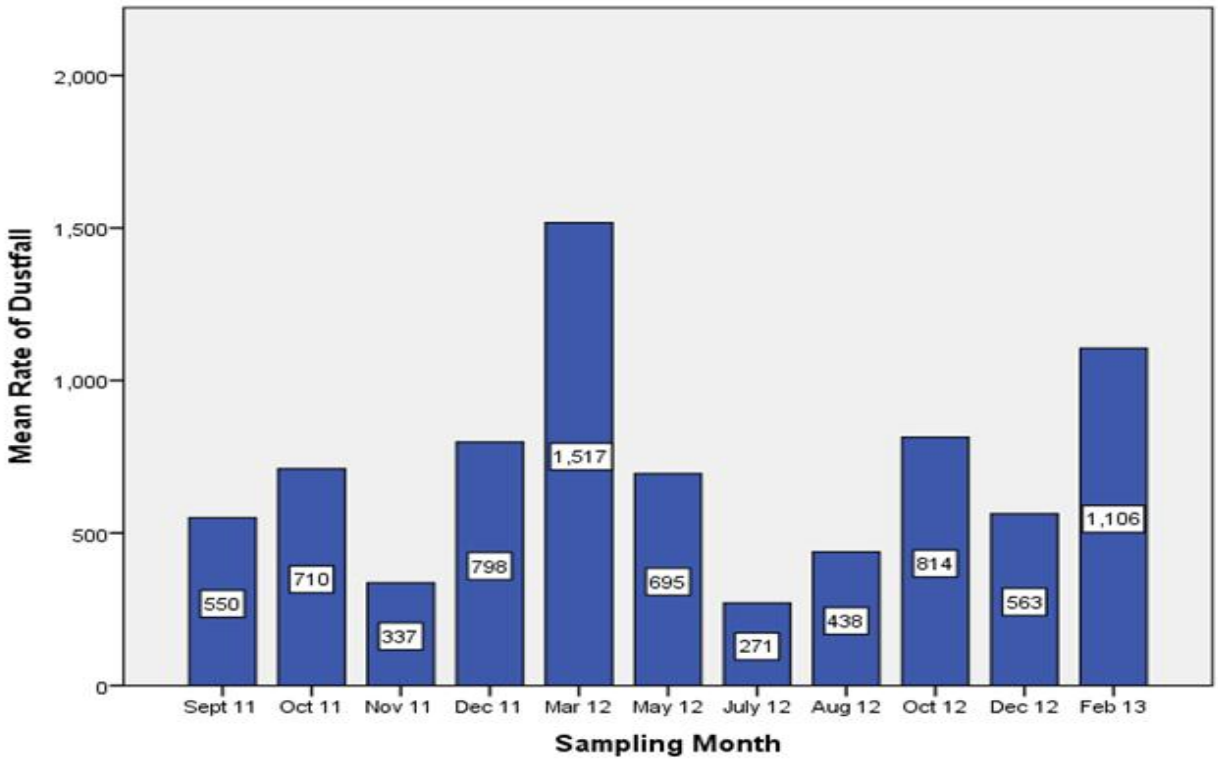

Figure 2: Mean dust fall deposition rates in $\mathrm{mg\,m^{-2}\,d^{-1}}$ during September 2011 to February 2013.

which caused significant reductions in visibility according to the Meteorological Office.

We have shown (Vella et al., 2015) that perchlorate levels in dust reached maximum values of about 120 ppm (July 2012) and 40 ppm (August 2012) during the period corresponding to lowest rates of deposition of dust fall. Concentration values dropped to < 20 ppm in the other months and were lowest (< 5 ppm) during February (2013) and March (2012).

Using this information together with the total dust fall data, one could deduce the quantity of potassium perchlorate used in fireworks displays locally if one knew the emission factor for perchlorate of Malta-made fireworks and adopted a box model with specific assumptions regarding the mass balance.

There is a paucity of information with regards to emission factors for perchlorate from fireworks generally and there is none pertaining specifically to Malta manufacturers: so one is obliged to use the best data that is available. Armstrong, Ridley, Guilbeault and Duff (2009) established that for different pyrotechnic articles containing between 2.5 and 28.0% by mass perchlorate, combustion in a sealed container resulted in formation of between 0.001 to 0.017% of residual perchlorate, although the authors observed that these values could be underestimates for real pyrotechnic devices. In another study by Oxley et al. (2009), the emission factors for perchlorate in propellant and explosive articles containing 56.0% and 5.6% by mass perchlorate, respectively, were found to be 0.0022 and 0.0210%; in road flares containing 10% by mass perchlorate, the emission factor was significantly higher, namely 0.17%. In Malta, flash crackers typically contain 63% by mass perchlorate (Camilleri, 2008) but since no information is currently available for the emission factor for the local products, we assumed a probably-inflated value of 0.17%.

Aerial pyrotechnic activity in Malta takes place almost exclusively within the built zone at distances of about 200 m from inhabited areas. Ground displays often occur within town squares or in open spaces within the township. Since the built environment constitutes 22% of the total surface area ($316\,\mathrm{km^2}$) of the Maltese Islands, we assumed that, to a first approximation which ignores losses to uninhabited and marine areas, the perchlorate-laden dusts from fireworks deposit equally over an area of about $69.5\,\mathrm{km^2}$. Using official perchlorate consumption data for 2012 i.e. 2.5 t, an estimated 4.25 kg of $KClO_4$ or 3.03 kg perchlorate (ClO_4^-) is emitted as unreacted oxidant over a period of about 3 months (July to September), which gives an average predicted value of $0.485\,\mathrm{\mu g\,m^{-2}\,d^{-1}}$. Since for July 2012, the average dust fall was $271\,\mathrm{mg\,m^{-2}\,d^{-1}}$, the calculated mass

fraction of perchlorate in the dust is 1.8×10^{-6}, corresponding to 1.8 ppm. Similarly, for August 2012, the rate of dust fall was $438\,\mathrm{mg\,m^{-2}\,d^{-1}}$ giving a predicted perchlorate concentration of 1.1 ppm.

The experimental mean perchlorate levels in dust for these two months were 120 ppm (July 2012) and 40 ppm (August 2012) which are 67 and 36 times larger, respectively, compared to the modelled values. Given the approximations in the calculation, the two values are reasonably close and clearly indicate that the real quantity of potassium perchlorate being released into the local environment is definitely much larger than the value reported to the National Office of Statistics. If we take, conservatively, the lower of the two estimates, then we conclude that the amount of potassium perchlorate imported annually for firework manufacture is most likely not less than 90 t.

If the value of the emission factor for Malta-made pyrotechnics for perchlorate is lower than the one assumed in this calculation and closer to the value determined by Armstrong et al. and Oxley et al., which pertained to pyrotechnic articles and not road flares, then the discrepancy between the modelled levels based on an import figure of 2.5 t and that actually measured in the dust would increase further. Moreover, even if the assumption that perchlorate is spread uniformly over about a quarter of the territory is relaxed to include the whole of the island's surface area (which is probably unreasonable), the resultant modelled import value (about 22 t) is still ten times larger than the official NSO record.

The fact that significantly more potassium perchlorate seems to be crossing jurisdictional borders than is apparently known to the authorities is of itself a concern, since the chemical is a precursor to powerful explosives and hence a useful commodity for terrorists. Importation of potassium perchlorate into Malta from European manufacturers does not, of course, require clearance by Customs although commercial trading involving the chemical has recently become subject to EU legislation. Regulation EU 98/2013 (OJEU (Official Journal of the European Union), 2013) requires specific official actions to be taken intended to keep tag of movements of (*inter alia*) potassium perchlorate between EU states or into the EU from third countries. This regulation exempts pyrotechnic articles.

Each firework factory is limited in the quantity of articles it can produce by the availability of oxidising agents: thus potassium nitrate regulates black powder production, which limits the quantity of lifting charges that can be manufactured and potassium chlorate, which is an ingredient in a number of manufactures. Both of these oxidising agents are controlled and each factory has access to 3200 kg (KNO_3) and 1000 kg ($KClO_3$) per annum of the chemicals. Given that there are about 30 factories in operation, it is likely that on average, each factory was employing about 3 t or more of additional oxidising agent over that function as provided by KNO_3 and $KClO_3$ and such would have given it potential to increase production by 170% over that without perchlorate use.

If perchlorate has indeed replaced chlorate completely in blasting mixtures, in response to the legal requirement, then the left-over but still utilized potassium chlorate is presumably being re-directed to other uses. Mixing potassium chlorate with charcoal produces a mixture (known as H3) which, due to its extreme sensitivity is illegal to produce but which serves to replace the safer but controlled black powder: this material is a critical component without which fireworks cannot be shot into the air and made to explode to form the familiar shapes associated with the art. If this is actually happening, then another factor is at play which increases the risk of accidental explosion during manufacture.

If utilization of perchlorate by the different factories is uneven, as is more likely, then the increase in consumption and hence in manufacture of articles by the bigger factories would be even larger than 170%. This surge in manufacture is not occurring in larger or improved amenities since fireworks factories have not upgraded or enlarged their facilities due to planning restrictions. This point is important in view of the fact that pyrotechnic manufacturing in Malta has had a rather poor safety record that has been principally attributed to human error, a factor aggravated by excessive and ever-rising production targets.

Apart from the effects on the pyrotechnic community itself resulting from excessive production, there are of course the equally serious effects on the general population. The presence of perchlorate in elevated concentrations in dust, including indoor dust, for at least 3 months per year is exposing the population to potential health risks from a contaminant with well-recognized toxic characteristics. The very young may be most at risk of exposure to perchlorate in dust in view of their propensity for ingestion of such matter. The presence of perchlorate in dust has potential to contaminate other environmental reservoirs such as drinking water and agricultural produce and this would serve to increase consumption of the chemical beyond that already likely present in several items of imported food (Lee et al., 2012).

Environmental pollution is often not easy to tackle when the causative agents are important due to their linkage to production and wealth creation or energy generation. Fireworks are a non-essential activity notwithstanding which however it has significant support from both the 1500-strong pyrotechnic community itself and a sub-set of the general population who fears any inter-

ference with the "right of its pastime ('*delizzju*')". The bulk of perchlorate is used for the making of maroons ('*tal-bomba*') and substantial reduction of these noise-making but otherwise colourless fireworks could go some way towards reducing contamination of the environment by perchlorate and other toxic wastes.

Could fireworks be made without the use of perchlorates? The problem is currently under investigation and compositions containing periodates (Moretti, Sabatini & Chen, 2012) and copper iodide (Klapötke, Rusan & Sabatini, 2014) and no $KClO_4$ have been found to perform well for certain applications. Also, organic high-energy materials such as 5-aminotetrazole in place of perchlorate also offer promise (Sabatini & Moretti, 2013). However, although future more environment-friendly ("greener") fireworks are likely to become technically possible, these will probably not be as cheap to manufacture as the chlorine-based pyrotechnics. Also, their adoption locally could present significant challenges to manufacturers, these being largely artisans with limited scientific training. Thus, to protect Malta's environment from further environmental contamination from fireworks, an immediate cap on the use of perchlorate is essential.

4 Conclusion

On the basis of the levels of perchlorate in dust fall and given that this chemical has no other local applications besides fireworks making and burning, a mass balance calculation shows that such manufacture is significantly higher than that which may be deduced from the official rate of importation of the precursors. Our results show that firework manufacture and burning has increased significantly since perchlorate use has become popular and even mandatory for the making of certain firework articles. It appears that factories may have increased their production by 170% over that based on the sole use of the controlled oxidising agents. Excessive manufacturing, coupled with the lack of proper amenity and possibly overcrowded work rooms could lead to accidents and thus obliterate any advantages provided by adoption of the safer chemical for the making of the fireworks. Reducing and limiting the use of perchlorate is the only solution for lowering the significant environmental impact from fireworks in this country which is needed to protect human health from possible deleterious effects from this chemical.

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Research Article

The re-emergence of the B1 cell compartment: Is this a pre-lymphoma stage?

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Abstract. Chronic Lymphocytic Leukemia (CLL) are in some cases stereotyped for immunoglobulin variants in different populations, suggesting emergence of B cell subsets following presentation of the same antigen. CLL cells may originate from $CD5^+$ naïve cells and from CD5 memory cells. Gene expression studies characterized a common cell of origin of the two clinical categories of CLL; the unmutated aggressive type and the mutated indolent type. The aim of this study was to investigate the presence of CD5 positive B cells in the elderly and their potential stimulation with exosomes derived from tumor cells. The findings from this study is aimed to create a model to identify instigating carcinomatous factors that may stimulate B1 cells to transform into a CLL-like model.

In this study we show that $CD19^+$ cells (B cells) in cord blood have a high expression of CD5. CD19/CD5 staining of blood samples from senior citizens showed the presence of B cells which also express the CD5 marker, though at a lower expression when compared to CLL cells ($CD19^+/CD5^{dim}$ B cells). Measurement of clonality using λ/κ flow cytometry staining show a monoclonal origin of the human $CD19^+/CD5^{dim}$B cells. Monoclonal B cell Lymphocytosis in the elderly is a potential cell compartment that represents the origin of B cell proliferative disorders. The origin of the B cell proliferative disease requires antigen stimulation. A preliminary experiment showed that sorted lymphocytes can be stimulated by exosomes isolated from 2 cancer cells lines, A549 (lung epithelial) and PC3 (prostate cell line). In comparison with phytohaemagglutinin (PHA) and phorbolmyristate acetate (PMA), known lymphocyte stimulators, the exosomes stimulated the proliferation of monocytic-like cells. Further characterization is required to know the origin of these cells. The result shows that one can speculate that exosomes present cancer-derived antigens and stimulate cell proliferation. Further studies are required to evaluate the potential transformation capacity of cancer-derived exosomes. In addition, various cytokines were measured in the sera of senior citizens to investigate a differential release of cytokines in the presence or absence of the $CD19^+/CD5^{dim}$ B cells. Cytokines examined were not significantly different between the 2 groups and further evaluation of cytokine levels is required.

Keywords: $CD5^+$ B cells, exosomes, CLL, lymphocytosis

1 Introduction

Development of lymphoproliferative disorders have been studied extensively, with a number of observations that elicit interesting mechanisms that are still not fully understood, such as the origin of subpopulations of B cells expressing the CD5 marker. Lymphomas are lineage specific and proliferate and transform into the specific sub-types reflecting the cell of origin (Caligaris-Cappio & Hamblin, 1999; Stevenson & Caligaris-Cappio, 2004). Viral infections are associated with transformation events in lymphomas, such as Epstein-Barr virus associated lymphoproliferative disorders (Rezk & Weiss, 2007) and some strains of *Helicobacter pylori* causing stomach maltomas and lymphomas. Treatment targeting the infection result in complete remission in most of the cases, suggesting that the lymphoproliferative disorder is driven by antigen presentation originating from the infective mechanism (Morgner et al., 2001). Activation of B cells using supernatant of mast cells, identified the role of exosomes as peptide presenting cells, resulting in blast formation,

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proliferation and IgM production, independent of cell-to-cell interactions (Skokos et al., 2001). This supports the role of antigens to stimulate, activate and transform B cells, and prompted us to use exosomes as vehicles for tumour derived antigen presentation in this study.

Of interest, CLL and mantle cell lymphomas are characterised by a subset of B cells that express the CD5 marker (Dono, Cerruti & Zupo, 2004). CD5 positive B cells constitute 80% of B cells in the human newborn decreasing to 10% of B cells in the adult human peripheral blood (Dalloul, 2009). The CD5 molecule is mainly associated with a repressive signal (Soldevila, Raman & Lozano, 2011), indicating that CD5 positive B cells are under tight proliferative control.

Various studies indicate that B cell stereotypic subsets (Damle et al., 2002; Stamatopoulos et al., 2007) might emerge from the presentation of antigens. Our hypothesis is that these stereotypic subsets might emerge from co-occurrence with solid tumours and the antigenic repertoire presented by apoptotic cancer cells. This is supported by the fact that antibodies produced by CLL cells can bind to cancer neoantigens from epidermoid cells, melanoma and liver cancer antigens (Chu et al., 2008, 2010) and that apoptotic cancer cells secrete exosomes (Fevrier & Raposo, 2004) into the microenvironment eliciting an immune response (Delcayre, Shu & Le Pecq, 2005). Immunogenic tumour-derived exosomes potentially stimulate an indolent subset of immune cells, resulting in transformation and clonal expansion as seen in CLL. In addition studies have shown that CLL mAbs recognize and bind the nonmuscle myosin heavy chain IIA (MYHIIA) exposed on the cell surface of a subgroup of apoptotic cells (MEAC) (Chu et al., 2008, 2010). Interestingly, 94% of CLL mAbs binding to MEACs expressed unmutated immunoglobulins, of which 88% belonged to a stereotyped subset.

In this study we identified a subset of B cells expressing low levels of CD5 and utilised cancer cell derived exosomes to study the potential stimulation effect. Further characterisation of these cells is required to allow proper proliferation and expansion. The ultimate goal of this study is to identify instigating carcinomatous factors that may stimulate B1 cells to transform into a CLL-like model.

2 Methods

2.1 Collection of material

50 adults and 20 neonatal blood samples were consented as per ethical approved project (University Research ethics committee, University of Malta). The criteria for selection of the senior citizens included the absence of dementia and cancer. The serum samples were collected, centrifuged and stored in the Malta Biobank together with the consent forms. Mononuclear cells were isolated from whole blood and stained accordingly for the flow cytometry or the magnetic cell sorting as detailed below.

2.2 Flow Cytometry

A cohort of individuals ($n = 50$) over 65 years of age were collected from Karen Grech Geriatric Wards. Following mononuclear cell isolation, the cells were incubated with antibody mixes (from BD Biosciences) consisting of fluorescein isothiocyanate (FITC) conjugated anti-CD5, PerCP-Cy5.5–conjugated anti-CD19, FITC–anti-κ and phycoerythrin (PE)–anti-λ light chain. At least 200,000 events were acquired on a FACS Calibur equipped with a 488 argon ion laser and 635 red diode laser (Becton Dickinson) and analysed with the CellQuest software system (Becton Dickinson). The ratio of κ^+ and λ^+ events was evaluated following gating of $CD19^+$ subsets. The κ/λ ratio was considered abnormal when it was more than 3:1 or less than 1:3, providing information on the monoclonal proliferation of $CD19^+$ B lymphocytes.

2.3 Magnetic cell sorting (MACS)

For stimulation experiments, $CD19^+$ cells from neonatal whole blood were sorted using the EasySep Human CD19 positive selection kit from Stem Cell Technologies, as per manufacturer's instructions. In summary, mononuclear cells were isolated using Ficoll Histopaque (SIGMA), followed by addition of a positive selection antibody cocktail. After mixing and incubation, the magnetic nanoparticles were added. The cell suspension was placed into the magnet for 5 minutes and the tube was inverted to remove the unbound cells. These cells were labelled as $CD19^-$. Following a number of washes, cells were incubated overnight for subsequent experiments.

2.4 Cytokine level measurements

The level of cytokines in serum were measured by a multiplexed method using a Procarta immunoassay (Affymetrix) on a Luminex platform. The serum and antigen standards were prepared according to the protocol. The captured protein was incubated with antibody conjugated beads for 60 minutes. To detect the captured antibody conjugated beads these were incubated with biotinylated detection antibody for 30 minutes. The beads were then incubated with streptavidin-SE (sape) for 30 minutes and the signal from the beads was read on the Luminex instrument.

The filter plate was prepared, including standards, samples and a blank. Antibody polystyrene beads were added with antigen standards and serum samples. Following incubation at room temperature the plate was mixed for 60 minutes at 700 rpm, washed and incubated with the detection antbodies. Following addition of SAPE and washes the fluorescence was read using the

Luminex Analyser.

2.5 Exosome isolation

The prostate cancer cell line, PC3 and the epithelial lung cancer cell line, A549 were cultured and expanded to have at least 1×10^8 cells. The cells were serum deprived for 24 hours and 100 ml of supernatant was harvested from each cell line.

Exosomes were isolated using an optimized protocol kindly provided by Professor F. Cappello (University of Palermo) (Merendino et al., 2010). The supernatant was centrifuged at 2000 revs for 5 minutes to remove the cells and cell debris, and transferred to ultracentrifuge tubes. Following a 2 hour spin at 110,000 g in a ultracentrifuge (RotorT25) the resulting exosome pellet was collected and suspended in 150 µl of PBS. The exosome suspension was stored at 4 °C until use. Figure 1 shows the resulting electron micrograph of the exosomes isolated from the A549 cell line.

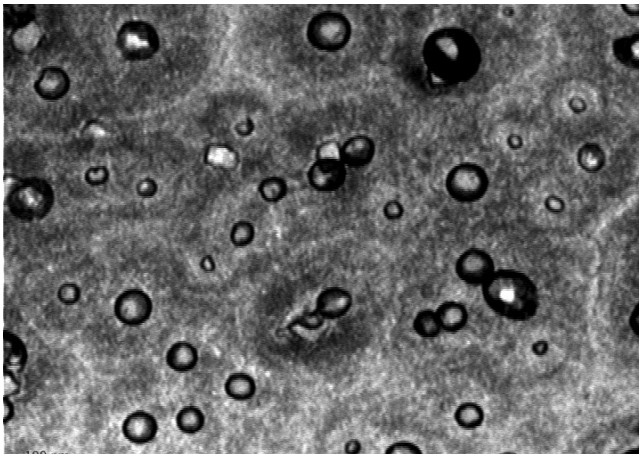

Figure 1: Electron micrograph showing exosomes isolated from A549 cell line. The image was taken at Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, Sez. Anatomia Umana, Università degli Studi di Palermo [Prof. Francesco Cappello].

2.6 Stimulation experiments

The cells from the magnetic sorting preparation were used for stimulation experiments. Both the $CD19^+$ cells and the $CD19^-$ cells were used in this experiment. Stimulation was done using known lymphocyte stimulators, Phytohemagglutinin (PHA) and Phrobolmyristate acetate (PMA) or with exosome suspension isolated from cancer cell lines. To remove macrophages, the cells were incubated overnight and adherent cells were removed by transferring suspension cells to a new culture flask. The cells were divided into separate wells and treated with a final concentration of 50 µg ml^{-1} PHA, 80 nM PMA, and with exosome suspension derived from 2 cancer cell lines (PC3: prostate cell lines; A549: lung cancer cell lines), or left untreated. PHA was added to cells every third day and exosomes once on day 1.

3 Results

3.1 Characterisation of a $CD19^+$ $CD5^{dim}$ B cells subset

The $CD19^+$ fraction of the mononuclear cells derived from neonate cord blood, was positive for CD5 (ROI 1; Figure 2A). Of interest, the cells lacked a CD5 positive ($CD19^-$) T cell fraction. From a cohort of 50 senior citizens, 25 samples were selected on the basis of the number of $CD19^+$ events (> 100 events). In addition to the $CD19^+$ B cells and the $CD5^+$ T cells, immunophenotyping identified a $CD19^+$ $CD5^{dim}$ fraction (Figure 2B). The percentage of B cell subtypes in the cohort are summarised in Table 1. In the age group between 65 and 75 years, less than 20% of total B cells ($CD19^+$) stained for CD5 ($CD19^+$ CD5dim), while other age groups ($n = 22$) showed samples with higher percentages (Figure 3). Of interest, one of the senior citizens samples showed that more than half (57.12%) of the B cells were typed as

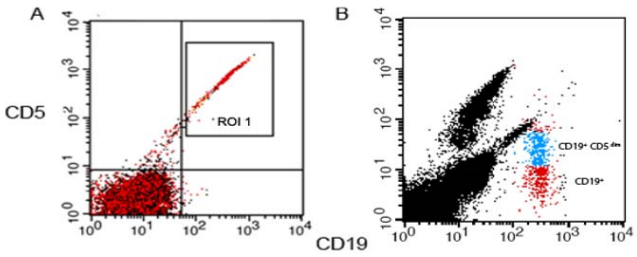

Figure 2: Flow cytometry of stained (CD5 and CD19 antibodies) mononuclear cells derived from (A) neonates and (B) adult (age of 79). The quadrants in (A) were set using isotype controls. [ROI 1 = Region of Interest 1].

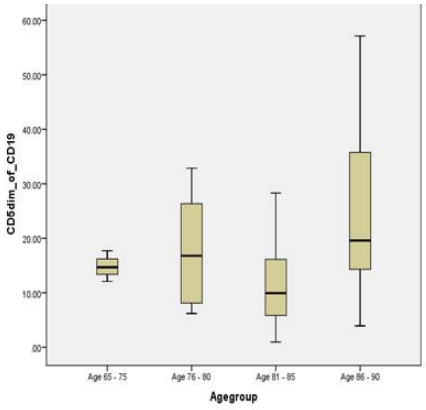

Figure 3: Box plot showing distribution of $CD19^+$ $CD5^{dim}$ percentage across age groups. Age group 65–75 ($n = 3$); age group 76–80 ($n = 8$); age group 81–85 ($n = 7$) and age group 86–90 ($n = 7$).

Table 1: Percentage of B cell subtypes in the senior citizen cohort.

Sample	Age	$CD5^{dim}$ [$CD19^+$ gated]		$CD5^{neg}$ [$CD19^+$ gated]	
		Events	% of $CD19^+$	Events	% of $CD19^+$
A8	87	381	57.12	285	42.73
A16	86	68	40.48	107	63.69
A22	77	68	32.85	168	81.16
A13	90	68	31.05	168	76.71
A21	76	41	30.15	88	64.71
A9	81	73	28.29	180	69.77
A49	79	141	22.56	362	57.92
A3	84	87	21.59	308	76.43
A32	80	39	20.42	140	73.3
A10	90	141	19.58	527	73.19
A42	73	28	17.72	120	75.95
A45	65	64	14.68	352	80.73
A39	87	20	14.39	118	84.89
A6	90	68	14.23	168	35.15
A11	76	50	13.16	289	76.05
A27	65	33	12.09	237	86.81
A14	84	11	10.68	80	77.67
A1	85	30	9.93	271	89.74
A38	85	11	9.91	90	81.08
A28	79	24	8.25	257	88.32
A50	78	39	7.93	377	76.63
A23	77	14	6.19	210	92.92
A44	89	9	3.93	210	91.7
A51	83	3	1.76	156	91.76
A19	83	3	0.95	313	98.74

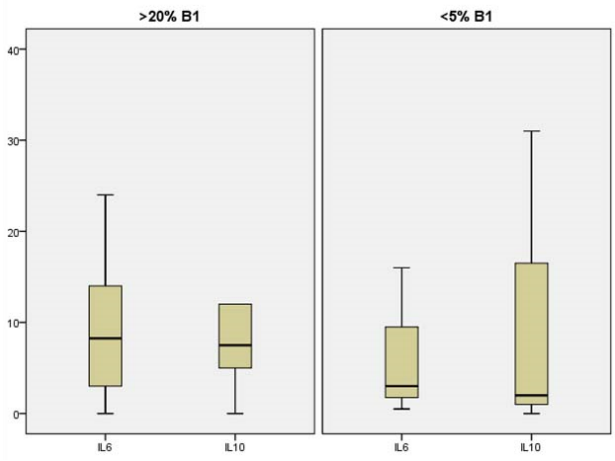

Figure 4: Distribution of IL6 and IL10 levels. The left panel shows the results obtained for individuals which have a $CD19^+/CD5^{dim}$: total $CD19^+$ ratio higher than 0.2 (> 20% B1), while the right panel individuals with a $CD19^+/CD5^{dim}$: total $CD19^+$ ratio lower than 0.05 (< 5% B1). [B1 = $CD19^+/CD5^{dim}$ cells].

$CD19^+$ $CD5^{dim}$. In this sample, flow cytometry using λ/κ staining showed a monoclonal origin of the human $CD19^+/CD5^{dim}$ cells.

3.2 IL6 and IL10 levels are not affected by presence of $CD19^+$ $CD5^{dim}$ cells

Given that the variance between age groups showed an increase of $CD19^+/CD5^{dim}$ cells with older age groups, and that the age group between 65 and 75 were all below 20% of B cells (Figure 3), we used this as a threshold to compare cytokine levels between those individuals with a percentage higher than 20% and individuals in which the $CD19^+/CD5^{dim}$ cells are less than 5% of the total B cells. Both IL6 and IL10 showed a high variance in the different groups and although there is no significant difference, the average of cytokine levels is higher in individuals having a $CD19^+/CD5^{dim}$: total $CD19^+$ ratio higher than 0.2 (> 20% B1; Figure 4).

3.3 Cancer cell line derived Exosomes stimulate mononuclear cell expansion

The cord-blood derived $CD19^+$ fraction, was cultured in the various stimulants or left unstimulated. In ad-

dition, the remaining fraction following separation was also used in the experiment. The $CD19^+$ fraction was kept in culture only for 3 days and hence further experiments were not possible. Hence, further experiments require the isolation of this fraction from a monoclonal lymphocytosis individual characterized in the screening described above. The other fraction was viable for the whole duration of the experiment. The fraction consisted of mononuclear cells with $CD19^+$ cells depletion. Proliferating cells under PHA stimulation were confirmed to be $CD5^+$, while cells under exosome stimulation are not $CD19^+$ or $CD5^+$ (Figure 5A–5C). Infact, electron microscopy show clearly that the proliferating cells are larger and cytospins suggest selection for the monocytic lineage. Of interest, at day 12 (Figure 5D–5F) cells in the presence of exosomes show proliferation of lymphocytes and monocytic cells. Proliferation of lymphocytes was absent at day 12. Hence eventual experiments should be designed to add exosomes at various intervals. All cultures were stopped at day 21.

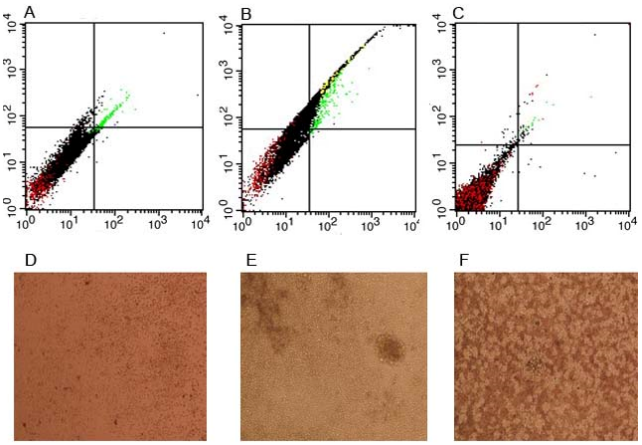

Figure 5: Flow cytometry of CD5/CD19 staining mononuclear cells (A-C). A: Isotype control; B: PHA stimulated cells and C: cells exposed to exosomes derived from PC3. Lower panel (D-F) shows light microscopy images of D: unstimulated cells; E: PHA stimulation at day 12; F: cells exposed to PC3-derived exosomes at day 12.

4 Discussion

Previous studies show that the presence of CD5 positive B cells increases with age in senior citizens (Geiger et al., 2000); CD5 positive B cells are detected in human malignancies (Wang, Amato, Rabah, Zheng & Fernandes, 2002) and that the presence of CD5 positive B cells in relatives of Chronic lymphocytic leukemia (CLL) patients, increases the risk of acquiring the disease (Rawstron et al., 2002). This suggests that these cells are potential precursors that require a transformation event, to progress into a malignant phenotype. To study transformation to malignant phenotype, a source of physiologically occurring CD5 positive cells is required. CD5 expressing B cells include (1) the life-long pool of B1 cells homing primarily to the peritoneal cavity, and having the capacity of self renewal; (2) the $CD5^+$ B cell subpopulation in neonatal blood (Erkeller-Yuksel et al., 1992); and (3) monoclonal lymphocytosis in the elderly (Ghia et al., 2004). Hence we decided to immunophenotype cord blood and a cohort of senior citizens. Of interest all $CD19^+$ cells in neonatal derived lymphocytes highly express the $CD5^+$ marker. In addition we show the presence of a subset of $CD19^+$ B cells with low expression of CD5, $CD19^+/CD5^{dim}$. The percent of $CD19^+/CD5^{dim}$ to total $CD19^+$ increases with age (Figure 3), with percentages higher than 40% found in 2 individuals over 80 years of age. Weak expression of CD5 in $CD19^+$ B cells were previously described in a patient cohort of CLLs (Wang et al., 2002), showing heterogeneity of CD5 expression levels. The significance of the differential level of CD5 expression in B cells is unknown, and hence understanding their origin and function towards initiation or development of B cell malignancy is imperative.

Survival of CD5 expressing B cells depends on CD5-dependent IL10 production (Gary-Gouy et al., 2002). In addition to B1 cells, a subset of CD5 positive B cells produce IL10 and have a regulatory function (Yanaba et al., 2008). Although the $CD19^+/CD5^{dim}$ cells characterised in this study have a low expression of CD5, we measured the level of IL10 and other cytokines in the sera of senior citizens. As shown in Figure 4, the IL10 level varies considerable, but there is a trend to a higher mean level of IL10 in elderly with higher proportion (percent of total B cells) of $CD19^+/CD5^{dim}$ cells. IL6 was selected for analysis since with increasing age, this cytokine is more abundant and provides an inflammatory response required for the formation of various cell clones and in the transformation of the benign condition, MGUS into Multiple Myeloma (Fayad et al., 2001). Our interest is that IL6 might also be involved in the transformation of MBL into a malignant CLL condition. Further analysis is required to quantify IL10 release in sorted cells. Of interest, the stimulation of B cells with phorbolmyristic acetate (PMA), increases the expression of CD5 (Youinou, Mackenzie, Jouquan, Le Goff & Lydyard, 1987). This suggests that cells can acquire the expression of CD5 through an activation stimulus. In this study we used neonatal-sorted $CD19^+$ $CD5^+$ cells to address the potential activation of CD5 positive B cells using well known activators, such as PMA, and cancer cell- derived exosomes as a vehicle to present cancer antigens. In the stimulation experiment, we used both positively selected $CD19^+$ cells and the mononuclear cells depleted of $CD19^+$ cells, derived from neonatal blood.

The positive selected $CD19^+$ cells lost viability after 3 days, and the stimulation experiment was performed using the cells depleted of $CD19^+$ cells ($CD19^-$ fraction).

In comparison with PHA and PMA (known lymphocyte stimulators), the exosomes isolated from 2 cancer cells lines, A549 (lung epithelial) and PC3 (prostate cell line) stimulated the proliferation of monocytic-like cells. Characterization is required to know the origin of these cells and further studies should utilise $CD19^+/CD5^{dim}$ cells from elderly individuals, ideally from the 2 individuals that have a high percentage of cells expressing the CD5 marker.

5 Conclusion

Creating a cellular model of B cell transformation following antigen presentation will provide a tool to study the origin of CD5 positive cells and to understand the role of tumour derived-exosomes in antigen presentation and activation of B cells. In this paper we show the presence of a specific population of CD5 positive cells that re-emerge in the elderly individuals, and the capacity of tumour derived exosomes to stimulate lymphocytes and monocyte-like cells. Further studies are required to expand the $CD19^+$ $CD5^+$ cells. Understanding stimulation and proliferation of these cells will be useful as a CLL-like model.

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Research Article

Influence of Antimony on Structure and Physical Properties of Molten Tin

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Abstract. Structure of liquid Sb-Sn alloys were studied by means of viscosity measurements and X-ray diffraction. Structural factors and pair correlation functions were analysed and interpreted using the random atomic distribution model. The features of temperature dependence of the viscosity coefficient were analysed taking into account X-ray diffraction patterns. The results allow us to conclude that Sb atoms substitute for Sn atoms, forming a typical atomic solution, which reveals chemical and topological short-range order. Moreover, certain atoms form Sb- and Sn-based Sb_nSn_m associates and self-associates.

Keywords: liquid alloys, Sb-Sn, viscosity, X-ray diffraction, antimony (Sb), tin (Sn)

1 Introduction

The physical properties of tin (Sn) and antimony (Sb) are similar. Owing to their low melting temperatures, Sn-based alloys can be used in the production of Pb-free solders. In particular, Sb-Sn alloys with other metals are widely used. Therefore, studying Sb-Sn alloys in the liquid state is of great importance.

The equilibrium phase diagram of the Sb-Sn binary system reveals the presence of SbSn and Sb_2Sn_3 metallic phases (Vassiliev, Lelaurain & Hertz, 1997). Measurements of physical properties (Anusionwu, 2006; Zu et al., 2006) and thermodynamic studies (Sommer, Luck, Rupf-Bolz & Predel, 1983) reveal microsegregation in liquid Sb-Sn alloys. Experimental density, surface tension and molar volume differ from the predictions of the regular solution model (Gasior, Moser & Pstrus, 2003). Partial studies on the viscosity and structure of liquid Sb-Sn alloys have also been performed (Sato & Munakata, 1956; Klym, Mudry & Kozyrenko, 1986). The understanding of the relationship between the structure and physical properties is limited and needs further quantitative characterisation. At this point, new experimental studies of dynamic viscosity and the structure of liquid Sb-Sn alloys seem worthwhile.

The phase diagram of Sb-Sn does not reveal unlimited solubility of components, but it indicates the presence of intermediate phases. Therefore, the effects of antimony atoms on the structure of tin are worth studying.

2 Experimental

The samples were prepared in an arc melting furnace filled with argon, from Sb and Sn of high purity (99.99%). The diffraction studies were carried out using a high-temperature diffractometer with a special attachment, which allows investigation of the liquid samples at different temperatures (up to 1800 K). Cu–K_α radiation, monochromatized by means of a LiF single crystal as a monochromator and Bragg–Brentano focusing geometry, were used. The scattered intensity was recorded as a function of the scatering angle within the range $1\,\text{Å}^{-1} < k < 7\,\text{Å}^{-1}$, with an angular step of 0.05° within the region of the principal peak and 0.5° for the remaining values. The scattered intensity was measured with an accuracy of at least 2%. In order to obtain the scattered intensities with such accuracy, the scan time was chosen as 100 s. The diffracted intensity was recorded using a NaI(Tl) scintillator detector together with an amplification system. The sample was placed

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in a round cup with 20 mm diameter. Intensity curves were corrected for polarization and incoherent scattering (Cromer & Waber, 1965) and were subsequently normalized to electron units by the Krogh–Moe method (Krogh-Moe, 1956). The obtained intensity curves were used to calculate structure factors (SFs) and pair correlation functions (PCFs). The main structural parameters obtained from SFs and PCFs were subsequently analyzed.

Sb-Sn alloys containing 10, 20, 30 and 40 at.% Sb, respectively, were chosen for viscosity studies. The composition of the samples, weighing about 30 g, was accurate to 0.02 wt%. Each sample was weighed before and after the measurements and no loss of mass was observed. Viscosity measurements were carried out using an oscillating-cup viscometer. The samples were placed in a graphite chamber with internal diameter of 14 mm, filled with helium of high purity and remained there throughout the entire experiment. The temperature was measured with a WRe5/20 thermocouple attached just below the crucible. A detailed description of the equipment was given in Mudry, Sklyarchuk and Yakymovych (2008). Before the measurements were taken, the samples were homogenized for about 5 hours at the highest temperature. The viscosity was measured during the cooling of the samples. The viscosity coefficient η was calculated from the measured logarithmic decrement, whereas the period of the oscillations were determined using the modified Roscoe equation (Vollmann & Riedel, 1996). The viscosity data were obtained with an accuracy of about 3%.

3 Results and Discussion

Experimental structure factors obtained for the liquid $Sb_{100-x}Sn_x$ $(x = 70, 90)$ alloys were compared with those of liquid Sb and Sn near their melting points (5 K above the melting temperature T_m) (Fig. 1). The main structural parameters are listed in Table 1.

The experimental $S(k)$ exhibits a shoulder on the right-hand side of the first peak. This shoulder points to the presence of structural units in which the atoms are covalently bonded.

The positions of the principal peak and the shoulder on the right-hand side of this peak coincide for the liquid $Sb_{10}Sn_{90}$ alloy and liquid Sn (Fig. 1). Upon the addition of 10 at.% of Sb, the second maximum shifted towards lower k values. Upon further increase in the content of antimony, up to 30 at.%, this tendency became more pronounced – the first and second maximum shifted further towards lower k values.

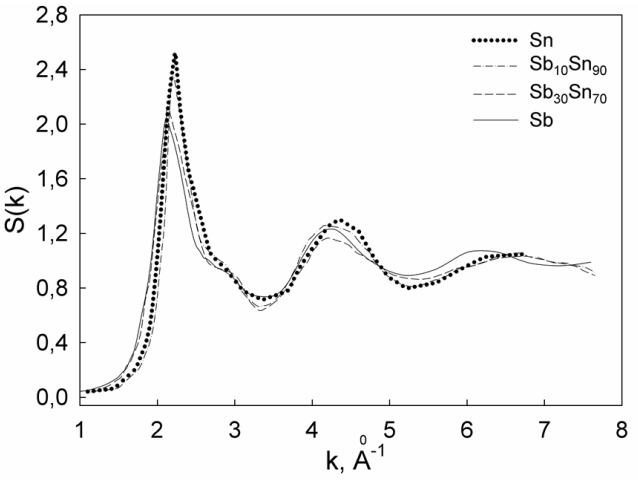

Figure 1: Structure factors of liquid Sb, Sn and liquid $Sb_{30}Sn_{70}$, $Sb_{10}Sn_{90}$ alloys.

By comparing the positions of the maxima (k_1 and k_2) for tin with the structure factors of doped melts, we conclude that Sb atoms added to Sn melt form a structure of their own. For the investigated molten alloys the ratio k_2/k_1 was close to the corresponding ratio of molten tin and molten antimony. The position of the experimental most probable interatomic distance r_1 showed no significant changes with composition. This suggests the presence of associates in the liquid $Sb_{30}Sn_{70}$ alloy, the composition of which is similar to the composition of the investigated melt, as well as the presence of self-associates of Sb_l and Sn_k. This is in agreement with Anusionwu (2006), Zu et al. (2006), Sommer et al. (1983). The experimental coordination number (Z_{exp}) was different from that calculated according to the regular solution model (Z_{RS}) for the liquid alloy at 10 at.% Sb. This was most likely caused by topological disorder-

Table 1: Structural parameters of liquid Sn and Sb-Sn alloys, where k_1 is the position of the first peak of the structure factor; k_2 is the position of the second peak of the structure factor; $S(k_1)$ is the height of the principal peak of the structure factor; r_1 is the experimental most probable interatomic distance; $r_{1(AD)}$ stands for the most probable interatomic distance calculated according to the random atomic distribution model; Z_{exp} is the experimental value of the coordination number; Z_{RS} is the coordination number calculated according to the regular solution model.

at.% Sb	k_1, Å^{-1}	k_2, Å^{-1}	k_2/k_1	$S(k_1)$	r_1, Å	$r_{1(AD)}$, Å	Z_{exp}	Z_{RS}
0	2.23	4.35	1.95	2.52	3.23	3.23	9.7	11.0
10	2.20	4.15	1.89	2.35	3.25	3.24	8.3	7.0
30	2.13	4.20	1.97	2.13	3.20	3.26	7.3	6.9

ing.

The dependence of the coefficient of viscosity on temperature for liquid Sb-Sn alloys with different concentrations is shown in Fig. 2. The experimental curves demonstrate Arrhenius-like behaviour in a wide range of temperatures far from Tm, suggesting that no significant structural changes occur with a change in temperature,

$$\eta = A \exp\left(\frac{E}{RT}\right), \qquad (1)$$

where η is the viscosity coefficient; A is a constant; E is the activation energy; T is the temperature; R is the gas constant.

We also calculated viscosity coefficients using the hard-sphere model (Dymond, 1974). The dependence of viscosity on temperature determined according to this model is compared with experimental data in Fig. 2. The hard-sphere model disagrees with the experimental data, which is most likely caused by the neglect of the attractive force in the formula for the hard-sphere potential (Dymond, 1974)

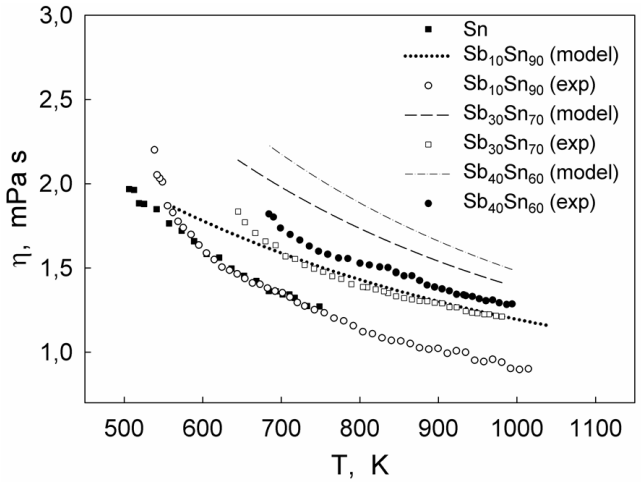

Figure 2: Viscosity of liquid Sn and of liquid Sb-Sn alloys.

The dependence of viscosity on the composition at two temperatures is presented in Fig. 3. Upon the addition of 10 at.% Sb, the viscosity was very similar to that of tin. For alloys containing 30 and 40 at.% of Sb, we noted an increase in viscosity.

Taking into account the profile of the equilibrium phase diagram, the aforementioned behaviour of viscosity was attributed to a structural change which occurred due to the preferential interaction of unlike atoms.

Thus, upon the addition of Sb atoms to tin, the most significant changes were pronounced in the profile of $S(k)$, the peak positions and the coordination number (Fig. 1, Table 1). In particular, $S(k_1)$ decreased and the second maximum shifted towards lower k values. A large discrepancy between the experimental results for

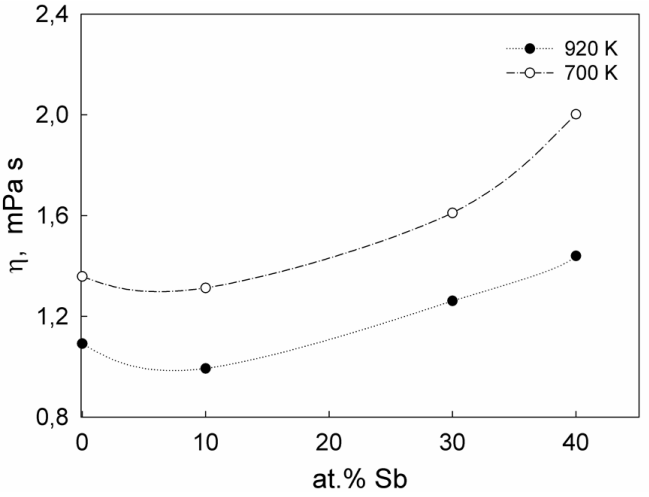

Figure 3: The concentration dependence of viscosity for liquid Sb-Sn alloys.

Z and the calculated results for liquid $Sb_{10}Sn_{90}$ suggests that for low concentrations of Sb in the melts near the melting point we deal with a micro-inhomogeneous structure. Such behaviour is in agreement with viscosity results obtained for liquid $Sb_{10}Sn_{90}$, which revealed a rapid increase in the values in the temperature dependence (Fig. 2) and divergence from Arrhenius-like behaviour (Fig. 4) in the temperature region near T_m.

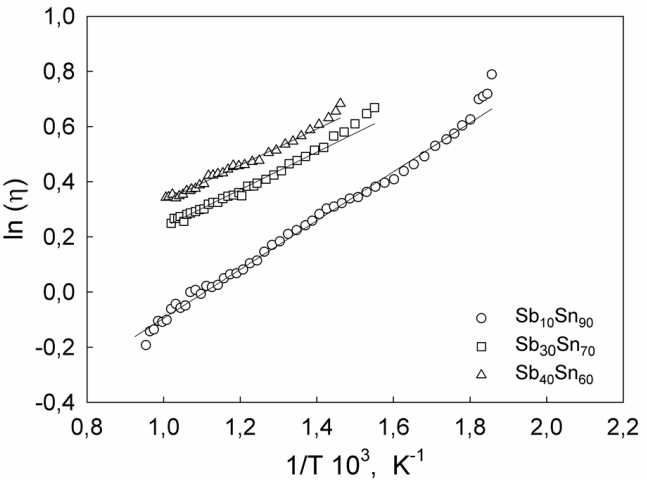

Figure 4: Dependence of viscosity of liquid Sb-Sn alloys on reciprocal temperature.

For higher concentrations of antimony, the interaction between the atoms of Sb and Sn was more pronounced and led to a change in the size of structural units. This was confirmed by an increase in the experimental values for viscosity of liquid Sb-Sn alloys, as compared with the viscosity of liquid Sn (Fig. 2). From this, the presence of associates and self-associates in liquid Sb-Sn alloys is inferred. Our data are in agreement with the res-

ults obtained in Sato and Munakata (1956), Klym et al. (1986).

The addition of a small amount of Sb neither significantly changed the structure (compared to pure Sn) at higher temperatures, nor led to the formation of a micro-inhomogeneous structure in the temperature region near the melting point.

The atomic structure changes upon subsequent addition of Sb atoms. An associated solution is formed, which is accompanied by a decrease in the values of the activation energy (Table 2). This is due to covalent bonding between Sb-Sb and Sn-Sn, which persists in the liquid state.

Table 2: Values of activation energy for liquid Sb-Sn alloys

at.% Sb	10	30	40
E, kJ mol^{-1}	7.37	5.66	5.45

4 Conclusions

Viscosity of liquid Sb-Sn alloys containing 10, 30 and 40 at.% Sb, respectively, increased with an increase in the content of Sb. Such behaviour was caused by the formation of clusters of Sb and their cooperation. The observed structural features and the viscosity of the liquid alloy with 10 at.% Sb suggests that atomic rearrangement occurred in the alloy in the temperature region near the melting point.

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Research Article

Numerical Modelling and Economics of Agricultural Land Degradation in the Maltese Islands

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Abstract. The study applies a new GIS-based numerical modelling approach to calculate the economic burden agricultural land owners suffer through soil erosion land degradation. Numerically modelled soil erosion volumes in Maltese agricultural areas were estimated at 766 278 m^3/yr costing 7.98 M€/yr to replace. The model calculates that the average owner incurs 1170 €/0.01 km^2/yr on soil replacement and soil improvement requirements. With average yearly economic revenue of 1720 €/0.01 km^2/yr, this cost benefit imbalance may force agricultural land owners to not replace eroded soils. Over time, as a result of soil erosion, an increasingly large proportion of agricultural land may no longer suitable for agricultural purposes. Over 50 years, 1.53 km^2 (0.5% of Maltese area) of agricultural land may be depleted of soil, incurring an average national agricultural revenue loss of 0.26 M€ per year.

Soil erosion rates, and associated economic implications, may be mitigated with cost effective management practices. Two such practices include conservation tillage, which offers various economic advantages to farmers, and the restoration of breaches in slope-facing rubble walls in areas subject to soil erosion. The latter may require an investment of 11.94 M€ at the National scale or €1,600 by the average agricultural land owner. Both measures contribute towards the sustainable use of Maltese agricultural areas and maintaining key associated ecosystems services.

Keywords: Malta, agriculture, rUSLE soil erosion, GIS land degradation modelling, economics of land degradation, land use management

1 Introduction

Land degradation is caused by various forces and leads to a significant reduction of the productive capacity of land (Amundson et al., 2015). Various human activities contribute and accelerate land degradation, chief amongst these are unsustainable land use practices and inadequate management of natural resources. Such activities degrade soil quality and reduce the ability of lands to provide various ecosystem services. Ecosystem services can be grouped into four main categories that often form the basis of various economic activities. These are the provisional services that include the production of food and water, regulatory services that control climate, supporting services that include nutrient cycles, and cultural services which offer recreational benefits (e.g. Barrios, 2007; Kibblewhite et al., 2008; Clothier, Hall, Deurer, Green & Mackay, 2011).

Extensive empirical evidence ties land degradation to reductions in the provision of ecosystems services (e.g. Pimentel et al., 1995). Such information has however rarely promoted policy action (Second Scientific Conference United Nations Convention to Combat Desertification (UNCCD), 2013). Rather, a systematic analysis of costs of land degradation and the benefits of preserving ecosystems services has been promoted decision-makers to take steps in achieving land degradation neutrality (e.g. Baumgartner, von Braun, Abebaw & Müller, 2015). This economic-based approach, termed economics of land degradation, provides an economic framework against which decision-makers can appreciate the value of taking action against land degradation (Yesuf, Mekonnen, Kassie & Pender, 2005).

The Maltese Islands are situated in the centre of the Mediterranean Sea, 93 km south of Sicily, 120 km east of the northern coast of Tunisia, and 355 km north of Tripoli (Libya). The Islands have a total land area of 320 km^2 consisting of three principal islands Malta, Gozo and Comino (Figure 1). The Maltese Islands have a semi-arid Mediterranean climate with an average an-

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nual rainfall of 524 mm and an average yearly temperature is 22.5 °C (Malta National Report, 2002).

Agricultural land use covers 48% of the Maltese islands and is its predominant land use (Rural Development Department, 2014). In 2011, the primary productive agriculture and fisheries sectors produced 1.8% of the National gross domestic product (GDP) and in 2010 the agricultural sector employed 10.6% of the financially active Maltese population (Axiak et al., 1998). Agriculture is therefore a key economic production centre and plays a key role in Malta's long-term food-provision (Ministry for Rural Affairs and the Environment, 2007).

Agricultural practices have a significant impact on an area's susceptibility to land degradation (Brasselle, Gaspart & Platteau, 2002). Appropriate management may sustain key ecosystem services and agricultural productivity while inadequate measures may degrade natural resources and reduce crop yields (Axiak et al., 1998).

The Maltese agricultural sector faces significant economic, social and physical challenges that limit agricultural revenue. Such challenges include the relatively small agricultural parcel sizes (an issue exacerbated by land fragmentation) and poor soil quality (Camilleri, 2005). In marginally profitable or entirely uneconomic situations agricultural land is abandoned. In Malta, such scenarios are common in valley margin terraced slopes. These areas require regular maintenance of rubble walls, are difficult to access and are of small size (Rural Development Department, 2014).

In Malta, significant expanses of sloping valley margins were reclaimed for agricultural use. The creation of valley margin terraced slopes involved the use of rubble material for levelling, infilling with soil and the construction of rubble walls to retain soil (Rural Development Department, 2014). The resulting anthropogenic landscape may be maintained under continued agricultural management. However, abandoned terraced fields do not receive the required rubble wall maintenance and consequently soils retained by these structures are rapidly eroded, transported and deposited downslope. This dynamic is eroding the thin soils artificially deposited in the flanks of valleys, and over time reducing the agricultural capacity of such areas. This dynamic demonstrates the importance and need for continued agricultural land management.

The paper aims to quantify the loss in Maltese agricultural provisionary services resulting from soil erosion and its economic consequences. The study provides an 5 economics of land degradation argument that highlights the costs and benefits of action versus inaction towards achieving sustainable agricultural land management. Various cost effective soil conservation methods and management policies aiming to achieve land degradation neutrality are proposed for areas subject to or susceptible towards agricultural land degradation.

2 Methods

2.1 Soil erosion (rUSLE)

The revised Universal Soil Loss Equation provides estimations of potential erosion rates, calculated from empirical and functional relationships between various factors [Equation (1)] (Renard, Foster, Weesies, McCool & Yoder, 1997). Sultana (2015) applies the rUSLE to estimate soil erosion by water in the Maltese Islands for the year 2013. Sultana applied rUSLE into a GIS-based model where input parameters - rainfall erosivity (R), soil erodibility (K), slope length and steepness (LS), cover and management practices (C) and conservation practices (P) (Wischmeier & Smith, 1978) - were prepared separately and stored as GIS vector layers with a cell size of 50×50 m. The aforementioned rUSLE factors were then converted to raster layers with a grid resolution of 50 metres. The reader is referred to Sultana (2015) for a detailed explanation of methods employed and justification of values applied for RUSLE factors.

$$A\left(\mathrm{t\,ha^{-1}\,yr^{-1}}\right) = R \times K \times LS \times C \times P, \qquad (1)$$

where, A = average annual soil loss ($\mathrm{t\,ha^{-1}\,yr^{-1}}$), R = rainfall/runoff erosivity ($\mathrm{MJ\,mm\,ha^{-1}\,h^{-1}\,yr^{-1}}$), K = soil erodibility ($\mathrm{t\,h\,MJ^{-1}\,mm^{-1}}$), LS = slope length and steepness (dimensionless), C = cover management (dimensionless), and P = support practice (dimensionless).

This paper focuses on agricultural areas. In view of this, soil erosion rates were exclusively calculated for agricultural areas. The five RUSLE factors – R, K, LS, C and P - were calculated on a cell-by-cell basis following equation (1) with the ArcGIS Spatial Analyst extension. The resulting layer defines average annual soil loss ($\mathrm{t\,ha^{-1}\,yr^{-1}}$) for each cell in the study area for the year 2013.

2.2 National soil depth

Soil depth, surface to bedrock, was measured at a grid distribution of between 0.5 to 1 km (Figure 4) using soil augers. Soil depth was measured in three hundred and thirty locations. In each location, soil depth was measured four times, each measure spaced 1 m east of the previous sampling point. The soil depth vales for each location were averaged and are displayed as point values in the average soil depth map (Figure 4). Average soil depth point values were used to interpolate soil depth between points using the ArcGIS kriging technique. The interpolation technique weighs the surrounding measured soil depth values to derive a predicted value for the unmeasured neighbouring locations. The weights applied in the kriging operation in this study are based

Figure 1: Map of the Maltese Islands (from Ezilon, 2009).

on the distance between the measured points, the values of the closest 8 point values and the prediction locations.

2.3 Soil volume and depth eroded per year

Eroded soil volume was calculated for each cell (50×50 m) [Equation (2)] from the eroded soil mass (variable tonne) obtained for each cell (50×50 m) through the rUSLE method [Equation (1)], and a common average soil density $\left(1.173\,\mathrm{g\,cm^3}\right)$ obtained as an average of 320 national bulk density values (MALta Soil Information System, 2003).

$$V = m \times D, \tag{2}$$

where V = volume $\left(\mathrm{m^3}\right)$, m = mass (kg), and D = density $\left(\mathrm{kg\,m^{-3}}\right)$.

Eroded soil depth was calculated for each cell (50×50 m) [Equation (3)] from the eroded soil volume [Equation (2)] with a common fixed area (50×50 m) for each GIS cell.

$$h = V/A, \tag{3}$$

where h = height (m), V = volume ($\mathrm{m^3}$), and A = area ($\mathrm{m^2}$).

Following the above method, soil erosion of $1\,\mathrm{t\,ha^{-1}\,yr^{-1}}$ (per 50×50 m unit cell) [Equation (1)] is equivalent to a volumetric loss of $0.852\,\mathrm{m^3}$ $\left(1000\,\mathrm{kg}/1173.1\,\mathrm{kg\,m^{-3}}\right)$ [Equation (2)] or a depth loss of $0.008\,52\,\mathrm{cm}$ $\left(8.524\times10^{-10}\,\mathrm{km^3}/0.1\,\mathrm{km^2}\right)$ [Equation (3)]. The volumetric and depth values were applied in cost estimates associated with soil erosion.

3 Calculations and Results

3.1 rUSLE soil erosion model

The rUSLE soil erosion map (Figure 2) shows the spatial distribution of soil loss $\left(\mathrm{t\,ha^{-1}\,yr^{-1}}\right)$ in agricultural

areas in the Maltese Islands for the year 2013 (Sultana, 2015). The soil erosion values were classified into eight categories of increasing soil loss severity: < 1 (none), 1 to 2, 2 to 5 (very low), 5 to 10 (low), 10 to 25 (moderate), 25 to 45 (high), 45 to 75 (very high), $> 75\,\mathrm{t\,ha^{-1}\,yr^{-1}}$ (severe). Erosion severity thresholds are in line with those presented by numerous authors (e.g. Šúri, Cebecauer, Hofierka & Fulajtár, 2002; Iraldo et al., 2013).

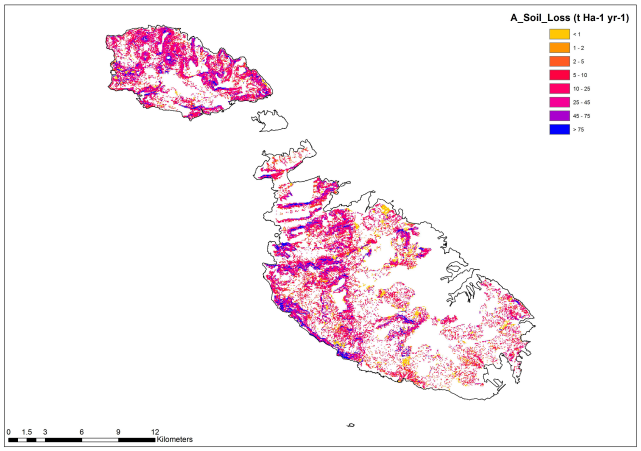


Figure 2: Average soil loss $\left(\mathrm{t\,ha^{-1}\,yr^{-1}}\right)$ in the Maltese Islands in 2013 following RUSLE equation (Sultana, 2015).

3.2 Soil volume lost/year and associated costs

The total calculated [Equation (2)] volume of eroded soil for Maltese agricultural areas in the year 2013 amounts to $766\,278\,\mathrm{m^3}$ (Figure 3). The total volume of soil eroded in 2013 in the local councils most affected by soil erosion has been calculated (Table 1). This information identifies localities that are subject to most severe soil volume loss and helps prioritise management initiatives seeking to reduce soil erosion.

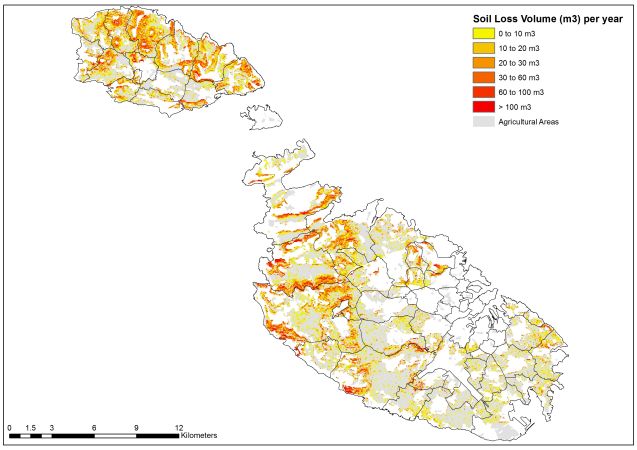


Figure 3: Soil erosion volumes $\left(\mathrm{m^2}\right)$ per cell $\left(0.0025\,\mathrm{km^2}\right)$ for the year 2013 in Malta.

3.3 Soil depth and soil depth lost 1, 10, 50 and 100 years

An interpolation between average soil depth points was carried out via GIS kriging and is calculated on the basis of the closest 8 point values. The resulting National soil depth map (Figure 4) is displayed below.

The depth of soil eroded yearly (2013) in agricultural areas has been calculated at a cell ($50\times50\,\mathrm{m}$) level. The value is obtained from rUSLE defined soil erosion rate $\left(\mathrm{t\,ha^{-1}\,yr^{-1}}\right)$ [Equation (1)], its conversion to soil erosion volume [Equation (2)], and its conversion to depth [Equation (3)]. The calculated yearly soil depth eroded (MSDE) and the National soil depths (NSD) (Figure 4) were superimposed and divided. The resulting value is a proportion (percent) indicating total soil depth eroded (TSDE) relative to initial soil depth [Equation (4)]. The greater the resulting value (the higher the percent value) the less soil remains.

$$TSDE = (MSDE/NSD) \times 100, \tag{4}$$

where $TSDE$ = Total Soil Depth Eroded (%), $MSDE$ = Modelled Soil Depth Eroded (cm), and SD = National Soil Depth (cm).

When the minimum soil depth for agricultural practice is reached, $< 15\,\mathrm{cm}$ soil depth (The National Environment, minimum standards for management of soil quality regulations, 2001), the area is identified as no longer being suitable for agricultural practice. The method defines the aerial extent of agricultural land that will, provided no soil is added, not contain sufficient soil depth to support agricultural practices. The agricultural area will thus be degraded to the point of agricultural unsuitability and be of no agricultural economic revenue (marked as black areas in Figure 5). The analysis has been carried out at various time scales, 1, 100 and 500 years (Figure 5). The resulting reduction in agricultural area has been calculated (Figure 5 and associated tables).

4 Discussion

Maltese central, south-eastern and north-eastern agricultural areas show the lowest erosion risk (Figures 2 and 3). These areas are characterised by relatively flat topographies and adequately maintained soil erosion structures. The ***Maltese north-western and Gozitan*** areas are most susceptible to soil erosion. These zones are characterised by a large range in erosion rates (Figure 2). Within the area, low erosion risk occurs in plateaus comprising low topographic gradients, and the application of good land management and soil erosion control measures. Plateau flanks and valley sides typically demonstrate exceptionally high erosion rates and are characterised by high topographic gradients, inadequate cultivation practices and poor erosion control

Table 1: Local councils and total soil volume eroded by council per year (m^3/yr) in the year 2013 (MT: Malta; GZ: Gozo).

Rabat MT	116502	Kercem	20590	Rabat GZ	9536	Marsaxlokk	4220
Mgarr	86966	Zebbug MT	15317	Ghajnsielem	9342	Iklin	4151
Siggiewi	55514	Mosta	14440	Marsascala	7949	Luqa	3698
Zebbug GZ	49557	Dingli	13834	Zabbar	7635	Xewkija	3593
Mellieha	47386	Gharb	13055	Qrendi	7193	Mdina	3466
Sn Pawl Bhr	43330	Swieqi	11621	Zurrieq	7019	Attard	3441
Nadur	35678	Munxar	11400	Ghargur	6922	Birzebbugia	3162
Xaghra	28408	Sn Lawrenz	11299	Sannat	6644	Ghaxaq	3103
Ghasri	27113	Mqabba	10515	Zejtun	5594	San Gwann	2960
Qala	24908	Naxxar	10295	Qormi	4607	Mtarfa	2483

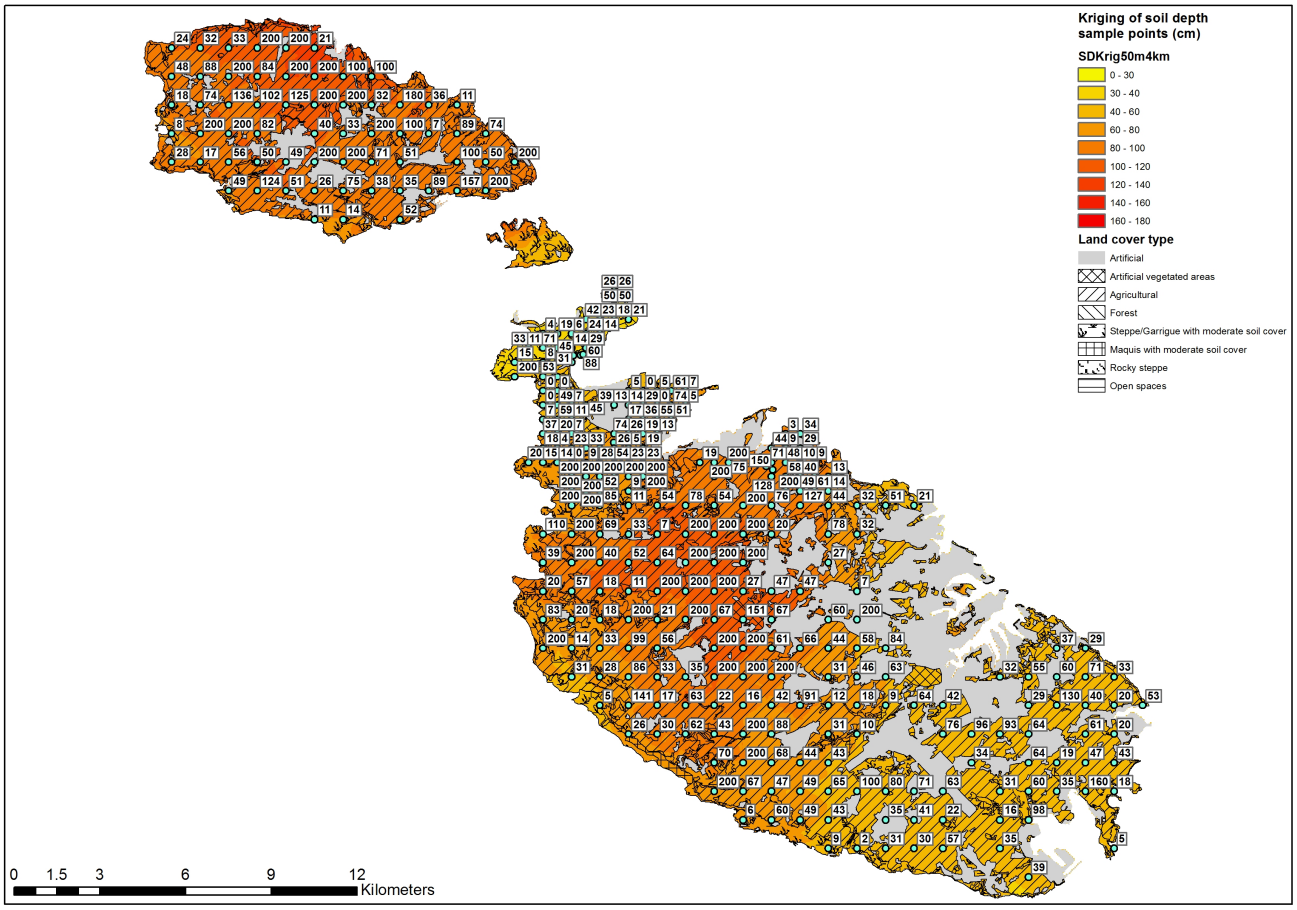
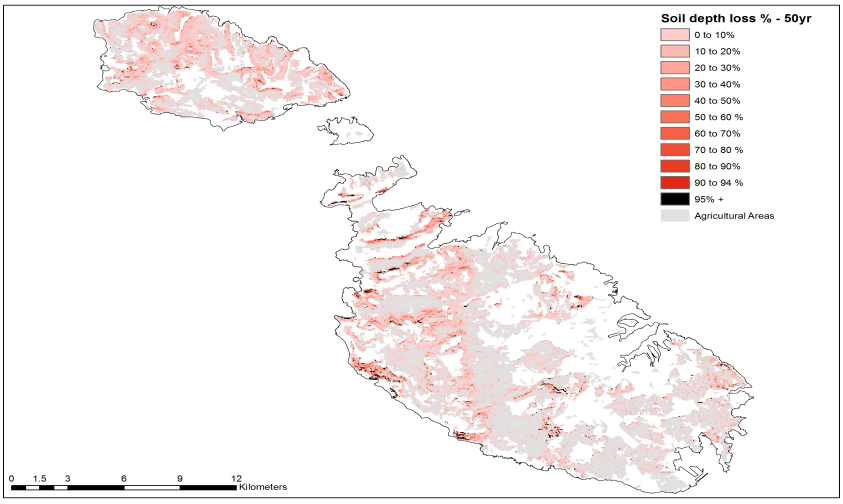


Figure 4: Malta soil depth map. Point values are average soil depth calculated in location. Values between points are interpolated soil depths using ArcGIS kriging technique. *Note, maximum measurable soil depth is* 200 cm*, point values of* 200 cm *indicate soil depths are greater than* 200 cm.

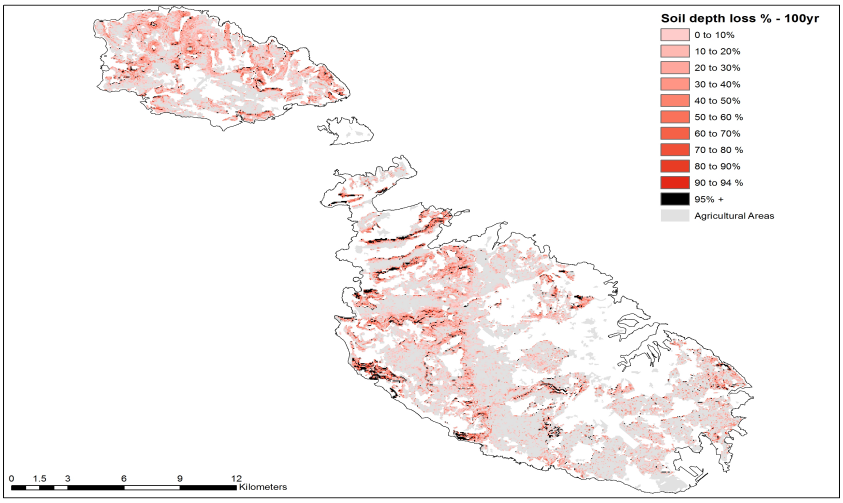
measures.

Maltese agricultural land is subject to various socio-economic conditions that constrain net farm income. Such hindering conditions include increased international agricultural price competition of cheaper costing imported foreign goods. Small agricultural holding size, exacerbated by land fragmentation, and an Agricultural Leases (Re-letting) Act, that does not facilitate the change of land ownership, further constrain potential agriculture income.

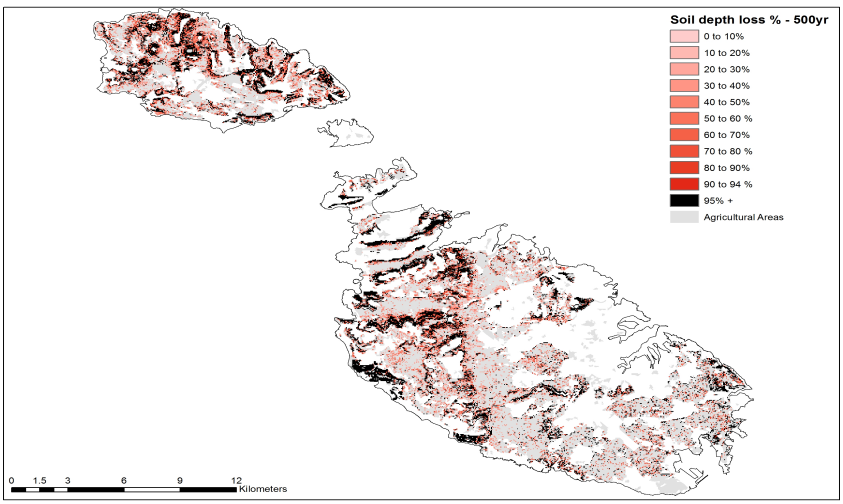
As a consequence of the aforementioned traditional and modern economic constraints, a number of agricultural areas, once financially viable, are now less so. Having lost their economic potential, marginally profitable agricultural areas were abandoned. In Malta, such areas are common in valley margin terraced slopes which



50 year % soil depth removed	Area (km^2)
0–10%	41.32
10–20%	17.19
20–30%	6.66
30–40%	3.20
40–50%	1.81
50–60%	1.14
60–70%	0.74
70–80%	0.52
80–90%	0.42
90–94%	0.10
95%+	**1.53**
% MT agric. area lost	**0.5**



100 year % soil depth removed	Area (km^2)
0–10%	19.62
10–20%	21.75
20–30%	10.83
30–40%	6.35
40–50%	3.97
50–60%	2.67
60–70%	1.97
70–80%	1.23
80–90%	1.05
90–94%	0.29
95%+	**4.90**
% MT agric. area lost	**1.6**



500 year % soil depth removed	Area (km^2)
0–10%	0.10
10–20%	1.82
20–30%	5.19
30–40%	6.10
40–50%	6.41
50–60%	5.58
60–70%	5.02
70–80%	4.34
80–90%	3.43
90–94%	1.36
95%+	**35.28**
% MT agric. area lost	**11.2**

Figure 5: 50, 100, 500 year (top to bottom) erosion maps showing % (of total) soil depth eroded. Area in black marks agricultural land with 95%+ soil eroded; considered as containing insufficient soil depth to support agricultural practices. Tables to the right of images indicate the total National land area affected by the soil loss percentage category.

contain soil retaining rubble walls. These structures require regular maintenance, which is no longer carried out when agricultural land is abandoned. As soil retaining rubble walls on sloping surfaces deteriorate they are breached and gravitational processes rapidly transport the retained soils downslope to more stable areas. As a consequence of the above interacting factors, soil erosion has been identified as a prevailing land degradation process that poses a significant threat to continued agricultural land use (Tanti, Role, Borg. C. & Calleja, 2002; Sultana, 2015).

Eroded soil can be deposited downslope, either in low gradient areas or in valleys, particularly where water retaining structures are present. Soil deposited in dams and reservoirs reduce water retention capacity and require dredging. These activities incur a cost; termed off-site cost (Section 4.1). To ensure continued sustainable agricultural land use, eroded soils need to be replaced. This process increases farming costs and reduces net agricultural earnings. In addition to soil replenishment, soil erosion degrades the remaining *in situ* soil requiring the addition of chemical soil supplements to maintain crop yields (Pimentel et al., 1995). These costs are termed on-site costs (Section 4.2).

4.1 Off-site costs

Between the years 2011 to 2013 the National valley management unit cleared 12 708 m^3 of rubble type material and 9492 m^3 of soil sediment eroded from catchment areas and deposited in valleys, dams and reservoirs. The sediment clearing works represent a fraction of the total sediment deposited yearly in various sediment depocenters. These works carry a direct cost, associated with the dredging of such material, and an indirect cost, tied to reduced drainage capacity and consequent flood related damages. While the National dredging works budget is not known, it is clear that should soil erosion rates be reduced, the budgetary allocations to maintenance works on previously dredged areas is less required. Funds could instead be redirected to dredging previously unmaintained channels.

4.2 On-site costs

4.2.1 Volume of soil erosion

A number of local private agencies were contacted by the authors enquiring on the price of 1 m^3 of soil. The author was informed that soil prices ranged depending on; soil quality (most often soil is described as a mixture of soil present in various construction sites), whether soil shall be transported to location by seller or buyer, and accessibility of deposition site (if less accessible, smaller transport vehicles and more journeys may be required). Quoted soil prices per meter cubed, including VAT, ranged from 7.34 €/m^3 when the buyer transports, 10.42 €/m^3 when the seller transports, and 12.80 €/m^3 when the seller transports soil to locations of limited accessibility (personal communication). A 10.42 €/m^3 soil price, likely to represent costs incurred by farmers, is applied in our calculations.

The calculated total soil volume eroded annually in National agricultural areas amounts to 766 278 m^3. Applying the 10.42 €/m^3 soil prices, the national cost for replacing eroded soil in agricultural areas is 7.98 M€/yr. Applying the same soil price, the average soil volume (m^3) eroded per unit area $(0.0025\,km^2)$ (Table 2, column B) in the most affected agricultural areas (Table 2, column A) has been calculated. The standard deviation of soil erosion volumes (m^3) for the listed affected agricultural area is also provided (Table 2, column C). The average soil volume (m^3) eroded per unit area value (Table 2, column B) allows agricultural land owners to calculate the average monetary costs (Table 2, column D) incurred to replace eroded soil per 0.0025 km^2 in zones affected by erosion (Figure 3). The replacement of eroded soil is necessary to maintain agricultural areas subject to erosion.

The 2010 Malta agricultural census indicates that 74% of total utilised agricultural area (UAA) consists of agricultural holdings covering less than 0.01 km^2, and 24% consists of medium-size holdings with a land area between 0.01 to 0.05 km^2 (Rural Development Department, 2014). Applying the 10.42 €/m^3 soil prices, the average monetary cost to replace eroded soil per average agricultural holding/parcel size $(0.01\,km^2)$ is calculated (Table 2, column E) for the areas most affected by soil erosion (Figure 3).

The average agricultural land owner, managing an average sized agricultural field $(0.01\,km^2)$ in an area subject to soil erosion (Figure 3), suffers a yearly expense ranging from 400 €/0.01 km^2/yr to 2585 €/0.01 km^2/yr (Mtarfa) averaging at of 994.56 €/0.01 km^2/yr on soil replacements (Table 2).

4.2.2 Erosion negatively affects soil quality

The impact soil erosion has on productivity and non-point source pollution is well known (Lal, 2003). Soil erosion by water removes valuable topsoil, rich in low density organic carbon, nitrates, phosphates and potassium. With increased soil erosion soil quality, associated with nutrient quantity, decreases. This has significant adverse impacts on agricultural yield (Verity & Anderson, 1990).

National soil erosion by water reduces soil quality and consequently reduces crop yield. Farmers aiming to maintain agricultural productivity incur costs to artificially restore eroded soil nutrients. National records indicate that Maltese farmers have spent 1.90 M€ (2010), 1.93 M€ (2011) and 2.00 M€ (2012) on fertilisers and soil improvers (National Statistics Office, 2012). Assum-

Table 2: Forty four (of the sixty seven) localities affected by highest soil erosion rates. A: localities affected by soil erosion; B: yearly average soil loss volume (m^3) per unit area $(0.0025\,km^2)$ in affected eroded areas; C: standard deviation of soil erosion volumes per affected area in the locality; D: average monetary costs incurred by the farmers to replace lost eroded soil per $0.0025\,km^2$ in zones affected by erosion; E: average monetary cost incurred by agricultural land owner to replace eroded soil per average agricultural holding/ parcel size cell $(0.01\,km^2)$ located within affected eroded areas. *Agricultural owners tending areas subject to soil erosion incur an average yearly soil replacement cost of* $248.64\,€/0.0025\,km^2/yr$ *(minimum* $100.04\,€/0.0025\,km^2/yr$*, maximum* $646.28\,€/0.0025\,km^2/yr$*) and an average yearly cost on soil improvers, necessary to artificially maintain soil quality, of* $43.67\,€/0.0025\,km^2/yr$.

A	B	C	D	E	A	B	C	D	E
Mtarfa	62.08	60.61	646.28	2,585.12	Sn Gwann	23.12	44.95	240.72	962.86
Mqabba	59.74	147.25	621.94	2,487.78	Sn Pawl Bhr	23.05	23.99	239.93	959.71
Marsa	50.20	32.30	522.54	2,090.16	Ghajnsielem	22.68	23.87	236.05	944.20
Swieqi	48.22	128.58	501.96	2,007.83	Santa Lucija	22.42	42.06	233.44	933.74
Fgura	38.31	22.25	398.83	1,595.33	Sannat	22.07	51.38	229.76	919.05
Mgarr MT	35.67	49.60	371.34	1,485.34	Qrendi	21.73	75.70	226.21	904.84
Sn Lawrenz	35.20	98.84	366.41	1,465.64	Zebbug MT	21.63	40.07	225.21	900.84
Rabat GZ	33.23	43.13	345.89	1,383.57	Mdina	21.53	21.90	224.12	896.48
Rabat MT	29.61	53.14	308.28	1,233.14	Kirkop	19.47	16.34	202.65	810.59
Mellieha	29.32	50.35	305.25	1,221.02	Kalkara	19.14	17.04	199.21	796.83
Nadur	28.96	34.87	301.47	1,205.88	Zejrun	18.59	60.01	193.48	773.91
Siggiewi	28.90	51.20	300.83	1,203.32	Gharb	17.83	20.17	185.66	742.63
Fontana	28.30	35.55	294.58	1,178.31	Iklin	17.66	31.55	183.89	735.56
Gharghur	27.80	25.86	289.40	1,157.60	Zabbar	16.74	18.45	174.30	697.18
Mosta	27.40	106.12	285.24	1,140.96	Luqa	16.43	17.87	171.08	684.32
Zebbug GZ	27.21	27.69	283.30	1,133.21	Birkirkara	16.26	19.57	169.23	676.93
Dingli	27.02	68.36	281.28	1,125.12	Xewkija	16.04	21.88	166.98	667.93
Munxar	25.79	36.62	268.48	1,073.92	Marsascala	15.40	19.78	160.37	641.46
Qala	25.26	26.15	262.98	1,051.91	Marsaxlokk	15.40	31.76	160.32	641.29
Xaghra	25.21	45.69	262.40	1,049.62	Qormi	15.15	17.65	157.74	630.97
Ghasri	25.06	31.65	260.86	1,043.44	Pembroke	15.03	16.35	156.48	625.91
Kercem	24.54	32.99	255.47	1,021.89	Xghajra	15.00	15.87	156.10	624.40

ing an even application of fertilisers and soil improvers throughout the Maltese utilised agricultural area (UAA) $(114.5\,km^2)$, an average cost of $17\,467.25\,€/km^2$ is calculated. The average cost on fertilisers and soil improvers incurred by the typical agriculture land owner $(0.01\,km^2)$ is $174.67\,€/0.01\,km^2/yr$. Should management practices, reducing soil erosion, be introduced, these costs could be diminished.

4.2.3 Costs of action; maintain state of affair in affected areas

To maintain the current state of affairs - in terms of quantity and quality - in agricultural areas affected by soil erosion, eroded soil must be replaced and soil quality maintained. The average agricultural land owner $(0.01\,km^2)$, managing an average sized agricultural field in an area subject to soil erosion (Figure 3), suffers an average yearly expense of $1169.24\,€/0.01\,km^2/yr$ on replacing eroded soil (minimum 400.18, average 994.56 and maximum $2585.12\,€/0.01\,km^2/yr$) and artificially maintaining soil quality (average $174.68\,€/0.01\,km^2/yr$).

The yearly cost incurred by the average agricultural farmer to replace eroded soils and artificially maintain soil quality in erosion affected areas amounts to $1169.24\,€/0.01\,km^2/yr$. The average yearly economic revenue from Maltese UAA is $1719.65\,€/0.01\,km^2$. Therefore the gain of an agricultural land owner who undertakes measures to address soil erosion would be $550\,€/0.01\,km^2/yr$. In view of this cost and benefit imbalance, various agricultural land owners may consider the price tied to replacing eroded soils in areas subject to soil erosion too high in relation to economic revenue. As a result, the owners of agricultural land in erosion prone areas (Figure 3) may choose not to replace soil lost through erosion. Over time, dependent on soil erosion rate and soil depth present, the agricultural area may be degraded to such an extent as to no longer be suitable for agricultural purposes. The effects of no soil replacement scenarios – *do nothing scenario* (Section 4.2.4) - on total agricultural area over various times scales are shown in Figure 5.

4.2.4 Costs of inaction; the do nothing scenario

Soil is formed over long periods of time and is therefore considered a finite resource. Nationally, soil is of moderate depth in agricultural areas (Figure 4). In situations where eroded soil is not replaced (do noth-

ing scenario), soil resource will eventually be depleted and consequently unsuitable for agricultural production. The time taken for soil depletion depends on *in situ* soil depth and soil erosion rate. To calculate the economic costs of the do nothing scenario, associated with the loss of agricultural land through soil erosion, a number of assumptions must be made (i) UAA produce equal income, (ii) no soil is added to the eroded agricultural areas, and (iii) only registered agricultural income is considered. Following these assumptions, an average agricultural cost can be attributed for a defined agricultural area.

Fresh vegetables and fruit that passed through organised markets in 2012 amounted to 41.24 Mkg yielding a wholesale value of 19.69 M€ (National Statistics Office, 2012). Malta UAA covers 114.5 km^2. Based on these values each square kilometre of Maltese UAA generated on average 171 965 €/yr in 2012 $\left(0.172\,\text{M€/km}^2\text{/yr}\right)$.

Results defining the aerial extent of agricultural land that will, provided no soil is added (do nothing scenario), be agriculturally degraded to the point of agricultural unsuitability (marked as black areas in Figure 5). Three time scales were assessed; 1, 100 and 500 years. Over 50 years, 1.53 km^2 (0.5% of Maltese area) of agricultural land is depleted of soil, incurring a loss of national agricultural potential amounting to 0.263 M€/yr; over 100 years, 4.9 km^2 (1.6% of Maltese area) of agricultural land is depleted of soil, incurring a national agricultural loss of 0.843 M€/yr and over 500 years, 35.28 km^2 (11.2% of Maltese area) of agricultural land is depleted of soil, incurring a national agricultural loss of 6.067 M€/yr.

4.3 Cost effective soil conservation measures

Soil erosion rates, and associated economic implications, can be mitigated with cost effective agricultural cover and management practices. Soil conservation practices may be cheaper to set up and maintain than the continuous replacement of eroded soils. This approach increases the economic viability of agricultural exploits within areas subject to soil erosion and ensures sustainable, continued, use of such areas.

Soil degradation is the result of soil displacement, erosion, or soil chemical and physical degradation (Land and Water Development Division Soil Resources, Management and Conservation Service & of the United Nations, 1998). Water driven soil degradation is intensified when vegetation bare sloping soil surfaces are exposed to rainfall that exceeds infiltration rate. Such scenarios increase surface-water runoff and many researchers observe a direct link between soil erosion rate and runoff intensity (e.g. Pruski & Nearing, 2002; Keppeler, Lewis & Lisle, 2003; Safriel et al., 2003). Soil conservation measures seek to dissipate water-runoff energy or increase water infiltration rate and consequently reduce the effects of soil erosion (Food and Agriculture Organisation, 1983, 1994; Hudson, 1992, 1981; Morgan, 1986; Schwab, Frevert, Edminster & Barnes, 1981).

4.3.1 No till technique

The majority of farmers plough their land prior to sowing their crops. Tillage with a mouldboard plough for instance, commonly used in Maltese agriculture, overturns the top 15 to 25 cm of soil exposing soil to erosion by wind and water (Huggins & Reganold, 2008). Montgomery (2007) argues tillage is a principal cause of agricultural land degradation.

Conservation tillage and no-till farming techniques seek to minimise soil disruption and retain at least 30% of the previous crop residues. Crop residue, left on the fields after harvest, helps increase water infiltration and reduces run-off. This process protects soil from erosion and promotes soil productivity. By reducing evaporation, crop residues also facilitate water conservation. In water scarce situations, greater water availability can lead to higher crop yields (Huggins & Reganold, 2008). In 2004 close to 40% of American cropland was farmed through conservation tillage. Reports from the United States Department of Agriculture indicate that such practices enriched agricultural soil organic material, reduced soil erosion, and improved soil water balance (Huggins & Reganold, 2008).

In addition to higher crop production, conservation and no-till methods provide farmers with direct economic incentives. Conservation and no-till techniques require fewer passes over a field and consequently, less fuel (50 to 80%) and less labour (30 to 50%) are required. This may significantly lowering production costs increasing agricultural return on investment (Huggins & Reganold, 2008).

4.3.2 Retaining rubble walls

Through the construction of terraced fields, upheld by retaining dry rubble walls, traditional Maltese agricultural practices have decreases field gradients and reduced soil erosion (Role, 2002). When properly constructed rubble walls are highly effective at retaining large volumes of soil while allowing appropriate soil drainage (Role, 2002).

As evidenced by field observations of rubble wall state (Sultana, 2015), terraced fields that are not regularly maintained develop rubble wall breaches. Gravitational processes then transport large volumes of soils to more stable down-slope areas. If breaches are not attended to, they rapidly widen, develop large collapses and soil mass erosion follows. Terraced fields and associated rubble walls must be perceived as a necessary soil conservation method essential for the sustained production of agricultural capital in areas subject to soil erosion (Figure 3).

A 2013 rubble wall state survey (Sultana, 2015) indicates that the majority of contour parallel rubble walls within terraced fields are in a moderate to poor state (Figure 6). Agricultural rubble walls in terraced field in a moderate state contain between 1 to 3 breaches that expose half the soil profile, and rubble walls in a poor state either contain more than 3 breaches or contain 1 breach that exposes the entire soil profile (Sultana, 2015).

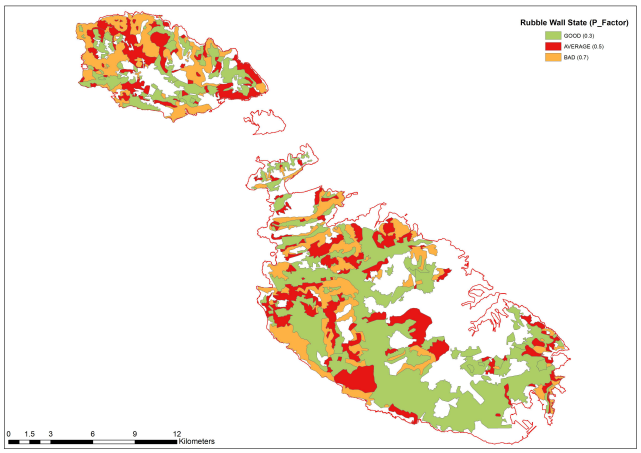


Figure 6: Map showing rubble wall state (Sultana, 2015). Note white areas represent urban areas that contain no rubble walls.

To be able to estimate the costs of restoring rubble walls in agricultural areas subject to soil erosion, a number of local rubble wall construction contractors were asked to supply a quotation based on the following specifications; (i) terraced fields (soil retained behind rubble wall) located in Rabat Malta, accessible through a countryside lane, (ii) rubble wall section to be built 5 m length, 0.5 m in width and 1.5 m in height, (iii) constructed following typical methods (outlined in Maltese L.N. 169 of 2004), and (iv) 50% of rubble material provided by contractor and transported to site. The quoted price averaged at €400 (personal communications).

Maltese sloping agricultural areas have been engineered into rectangular shaped terraced fields with contour parallel soil retaining rubble walls. Nationally, an area of 74.61 km^2 has been numerically identified as being subject to soil erosion. In the RUSLE model, amongst other factors, soil erosion is significantly influenced by management factor (rubble wall state) and slope factors (gradient). The affected areas likely represent sloping agricultural fields. Taking an average terraced field dimension of 100 m wide by 50 m deep and considering moderate state rubble walls, two breaches per terraced field, the Nation requires an investment of 11.94 M€ for the restoration of breaches in slope-facing rubble walls. The investment, while substantial, will reduce soil erosion in these areas thereby allowing agricultural practices to be continued and essential ecosystems services maintained.

The average Maltese farmer owns an agricultural field 0.01 km^2 in area. Taking an average terraced field dimension of 100 m long by 50 m wide, each farmer owns two tiers of terraced field containing a 200 m long section of slope-facing soil retaining rubble walls. Considering state rubble walls in a moderate state, two breaches per terraced field, the average agricultural farmer requires an investment of €1,600 for the restoration of four breaches in slope-facing rubble walls. The farmer may distribute these costs over time, and other than minor restorations from time to time, the farmer is unlikely to require such substantial investment in the future.

In view of the average yearly economic revenue from Maltese UAA $\left(1719.65\text{€}/0.01\,km^2/yr\right)$, the initial rubble wall restoration investment is considerable. The annual return on investment is however substantial. Rubble wall restoration will reduce an agricultural farmer's average yearly expense (in agricultural areas affected by soil erosion) by 1169.24 €/0.01 km^2/yr. These reduced costs are associated with spared costs to replace eroded soils and artificially maintain soil quality. Also, on longer time scales (Figure 5), the investment in rubble wall restorations will also preserve agricultural areas subject to erosion for long term sustainable use.

5 Conclusions

Land degradation is caused by various forces and leads to a significant reduction of the productive capacity of land (Amundson et al., 2015). Various human activities contribute and accelerate land degradation, which degrade soil quality and reduce the ability of lands to provide various ecosystem services.

Extensive empirical evidence Information tying on land and soil degradation to reductions in the provision of ecosystems services has however rarely promoted policy action. An economic-based approach, termed economics of land degradation, has been shown to be more successful. Such an approach provides an economic framework against which decision-makers can appreciate the value of taking action against land degradation.

Maltese agricultural land is subject to various socio-economic conditions that constrain net farm income. As a consequence a number of agricultural areas, once financially viable, are now less so. Having lost their economic potential, with such changes in socio-economic dynamics, the economic incentive for tending these marginally profitable agricultural areas fields was lost and the fields were abandoned.

In Malta, such areas are common in valley margin terraced slopes which contain soil retaining rubble walls. These structures require regular maintenance,

which is no longer carried out when agricultural land is abandoned. Sultana (2015) argues that various socio-economic factors contribute towards agricultural land abandonment and the consequent deterioration of soil retaining structures. As Once soil retaining rubble walls on sloping surfaces deteriorate they are breached and, natural dynamics dominate and gravitational processes rapidly transport the retained soils downslope to more stable areas. This process limits the national agricultural economic potential and also deteriorates associated ecosystem services.

The calculated total soil volume eroded annually in National agricultural areas at the nationally level amounts to 766 278 m^3. The national cost for replacing eroded soil in agricultural areas is 7.98 M€/yr. The yearly cost incurred by the average agricultural farmer to replace eroded soils and artificially maintain soil quality in erosion affected areas amounts to 1169.24 €/0.01 km^2/yr. The average yearly economic revenue from Maltese UAA is 1719.65 €/0.01 km^2 (Section 4.2.3). Therefore the gain of an agricultural land owner who undertakes measures to address soil erosion would be 550 €/0.01 km^2/yr. In view of this cost and benefit imbalance, various agricultural land owners may consider the price tied to replacing eroded soils in areas subject to soil erosion too high in relation to economic revenue. As a result, the owners of agricultural land in erosion prone areas (Figure 3) may choose not to replace soil lost through erosion. Over time, dependent on soil erosion rate and soil depth present, the agricultural area may be degraded to such an extent as to no longer be suitable for agricultural purposes.

Results defining the aerial extent of agricultural land that will, provided no soil is added (do nothing scenario), be agriculturally degraded to the point of agricultural unsuitability (marked as black areas in Figure 5). Three time scales were assessed; 1, 100 and 500 years. Over 50 years, 1.53 km^2 (0.5% of Maltese area) of agricultural land is depleted of soil, incurring a loss of national agricultural potential amounting to 0.263 M€/yr; over 100 years, 4.9 km^2 (1.6% of Maltese area) of agricultural land is depleted of soil, incurring a national agricultural loss of 0.843 M€/yr and over 500 years, 35.28 km^2 (11.2% of Maltese area) of agricultural land is depleted of soil, incurring a national agricultural loss of 6.067 M€/yr.

Soil erosion rates, and associated economic implications, can be mitigated with cost effective agricultural cover and management practices. Soil conservation practices may be cheaper to set up and maintain than the continuous replacement of eroded soils. This approach increases the economic viability of agricultural exploits within areas subject to soil erosion and ensures sustainable, continued, use of such areas. Two cost-effective agricultural management methods are proposed to reduce soil erosion, maintain soil quality and preserve agriculture associated ecosystem services. The first, conservation tillage and no-till farming techniques, seek to minimise soil disruption and retain at least 30% of the previous crop residues. This process protects soil from erosion and promotes soil productivity and also provides farmers with various direct economic incentives.

The second is the restoration and maintenance of soil retaining rubble walls. Taking an average terraced field dimension of 100 m wide by 50 m deep and considering moderate state rubble walls, two breaches per terraced field, the Nation requires an investment of 11.94 M€ for the restoration of breaches in slope-facing rubble walls. Should the restoration be carried out by the average agricultural farmer, an investment of €1,600 would be required. In both cases, while the initial rubble wall restoration investment is considerable, the annual return on investment is substantial. The investment, while substantial, will reduce soil erosion in these areas thereby allowing agricultural practices to be continued and essential ecosystems services maintained.

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Research Article

Economic and Labour Market Implications of Global Environmental Change on Agriculture and Viticulture in Malta

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Abstract. Agriculture contributes but a limited amount to Europe's gross domestic production, and the overall weakness of the European economy to climatic changes on agriculture is deemed low. Agriculture remains more considerable in the southern and south-eastern European states with regards to employment and economic contribution and these regions are expected to face decreases in yields of 10% or more as a result of the reduction of the growing season and decreased rainfall. In Malta, other than cereal production as a fodder crop, most other crops are supported with a degree of irrigation that may ultimately mitigate adverse climatic conditions. Local producers have indicated that cereals, olives and vines have so far demonstrated varying degrees of susceptibility to climatic factors, although, arguably management factors are also relevant. The development of drier and warmer conditions in the Mediterranean region would also create conditions that are favourable to pests. Analysis of potential output effects triggered by global environmental change indicates that some 6,300 hectares on which wheat, olives and vines are grown, or 55% of Malta's total utilisable agricultural area, could in effect be rendered economically unsustainable when productivity falls by about 23%. Such heavy losses could constitute a potential risk to the sustainability of rural farming systems and livelihoods in Malta.

Keywords: climate change; agriculture; Malta; production

1 Introduction

The latest report of the Intergovernmental Panel on Climate Change confirms that human interference with the climate system is occurring whilst extreme weather and climate events, including droughts and floods, have significant impacts on economic sectors, natural resources, ecosystems, livelihoods, and human health. With regards to biodiversity, there is evidence that many terrestrial plant and animal species have already shifted their area of activity, as well as numbers, as a result of past climate change, and they are also doing so now in many regions. It was confirmed that for rural areas, climate change will ultimately affect a number of economic, social, and land-use criteria (IPCC, 2014).

Socio-economic factors are important contributors to both the vulnerability and adaptability of human and natural systems and so assessing climate impacts on both human/cultural and natural systems requires a consideration of all factors influencing these systems, and their complex inter-relationships. Agriculture is considered responsible for an estimated one third of climate change. About 25% of carbon dioxide (CO_2) emissions are produced by agricultural sources, mainly resulting from deforestation, the use of fossil fuel-based fertilizers, and the burning of biomass. Most of the methane in the atmosphere comes from domestic ruminants, forest fires, wetland rice cultivation and waste products, while conventional tillage and fertilizer use account for 70% of nitrous oxides. The Climate Institute reports that the three main causes of the increase in greenhouse gases observed over the past 250 years have been fossil fuels, land use, and agricultural practices (Climate Institute, 2014).

In a 2014 statement, Hans Bruyninckx, Executive Director of the European Environment Agency (EEA), stated that climate change impacts are now visible in Europe and that the expected risks for this region are multiple and self-reinforcing. These include: augmented risk of flooding in coastal areas, further land erosion plus physical and financial losses due to accelerating sea-level rise; further possibilities of inland flooding along many

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river areas due to more heavy rainfall; increased economic, ecological and social impacts resulting from more extreme heat waves, as well as associated health issues and decreasing rural labour productivity. Additionally significant reductions in water availability, particularly in southern Europe, together with losses of crops and ecosystems plus the probability of escalating wildfires are to be expected (EEA, 2014, March 31).

Agriculture accounts for only a small part of the European Union's gross domestic product (GDP), and the overall vulnerability of the European economy to changes affecting agriculture is somewhat low (EEA, 2006). However it is also indicated that agriculture is much more significant in terms of land use, given that farmland and forest land cover approximately 90% of the EU's land surface, rural population and income. Agriculture's contribution is more noteworthy in southern and south-eastern European countries in terms of employment and GDP, and these countries will face decreases from their current yields by 10% or more as a result of the shortening of the growing season, drought and a likely trend for producers to alter practices and crop types resulting from climate change throughout Europe (EEA, 2008). In the context of the Maltese Islands, this scenario would imply the need for additional organic matter to improve water retention, together with the selection of hardier varieties of seed or breeds that exhibit more drought and heat stress tolerance and earlier maturity, such as the replacement of wheat by barley. Another probable good management obligation would be earlier, if not continuous, pest monitoring for disease control to compensate for milder winters with no cold break to destroy pests or, if possible, the provision of disease-resistant varieties.

In Malta's Second UNFCC National Communication published in 2010, regional climate model simulations showed that, over southern Europe as a whole, the winter wheat yields would not decrease where irrigation is not limited (MRA & UoM, 2010). There would however be negative impacts when water availability for irrigation was limited. The extent of the growing season was likely to decrease, together with increases in the breakdown of organic matter, plant water stress and reduced crop yields. Potatoes, for example, would benefit from elevated CO_2 levels and elevated temperatures; however, an overall reduction in crop yields was expected, particularly in semiarid conditions such as those in Malta. In the longer term, when the absence of water would become a critical factor, the final impact would result in the disappearance of the potato as a staple crop from Southern Europe.

This document also indicated that vines were likely to be impacted as a result of increases in mean temperature and from the dynamics of changing rainfall distribution and intensity. Impacts of increasing temperatures have already been felt in Spain and France, where vine growers are moving to higher elevations. This could however result in accelerated ripening that could inhibit the production of good quality grapes (MRA & UoM, 2010).

Basing their findings on the conclusion of the IPCC Fourth Report (IPCC, 2007), Parry et al. (2009) advised that global warming was unequivocal and affecting agricultural and forestry management. A number of effects of climate change on agriculture over the next 50 years were predicted. These include: higher levels of atmospheric CO_2; sea level rise; warmer temperatures and increased heat stress; changes in extent and cycles of precipitation; climate variability and storm intensity and variability. Each of these effects is reviewed in turn below.

1.1 Atmospheric carbon dioxide

There is a very high probability that CO_2 will increase from 360 ppm to 450–600 ppm over the next 50 years. In the case of the impact of increased atmospheric CO_2 on crop growth, physiological differences in plants will play on various uptake possibilities. Under conditions of CO_2 enrichment, crops may use less water while producing more carbohydrates, whilst requiring more nitrogen. When plants absorb more carbon, they grow bigger and faster. Depending on the photosynthetic mechanism at work, there are three types of plants: C3 plants, C4 plants and CAM plants. About 95% of the plants on Earth are C3 plants: these deploy a photosynthetic mechanism that takes carbon dioxide directly from the air during carbohydrate production. C4 plants are commonly seen in dry and high temperature areas and the 1% of plant species that have C4 biochemistry are much more efficient at capturing carbon dioxide because these can fix the gas at twice the rates of C3 plants without photorespiration. Increases in levels of carbon dioxide will benefit C3 species such as wheat and rice; however, warmer temperatures and drier conditions will tend to favour C4 species, such as maize, sugar and a large variety of agricultural weeds. Finally, CAM photosynthesis is a carbon fixation pathway that has evolved in some plants as an adaptation to arid conditions. CAM plants are more common than C4 plants and include cacti and a wide variety of other succulent plants; they are usually found in dry desert areas, with the pineapple as one commercialized exponent.

1.2 Sea Level Rise

Inundation of low-lying coastal areas from sea level rise and flooding from major storm events would create significant challenges for agriculture as this will result in loss of land, coastal erosion, flooding of agricultural areas and salinisation of groundwater. Sea level rise will not only pose a threat to agriculture in low-lying

coastal areas, but also promote its elimination, particularly in small islands where there are hardly any inland, non-coastal regions. The intrusion of seawater into Malta's coastal lowlands and aquifers, such as Burmarrad, Pwales and Armier, will create further hazards where drainage of surface water and ground water are impeded. This would oblige the usage of more salt-tolerant crops.

1.3 Warmer Temperatures and Increased Heat Stress

A rise of 1–2 °C in mean temperatures is considered to be a conservative estimate, but it would already contribute to faster, shorter and earlier growing seasons with increased evapo-transpiration and heat stress. Annual average land temperatures over Europe have been projected to continue increasing by more than the global average temperature during the 21st century (Füssel & Jol, 2012). Increases in land temperature in Europe are projected to rise by 1.0–2.5 °C by 2021–2050, and by 2.5–4.0 °C by 2071–2100. The largest temperature increases during the 21st century are projected to occur over eastern and northern Europe in winter and over southern Europe in summer (Füssel & Jol, 2012). The onset of hotter temperatures will also result in a quicker decomposition of organic matter, accelerate soil processes that determine fertility and provide more amenable situations for insect pests to thrive and propagate. This could necessitate additional fertilizer/pesticide applications to sustain current yields. In cases where soil moisture is scarce, both root growth and decomposition of organic matter would be comparatively more limited and would also increase their vulnerability to wind erosion.

Temperature is a crucial input in vine cultivation. If the temperature exceeds 35 °C for a significantly long period, red grapes would become stressed, having very low concentrations of tannins. Malta has a local advantage for producing red wine, unlike white which grows in a wider variety of places. Currently, there are all the right climatic conditions favouring red wine production, except for the lack of water, though this, so far, is controllable with a requirement of 800 m^3 (and sometimes even up to 1000 m^3) of water per hectare of vines annually. Rain prevails in winter and is, as yet not common in spring. However late rains and higher temperatures would tend to result in a greater frequency of vine diseases. Wide fluctuations in yield are becoming more noticeable; even down by 75%, but also even up to a fourfold drop in quality. A local expert emphasised that the maturity date is crucial, but this is being affected by early and bad breaks of season occurring in mid-February rather than spring. The prevalence of a cold spell after bud break has resulted in up to 50% losses in local Chardonnay production (Aquilina, 2014).

The global spatial pattern of climate change in the coming decades is expected to be largely similar to the pattern of recent changes that indicate a particularly strong warming in high latitudes, increasing precipitation in most tropical and high latitude regions, and decreasing precipitation in most sub-tropical regions. Global warming will lengthen the potential growing seasons in middle and higher latitudes, obliging earlier crop planting, but this again will depend on water availability (Füssel & Jol, 2012). In Malta, about half of the agricultural area is utilised for fodder production. The area under permanent crops with different fruits and vegetables is more limited with the resultant crops reflecting market prices in conjunction with water availability. Constraints in the form of elderly farmers, fragmented small fields, shallow soils, very limited organic matter, together with a high (alkaline) pH in the soil, have limited the introduction of new crops (Attard & Meli, 2008).

1.4 Precipitation

Precipitation changes across Europe show considerable spatial and temporal variability. During past decades, annual precipitation has been generally increasing across most of northern Europe, most notably in winter, but decreasing in parts of southern Europe. Most climate model projections show continued precipitation increases in northern Europe (most notably during winter) and decreases in southern Europe (most notably during summer). The number of days with high precipitation is projected to increase (Füssel & Jol, 2012). Seasonal changes in precipitation of ±10% are thus expected (Parry et al., 2009). Changes in seasonal precipitation will influence the quantity of rainfall, its storage in the soil together with evaporation and run-off. Diminishing rainfall can cause moisture stress in plants during all growth stages. The higher the incidence of evaporation, the more likely there will be soil salinisation with consequent diminishing yields and increased erosion, including drying of springs and aquifers.

Discussions with local producers indicate that variations in wheat production in Malta in recent years have generally been in the range of 10% drops, but even a 50% drop was recorded in one particular case. Similar yield losses were also apparent in olive production. Two years ago, there was an insignificant yield that could have been attributed to a typical off-year, with up to a 90% drop. In 2013, however, the olive crop yield was in the region of 33%: contributory factors include the occurrence of mild winters with limited rainfall as well as a mild summer, that probably also facilitated the presence of the olive moth.

1.5 Climate Extremes

Climate variability and associated extreme meteorological events will witness floods and droughts, causing extreme and possibly unrecoverable physical damage to crops (Füssel & Jol, 2012). Other than possibly exceeding the tolerance of these crops to temperature extremes, heavy rainfall will also result in increased soil erosion in these situations. It would be expected that erosion in agricultural areas would prevail in the regions of natural drainage depressions, where terraced soil is not contained by broken rubble walls, where land is very exposed to the elements and where there is a steep topography.

1.6 Storminess and Variability

The possibilities of increased levels and variability of major storms are considered low, together with the associated risk to damaging events that will affect crops and the timing of farm operations. Yet changes in wind patterns could also contribute to the spread of windborne pests and diseases. The presence of higher temperatures could further contribute to the vulnerability to fires following a drought period and thus further the desertification processes.

2 Main crop evaluation: Productivity and Profitability

The Malta National Census of Agriculture and Fisheries of 2010 indicates nine major crop types responsible for the highest land use, for a total national utilisable agricultural area (UAA) of 11,540 hectares in the Maltese islands (NSO, 2012) (see Table 1).

Table 1: Allocation of land area by crop type in Malta (NSO, 2012, Source:).

Crop Type	Land Area (ha.)
Forage/fodder crops	5,552.8
Vegetables	1,730.5
Kitchen Gardens	1,122.9
Potatoes	701.1
Vines	614.1
Fruit & berry	371.5
Olive	140.3
Citrus	111.3
Organic	26.1
Total	**11,540.0**

For all intents and purposes, forage constitutes a predominance of cereal crops, mostly wheat, harvested as hay for livestock fodder. Moreover, tomatoes, lettuce, watermelon and cauliflowers are the crops with the highest tonnage sold through official markets and these are taken as representative of vegetables and kitchen gardens. Peaches constituted the most common of orchard trees, oranges are the most grown citrus and strawberries are the most produced fruit (NSO, 2012).

Except for dry land cereal production, most other crops are supported with a degree of irrigation that may ultimately mitigate adverse climatic conditions. Local producers have indicated that cereals, olives and vines have so far demonstrated varying degrees of susceptibility to climatic factors, although, arguably management factors are also relevant.

Through specific feedback from local farmers, for the calculation of crop output, a 10% decrease in production is assumed as the first financial cost of climate change. Consequently, wheat production, which currently leaves a return of circa €221.52/ha, is reduced to a return of €123.75/ha when a 10% decrease as a result of climate change is assumed: effectively a 44% decrease in returns results. There is a prevailing situation where, with a 23% decrease in production, no revenue would result and the whole operation would be commercially non-viable.

On a similar basis, olive production gives a return of €6,777.28/ha which could decrease to €3,634.48 when a 10% fall for climate change is applied – again, a 46% decrease in returns where, with a 22% decrease in production, no revenue would result. The most immediate issue for the olive affected by climate change is rainfall. Less rainfall means stunted olive oil production.

Climate does not only affect olive trees directly. Changing temperatures also influence insect diversity and frequency for a given area. Rising carbon dioxide levels will exacerbate most insect and pest problems (Trumble & Butler, 2009). Downward trends, where yields are likely to drop by up to 25%, are generally expected due to poor harvests in drought-affected areas (Italian Food.net, 2014). A decrease of this extent will locally result in no profitability. Feedback attained from local oenologists indicates a possible 50% loss pertaining to a false early break as well, but only a 25% return due to higher temperatures. Other than these limitations, in the case of wine production, two further economic scenarios prevail as to whether IGT (Indikazzjoni Ġeografika Tipika) or DOK (Denominazzjoni ta' Oriġini Kontrollata) wine would be produced (MRA, 2015). These are correlated with production volumes per hectare at 18,000:12,500 for IGT:DOK. In effect, IGT allows additional production. Additionally, there is a price range by vintners of €0.65 to €0.80 per kilo, depending on the quality of the grapes and the production regime. Results indicate that IGT production operates with returns of €719.50 for the €0.80/kg price, but with a loss at €0.65/kg or lower. With an assumed 10% climate change deduction, the €0.80/kg return would then also operate at a €677.28 loss. All DOK production entails a loss and IGT would break even with but a 5.9% fall

for the €0.80/kg return. Thus, maintaining the volume of production is crucial for securing economies of scale and, therefore, economic survival.

Forecasts in percentage decreases in production suggest that some 6,300 hectares pertaining to the wheat, olive and vine crop types, as per Table 1, or 55% of Malta's total utilisable agricultural area, could be effectively rendered economically unsustainable should productivity levels fall by about 23%, given that for wheat, olives and vines, the break-even factors for production are 22.7%, 21.5% and 5.9% respectively. This impact could further exacerbate the push factor that discourages farming as a viable occupation. Thus, the onset of drier and warmer conditions in the Mediterranean region could lead to more favourable pest conditions and reduced yields: this has become a potential risk to the very existence of rural farming systems in Malta.

However, it is not only the farmers that will face difficulties. The production of fodder is closely linked to the dairy industry. Instead of purchasing local round bales that cost €30 and that weigh approximately 200 kg or €150 /tonne, utilisation of imported hay at 2014 prices of €250 /tonne (personal communication with various livestock breeders) will be necessary – effectively a €100 /tonne additional expense that could translate into a serious setback to profitability for livestock breeders, milk producers and finally consumers. In Malta, however, inextricably linked with the dairy industry are the other livestock industries via the provision of animal feed. Given that the local situation necessitates the utilisation of specialised smaller ships to provide the basic components for animal feed, any reduction in ordered quantities would necessarily result in higher tonnage costs.

In the case of the local production of fodder, grapes and olives, farmers could seek to cut down on expenses by minimising inputs, particularly fertiliser; but, this again would affect both yield and quality, particularly for wine and oil. The production of DOK wines has suffered a setback since market prices (as also determined by vintners) fell from the €0.93–€1.05 level to the current €0.65–€0.80 range. Attempting to increase production through additional irrigation will affect the brix or sugar content of the crop. Faced with diminishing returns, farmers could be more in favour of vine grubbing or removal after their 10-year commitment for state-aid development expires. Improvements in olive production shall require awareness to climate change issues to help farmers respond with better practices. Ultimately, oil and wine production command a niche in the local market that, if locally unavailable, would again oblige additional imports.

Looking at the value of gross production for the sector as at 2013 as indicated in the Economic Accounts for Agriculture (NSO, 2014), and comparing the milk, forage and wine components of €29,244,000 to the €138,222,000 total value of production, these three areas constitute at least 21% of the value of Malta's agricultural production. Should negative effects of climate change prevail, it may thus be assumed that at least this sector of the industry could be adversely impacted. One suitable response to damage to crops, with associated more difficult timing and management of agricultural operations, could include coordinated collective efforts at raising awareness that in turn promotes creative, practical and profitable responses, possibly including renewable energy, nutrient inputs, and soil management to the farming community. Agricultural adaptation options are grouped according to four main categories that are not mutually exclusive: (1) technological developments; (2) government programmes and insurance; (3) farm production practices; and (4) farm financial management (Smith & Skinner, 2001).

At EU level, through support by the European Agricultural Fund for Rural Development (EAFRD), there is the promotion of resource efficiency that involves extending two forms of support (ENRD, 2015). First, is the encouragement of a shift towards a low carbon and climate resilient economy in the agriculture, food and forestry sectors through better risk management. Second, is the support of market signals that promote climate change mitigation and adaptation through agro-environmental payments, as well as through support for areas facing natural or other constraints. As at 2010, in Malta there were some 12,530 agricultural holdings covering 11,450 hectares of utilised agricultural area (UAA), where 73.5% of these holdings have a UAA of less than 1.0 hectare each with some 18,539 persons actively engaged in agricultural activity. The need thus arises for a combined effort to effectively monitor and tackle productivity, profitability and sustainability, including mitigation of climate change.

3 Conclusion

Better risk management via improved resource efficiency may prove to be the only hope for the conservation of rural farming practices in south and south-eastern European countries faced with what is an already foreseeable battery of climate change impacts. The challenges are exacerbated on small island states like Malta, bereft of large non-coastal regions and with limited spare capacity to soak up and compensate for weaknesses and stresses that may result in other industrial sectors. Rural farming practices in Malta are likely to be considerably challenged by global environmental change.

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Research Note

Geology and wines of Pachino – Portopalo area. Preliminary outlines

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Abstract. This research, still in a developmental phase, analyses geological peculiarities and the relationships between them and the vineyards typical to the Pachino-Portopalo area.

The actual knowledge does not yet allow the drawing of tourist routes from which a geo-tourist would be able to join together Geosites and Oenosites. This will be one of the main topics of future research, oriented to produce substantial contributions to the sustainable development of this area.

Keywords: Geological Heritage, Geosites, Oenological Heritage, Oenogeological zonation, Oenogeological itineraries, Sustainable Development

1 Introduction

The southeastern extreme side of Sicily is characterised by the coexistence of many peculiarities that are very interesting, including geological landscape, historic-cultural aspects and eno-gastronomic features.

The authors of this paper have gained experience in the fields of history and geological evolution of this area of Sicily, particularly in the unique relationships between the geological substratum, the chemical and granulometric nature of its soils, and the unique mark that these soils attribute to the vine.

This preliminary contribution aims to establish a geological-oenological zonation of the Pachino-Portopalo area to draw geo-tourist routes for the joining of Geological resources with the oenological heritage.

2 Geological – Structural Frame

The basic volcanic rocks (Fig. 1: **CV**), outcropping between the cliff in the east of Portopalo and the area northwest of Pachino (Carveni, Romano, Capodicasa & Tricomi, 1991; Carveni & Capodicasa, 2011), are the oldest local rocks. The Cretaceous age has been inferred due to the fact they are comprised of a "Globotruncana calcilutites" layer and a "Rudiste limestones" layer of Maastrichtian age at the top (Fig. 1: **Cca**). An erosional surface, that truncates volcanic sequence, separates these units.

Limestones with Nummulites (Fig. 1: **Eca**), of Lutetian age, are characterised by a clear unconformity and rest on Cretaceous units. Clayey marls, with a breccias layer at the base, lie unconformably on older units and outcrop extensively in the north and west of Pachino. The age of these deposits are Messinian (Fig. 1: **Mm**). Moreover, there are calcareous marls in transgression on some of the older units. These are the classical "Trubi" (Fig. 1: **Pm**), of Infrapliocenic age, in heteropy with a calcarenitic facies. Finally, there are reddish calcarenites, of Thirrenian age, rich in organogenic component (Fig. 1: **Qca**).

Late and actual alluvial deposits outcrop at the borders of rivers (Fig. 1: **a**), and active dunes and fossil dunes are irregularly present along the coast (Fig. 1: **s**).

3 Geomorphological Frame

The morphological formations are a "consequence" of long and complicated palaeogeographic evolution; the more interesting structures are a product of the effusive processes of the Cretaceous age and the erosive action of the sea. Superficial eruptive structures, lined up

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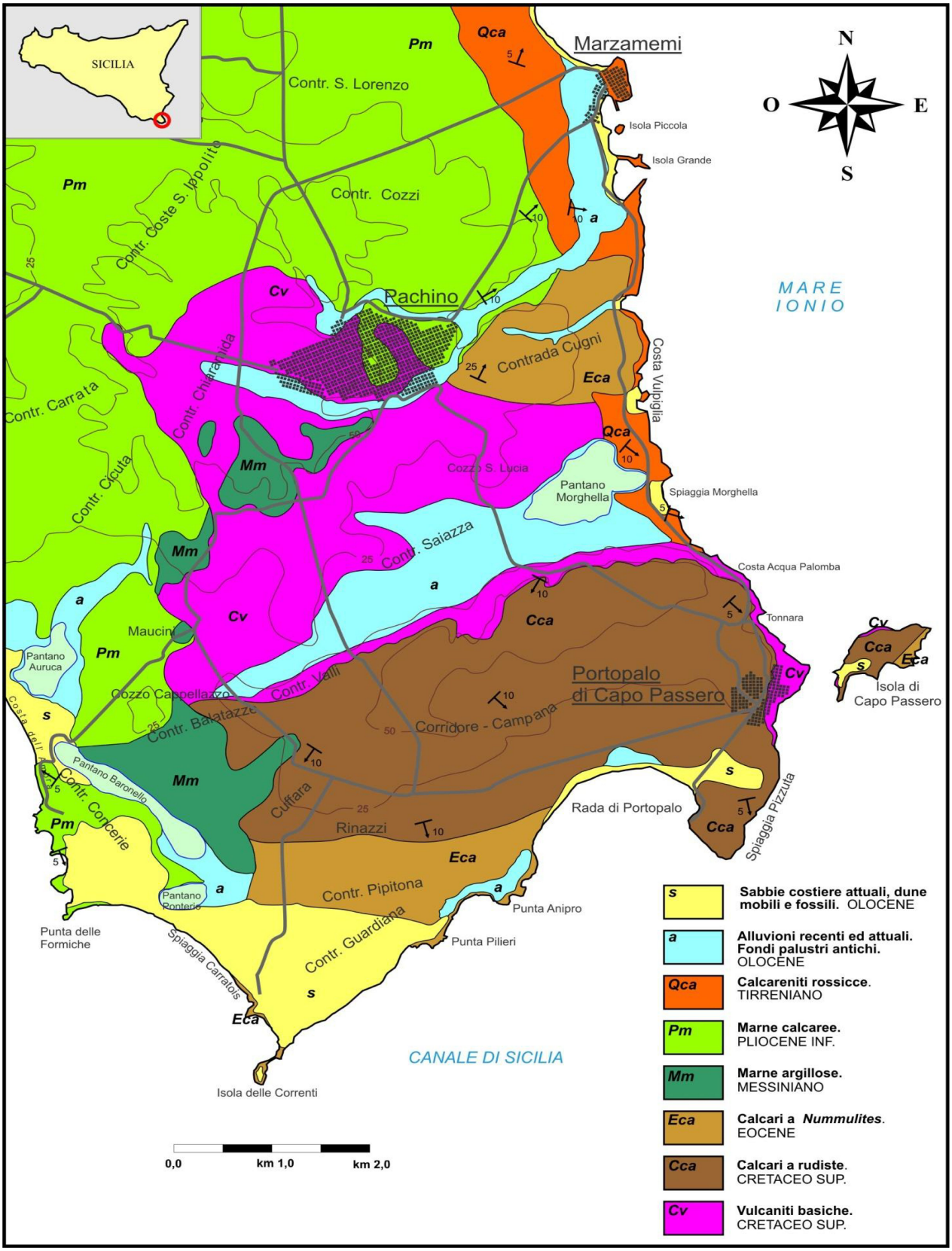

Figure 1: Schematic geological map (Carveni & Capodicasa, 2011).

along directrices of tectonic weakness, and many dykes with similar orientation, belong to effusive processes. Slopes, flat spaces of sea abrasion, marine terraces, marine caves, pot holes and coastal ponds are related to the erosive action of the sea.

Even karst forms, epygeal and hypogeal, are present.

The rise in sea level during the historical periods is shown by the presence of archaeological sites and historic installations, now partially or totally submerged.

The morphological characteristics may not be related to a unique pattern of evolution due to lithological variability of the outcropping rocks and their different exposure to the atmospheric elements during the geological time frame.

The northern sector, dominated by marly rocks, is marked by a series of little hilly raisings with rounded outlines, separated by large valleys. The erosional processes shown are extremely contained.

In the central sector, where outcrops are widely volcanic rocks, there are some volcanic deeply eroded structures. As a whole, the area is a wide valley that, in its terminal part, enlarges to form the coastal salt-marsh of Marghella. The hydrographic net is represented by short torrents.

The southern sector is characterised by typical tabular morphology of calcareous landscapes. A large plateau, delimitated at the north and east by fault, descends gradually and gently towards south.

The littoral area is characterised by low and rocky coasts, from high cliffs on the sea and creeks, sometimes very narrow and deeply incised, large and occupied by sandy deposits.

The coastal inland is bordered by ponds: they are hollows divided from the sea by sandy borders and/or calcarenitic bars.

4 Vineyards and Substrata

The vineyard that is more diffusely cultivated to produce wine grapes, more than Frappato and Pignatello, is the Nero d'Avola. More recently, the Moscato Bianco has also been introduced.

The substrata of the soils are prevailingly constituted by Cretaceous volcanic rocks and Messinian marly-clayey sediments.

Studies and analyses to map out Geosites, Geomorphosites, Oenosites and itineraries conjugating Geological and Oenological Heritages are in progress. These surveys are very important for the sustainable development of such areas (Aquino, D'Orefice, Esposito, Valletta & Vitale, 2011; Sibi & Valletta, 2013).

The climate is Mediterranean arid, with long periods which are almost totally dry (from May to October). The arid climate can be sporadically interrupted by storms, precipitation and months with a temperate climate, characterised by alternating short rainy periods with long periods of insolation.

5 Conclusions

This study concerns an area whose geological, structural and geomorphological features are well known. At this stage of the study we have taken a particular interest in the Oenological Heritage, especially the type of vineyards and the connection between substrata and soils. This study is only preliminary research for the most significant Geo-Oenological routes.

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We would like to dedicate this article to Lucilia Gregori, a dear friend and colleague who died prematurely in 2012.

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Research Article

Dativizable or Non-dativizable: That is the question? A syntactic-semantic analysis of English (non)-dativizable constructions in the production of a set of 2L1 English/Spanish simultaneous bilingual twins

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Abstract. This paper analyzes the syntactico-semantic factors which trigger Dative shift in English dativizable verbs, i.e. those verbs that allow alternation between double object and prepositional complement constructions. It also focuses on non-dativizable verbs that restrict their subcategorization framework to either double object or prepositional complement constructions. This syntactico-semantic relation between dativizable and non-dativizable structures is addressed in acquisition by examining the incidence for the two verb types in a set of English/Spanish 2L1 bilingual twins. Our results show that the syntactic and semantic features that dativizable and non-dativizables present go hand in hand with the age of first occurrence and the language development of the participants. Hence, dativizable to-dative double object constructions (DOC) are the utterances produced the earliest at the age of 2, as opposed to dativizable *to/for*-datives and non-dativizable constructions, which begin to emerge at around the age of 3. Finally, our results also suggest that the high adult input frequency explains the twins' early production of dativizable structures and that, in the same way, the children's low exposure to non-dativizable utterances correlates with the later occurrence in the twins' spontaneous production.

Keywords: dativizable, non-dativizable, DOC, to/for-dative, bilingual acquisition, L1 English

1 Introduction

Over the last few decades, the so-called (non)-dativizable constructions have been broadly studied from different syntactico-semantic perspectives. As illustrated in (1), the verbal subcategorization of these structures can project their arguments as double object complements (i.e. indirect object (Oi)-noun phrase (NP) along with a direct object (Od)-NP), or as an object complement followed by a prepositional object preceded by the preposition *to* or *for*, as shown in (2). Despite the fact that dativizable verbs have both verb complementation possibilities, there are some others, as shown in (3), that lack this syntactic alternation (non-dativizable verbs, henceforth).

(1)	a. He bought me a beer	(dativizable *for*-dative double object)
	b. They offered her some food	(dativizable *to*-dative double object)
(2)	a. He bought a beer for me	(dativizable *for*-dative)
	b. They offered some food to her	(dativizable *to*-dative)
(3)	a. He told me that story	(non-dativizable double object)
	b. The teacher explained it to his students	(non-dativizable *to*-dative)
	c. I thanked her for her help	(non-dativizable *for*-dative)

The possibility of a single verb to project their verbal argument complements both as double objects (DOCs) (examples in (2)) and as object plus prepositional constructions (examples in (1)) has triggered the debate in the literature of dativizable constructions as to which structure is syntactically base-generated and which one is derived (see section 2). Contrarily, the so-called non-dativizable verbs (examples in (3)) restrict their sub-

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categorization framework, as either DOCs or prepositional structures, they lack a dativizable counterpart.

In view of the potential argument alternation or dative shift of some verbs, semanticists have also posed some issues concerning the meaning that dativizable and non-dativizable constructions convey. Some linguists claim that there is no difference in the meaning when verbs allow argument alternative choices (Aoun & Audrey Li, 1989; Hale & Keyser, 2002; Krifka, 2003; Levin, 1993; Oehrle, 1976; Pinker, Lebeaux & Frost, 1987; Pylkkänen, 2002) unlike others who argue that there are semantic distinctions between dativizable DOCs and dativizable prepositional constructions (Green, 1974; Krifka, 2003; Greenbaum, Leech, Svartvik & Quirk, 1985).

Taking these syntactico-semantic issues into account, the aim of this study is to disentangle the syntactic and semantic dichotomies that exist in the literature of English dativizable and non-dativizable constructions by looking at their production in spontaneous speech. The participants of this research are a set of English/Spanish simultaneous bilingual twins, Simon and Leo. As mentioned above, only one of the L1s of these children is examined, namely, English.

This paper is organized as follows: section 2 reviews previous works both from the side of linguistic theory discussing the factors governing the syntax and semantics of these constructions, as well as those from the point of view of the acquisition of dativizable and non-dativizable constructions. Section 3 includes the hypotheses that guide this study. Data selection and classification criteria are presented in section 4. The data analysis is developed in section 5 on the basis of the following variables: (a) age, (b) MLUw, and (c) input. Section 6 presents the conclusions and points to directions for further work.

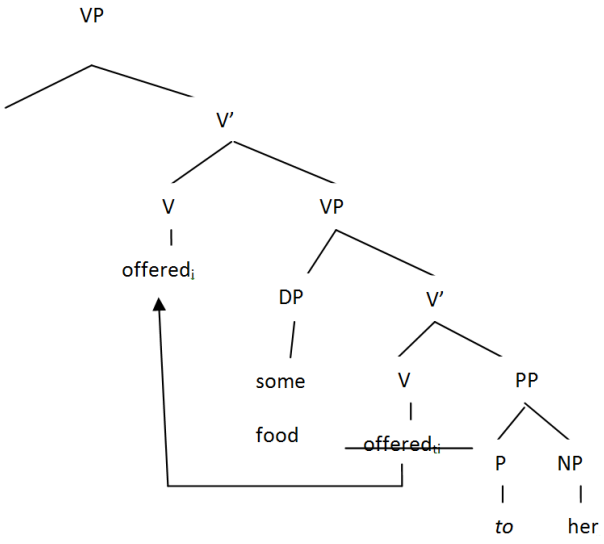
2 Theoretical background and previous acquisition studies

2.1 Syntactic approach to (non)-dativizable constructions

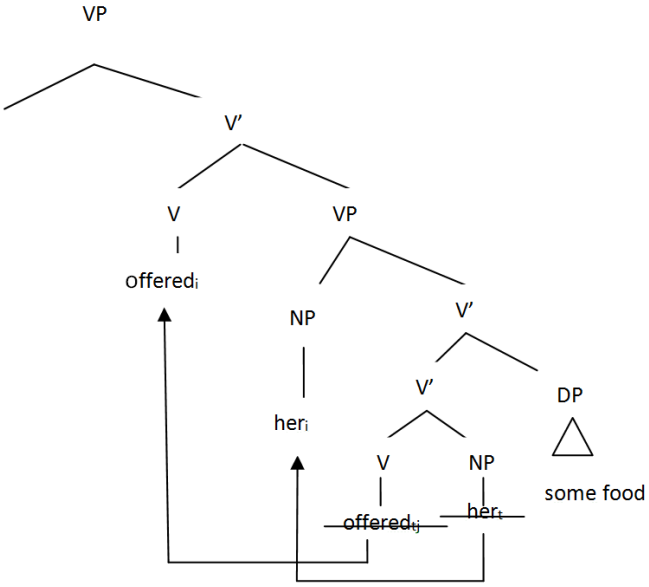
The so-called dativizable constructions have led to a dichotomy as far as their syntactic derivation is concerned. Some linguists (Aoun & Audrey Li, 1989; Snyder & Stromswold, 1997) claim that dativizable double object constructions are the basic structure from which dativizable *to/for*-datives derive. Alternatively, there are those who argue that dativizable DOCs are syntactically generated from *to/for*-dative structures (M. C. Baker, 1997; Chomsky, 1955; Larson, 1988, 1990).

Regardless of the semantic principle of Uniformity of Theta Assignment Hypothesis (M. C. Baker, 1988, UTAH,) which states that *"identical thematic relations between items are represented by identical structural relations between these items at D(eep)-structure"*, the underlying symmetry of thematic roles between dativizable DOCs and dativizable *to/for*-datives is not displayed in their S(urface)-structure. According to Larson (1988), *to/for*-dative constructions are the basic dativizable constructions from which dativizable *to/for*-dative DOCs derive. The verbal head in dativizable *to/for*-datives subcategorizes for a prepositional phrase (PP), headed by the preposition *to* or *for*. As illustrated in (4), the verbal head *offered* triggers head-to-head movement and rises to the specifier of the higher V' in order to meet Case and theta requirements, leading to what Larson terms the non-shifted version. In turn, Larson claims that dativizable DOCs derive from dativizable *to/for*-datives in a passive-like process, shaping, what he terms, the shifted version. More specifically, and as shown in (5), the preposition *to*, which is a case assigner in the non-shifted version in (4), absorbs its case being assigned to its prepositional complement *her*. This absorption of Case triggers NP-movement of *her* to the specifier of the lower VP. Similarly, the verbal head rises into the specifier of the higher V' in order to assign Case and theta role to its adjacent argument *her*. Due to this verbal movement, the Od in the non-shifted version is caseless; hence, it reduces its argument position to a non-thematic adjunct position, analogous to passive *by*-phrases, as depicted in (5).

(4) They offered some food to her (dativizable *to*-dative)



(5) They offered her some food (dativizable DOC)



Alternatively, it has been argued that dativizable *to/for*-datives are derived from dativizable DOCs by a passive-like process, where, as opposed to the previous argument, the Oi occupies an adjunct position via NP-movement (Aoun & Audrey Li, 1989; Snyder & Stromswold, 1997). Hence, example (4) derives from (5). In fact, as will be discussed in section 2.3, Snyder and Stromswold (1997) argue that the lexical item *to* is the factor delaying the acquisition of dativizable *to*-dative constructions.

Unlike dativizable verbs whose arguments can be projected as double object complements or as objects followed by prepositional complement constructions headed by the lexical item *to/for*, non-dativizable DOCs and non-dativizable prepositional verbs do not have the syntactic strength to trigger dative shift. As illustrated in (6a), the non-dativizable verb *explain* can only subcategorize *to*-dative complements, hence the ungrammaticality of (6b).

(6)	a. The teacher explained it to his students	(non-dativizable *to*-dative)
	b. *The teacher explained his students it	(ungrammatical non-dativizable *to*-dative)

Larson (1990) accounts for the argument restrictions of non-dativizable *to*-dative verbs as the impossibility of the oblique preposition to undergo case marking. Consequently, unlike his argument concerning the derivation of dativizable DOCs (Larson, 1988), the absorption of the preposition *to* in prepositional non-dativizables via a passive-like process would violate the recoverability of deletion or Dative shift.

Furthermore, the syntactic alternation of non-dativizable constructions is mainly restricted in the lexicon. Thus, verbs of Latin origin, as exemplified in (8) with the verb *donate*, can only take an [NP *to*-NP] complement. Furthermore, this factor that prevents the alternation of non-dativizable structures is also linked to verbal semantics. In particular, only morphologically native verbs, as illustrated in (7), are capable of triggering dative shift (Mazurkewich & White, 1984; Green, 1974; Oehrle, 1976).

(7)	a. He gave me $50	(DOC dativizable native verb)
	b. He gave $50 to me	(*to*-dative dativizable native verb)
(8)	a. He donated $50 to me	(non-dativizable *to*-dative Latinate verb)
	b. *He donated me $50	(ungrammatical non-dativizable DOC, Latinate verb)

2.2 Semantic approach to ditransitive constructions

Regarding semantic factors in (non)-dativizable ditransitive constructions, different studies have pointed out that there are divergences in meaning between both structures (Aoun & Audrey Li, 1989; Hale & Keyser, 2002; Krifka, 2003; Levin, 1993; Oehrle, 1976; Pinker et al., 1987; Pylkkänen, 2002). These denotative differences, as will be shown below, do not go hand in hand with the syntactic derivation of both structures argued in section 2.1. Nevertheless, there are arguments that point to a common underlying semantic ground between dativizable and non-dativizable constructions (Green, 1974; Krifka, 2003; Greenbaum et al., 1985).

On the one hand, some linguists assume that there is a transfer of possession relation (i.e., a cause-HAVE relation) hidden between the Oi and the Od in dativizable DOCs (Aoun and Audrey Li, 1989; Hale and Keyser, 2002; Krifka, 2003; Levin, 1993; Oehrle, 1976; Pinker et al., 1987; Pylkkänen, 2002). As depicted in (9), the Oi *her* is narrowly related in a possession relation with the Od *some food*. In other words, the causal agent *they* causes the possessor *her* to have *some food* (theme).

(9)	a.	They	offered	her	some	food	(dativizable DOC)
	b.	*Casual agent*		*possessor/goal*		*possessum/theme*	*thematic roles*
	c.	*'X*	*causes*	*Y*	*(to HAVE)*	*Z'*	*semantic structure*

It should be noted that the possession transfer relationship in dativizable DOCs does not infer that the Oi ends up *possessing* the Od (Aoun & Audrey Li, 1989; Hale & Keyser, 2002; Krifka, 2003; Levin, 1993; Oehrle, 1976; Pinker et al., 1987; Pylkkänen, 2002). As illustrated in (10), the causal agent *I* sends a letter to *Bill* but we cannot guarantee that *Bill* will receive it. Thus, even though dativizable DOCs imply a possession relation, the fact that the possessor ends up possessing the theme is not always accomplished.

(10) I sent Bill that letter (but he never got it)

On the other hand, dativizable *to/for*-dative constructions are claimed to denote literal or metaphorical motion towards a goal (for example, they express a mental movement as in the verbs *show* or *tell*). In other words, they suggest a cause-GO/cause-GOAL relation between the cause/patient and the path/goal thematic roles. As exemplified in (11), the causal agent *they* caused the cause/patient *some food* to go to the path/goal *her*.

(11)	a.	They	offered	some food	to her	(dativizable DOC)
	b.	*Casual agent*		*cause/patient*	*path/goal*	*thematic roles*
	c.	*'X*	*causes*	*Y*	*(to GO to) Z'*	*semantic structure*

There are also arguments that point to a common underlying semantic ground between dativizable structures (Bruening, n.d.; Green, 1974; Krifka, 2003; Rappaport Hovav & Levin, 2008; Greenbaum et al., 1985). Hence, dativizable DOCs and dativizable *to/for*-dative constructions are equally regarded as verbs of transfer of possession (cause-HAVE relation) and verbs of motion (cause-GO/cause-GOAL). As depicted in (12), where *John* gives *Mary a book*, a twofold explanation can be given: (a) the book is moved from John's possession into Mary's possession (transfer of possession meaning, taking into account dativizable DOCs as the basic semantics) or, (b) possession of the book was transferred from John to Mary (cause-Go/cause-Goal relation, taking into account dativizable *to/for*-datives as the semantic referential point).

(12) a. John gave Mary a book
b. John gave a book to Mary

Non-dativizable semantic restrictions are correlated with their corresponding dativizable counterparts (Comrie, Malchukov & Martin H., 2010; Oehrle, 1976). More specifically, non-dativizable DOCs are rooted in the absence of motion along some path in the same way as non-dativizable *to/for*-dative constructions lack a transfer of possession meaning. As can be seen in (13), the causal agent *John* causes the patient theta role *Max* to metaphorically possess *a kick* (path/goal).

(13)	a.	John	gave	Max	a kick	(non-dativizable DOC)
	b.	*Causal agent*		*cause/patient*	*path/goal*	*thematic roles*
	c.	*'X*	*causes*	*Y*	*(to GO to) Z*	*semantic structure*

Thus, the compositional semantics of the verbal phrase (VP) in non-dativizable DOCs is not compatible with the direction/path feature encoded in dativizable *to/for*-dative constructions. In other words, the example in (14) cannot be understood, according to Malchukov and Oehrle's principles, as John's causing a kick to go to Max; thus its ungrammaticality.

(14) *John gave a kick to Max (ungrammatical non-dativizable DOC)

2.3 Previous studies on the acquisition of (non)-dativizable constructions

A small number of studies have been carried out in order to examine the order of acquisition of dativizable structures. Gropen, Pinker, Hollander, Goldberg and Wilson (1989) analyzed the spontaneous speech of 5 L1 English children in the Brown corpus in CHILDES (MacWhinney, 2000, Child Language Data Exchange System,). They found out that children start producing dativizable DOCs and dativizable prepositional constructions simultaneously in the second year, with neither structure subsequently uttered after the other. However, Snyder and Stromswold (1997) replicated Gropen et al.'s study including more participants and proposed that dativizable DOCs and dativizable *to*-datives depend on two parametric properties: property A allows the grammar to produce dativizable DOCs as opposed to dativizable *to*-datives which merge the property of dativizable DOCs with another property, termed by Snyder and Stromswold as property B. Taking these observations into account, they implemented a study of the data from L1 English children to analyze the correlation in the acquisition of both structure types. Their results showed that dativizable DOCs are crucially acquired before dativizable *to*-datives as indicated by a sign test ($p = .00098$) and a t test ($t(11) = 4.15, p = .002$). However, this order effect in acquisition did not correlate with the children's input.

The acquisition of the dative alternation has also been correlated with the presence or the lack of negative evidence of these structures in the child's input (C. L. Baker, 1979; Gropen et al., 1989). In particular, C. L. Baker (1979) claims that children are conservative in their productions since their output will be affected by the input they have received. Hence, non-dativizable constructions like those in (15a) will be generalized as dativizable structures (see example (15b)) because the child has heard non-dativizable utterances less frequently in his/her input. In other words, children are not aware of non-dativizable verbal constraints in their emerging language development since children apply them to verbs that do not allow dative shift in the adult grammar (Mazurkewich & White, 1984).

(15) a. I donated a book to the library (non-dativizable to-dative)
b. *I donated the library a book (ungrammatical non-dativizable DOC)

Viau (2007) carried out a study dealing with the semantics of dativizable structures in L1 English acquisition. He attempted to demonstrate that dativizable DOCs denote transfer of possession by analyzing the correlation in acquisition between dativizable DOCs and *have* (the verb of possession par excellence), between prepositional constructions and the verb *go* (the verb of motion par excellence) and between dativizable DOCs and dativizable prepositional constructions and causative verbs. His results showed that dativizable DOCs convey transfer of possession since they are concurrently acquired with the verb *have*, in the same way as dativizable prepositional constructions denote motion shown in the correlation in acquisition with the verb *go*. In turn, dativizable DOCs and dativizable prepositional constructions display a correlation in acquisition with *causative* verbs. Despite the latter results, dativizable prepositional structures were acquired later than dativizable DOCs.

It should be noted that there is not much research on the acquisition of non-dativizable constructions. Thus, further investigations should be carried out in order to fill the gap of studies that deal with this type of constructions.

3 Hypotheses

Taking into account these previous studies on the linguistic description of the structures under analysis, as well as those on their acquisition, the following possible scenarios might be postulated in the acquisition data of (non)-dativizable constructions:

- Hypothesis 1: If dativizable *to/for*-datives are transformationally derived from dativizable DOCs via a passive-like process, then dativizable *to/for*-datives are expected to be acquired later than dativizable DOCs.
- Hypothesis 2: If, on the contrary, dativizable DOCs are transformationally derived from dativizable *to/for*-datives via a passive-like process, then dativizable *to/for*-dative are expected to be produced earlier than dativizable DOCs.
- Hypothesis 3: Regardless of the derivational accounts between dativizable *to/for*-datives and dativizable DOCs, dativizable *to*-dative and *for*-dative constructions are expected to be concurrently acquired since, despite the syntactic status of the *to*-PP and *for*-PP as subcategorized constituents or as adjuncts, respectively, they are considered to be a syntactic block of prepositional ob-

ject dativizable constructions as they are able to trigger dative shift resulting in DOCs.

- Hypothesis 4: If dativizable *to/for*-dative constructions require a lexical item *to/for* in order to meet semantic motion requirements (cause-GO), then it is expected that children acquire them at a later stage, as opposed to dativizable DOCs, which, in contrast, do not require an additional constituent to accomplish the semantic attribution of transfer of possession (cause-HAVE).
- Hypothesis 5: If semantics plays a role, despite restrictions in the subcategorization framework of non-dativizable constructions, a correlation in acquisition is expected between those constructions that share a semantic analogy, i.e., between dativizable and non-dativizable DOCs, as they have a cause-HAVE relation, and between dativizable and non-dativizable *to/for*-datives, as they imply a cause-GO relation.
- Hypothesis 6: If non-dativizable constructions are subject to syntactic and semantic constraints, then a later acquisition is expected for non-dativizable constructions as opposed to dativizable structures, which do not offer any limitations in their subcategorization framework or in their semantics.
- Hypothesis 7: If input is taken into account, the order of acquisition will correlate with the frequency with which a child is exposed to (non)-dativizable constructions.

Thus, the acquisition data, which revolve around the above-mentioned hypotheses, aim at shedding some light on the actual analysis of (non)-dativizable structures. That is, the results of this study will reflect how acquisition data can explain the syntactic and semantic properties of those constructions.

4 Methodology

4.1 Data selection

In order to provide an answer regarding the relative order of production of English dativizable and non-dativizable DOCs and dativizable and non-dativizable *to/for*-dative constructions, we have extracted child data from the FerFulice corpus available in the CHILDES project (MacWhinney, 2000, Child Language Data Exchange System,). More specifically, we have conducted our study by analyzing the production of a set of Spanish/English simultaneous bilingual twins, who were born and raised in a Spanish geographical background. Parents address children using the one-parent one-language strategy (Melanie, the mother, engages in conversations with the children in English as she is an English native speaker, whereas the father, Ivo, addresses them in Spanish as he is a Spanish native speaker) (Fernández Fuertes & Liceras, 2010, more information on the children's background as well as on the data collection process appears in). It should be noted that the analysis of this study has focused on the spontaneous production of English (non)-dativizable utterances so that only one of the L1s of these children is analyzed, i.e. English. The age range that covers this longitudinal corpus ranges from 1.0 to 6.5 years old and for this study the corpus has been analyzed in its entirety.

The search for both dativizable and non-dativizable ditransitive instances has been manually done by taking into account the following patterns of subcategorization: (a) S+V+NP+NP (for (non)-dativizable DOCs) and (b) S+V+NP+*to/for*-NP (for (non)-dativizable *to/for*-dative constructions). We have implemented the same process for the twins and for the child-directed speech (to analyze the adult input).

4.2 Data classification

Data have been classified regarding the type of participant, the children's age and the Mean Length of Utterance measured in words (Brown, 1973, MLUw;). Besides, concerning the type of construction, we have considered those structures which trigger Dative shift (i.e. dativizable structures), classifying them as those verbs which subcategorize for an Od along with a *for*-dative PP and, which are dativizable as DOCs, as shown in (16). Similarly, we have also taken into account dativizable constructions which allow for a *to*-dative PP in their subcategorization framework, and which, in turn, trigger Dative shift of their arguments as dativizable DOCs, as depicted in (17).

(16)	a. He bought a house for me	(dativizable *for*-dative)
	b. He bought me a house	(dativizable *for*-dative DOC)
(17)	a. He gave a present to me	(dativizable *to*-dative)
	b. He gave me a present	(dativizable *to*-dative DOC)

Apart from those constructions that are dativizable as both *to/for*-dative and dativizable DOCs, we have also analyzed utterances which do not allow for Dative shift, that is to say, non-dativizable constructions. Examples in (18) and (19) show *to/for*-dative and non-dativizable structures, respectively.

(18) a. I have something for you (non-dativizable *for*-dative)
b. He said that to the mice (non-dativizable *to*-dative)
(19) They called her Snow White (non-dativizable DOC)

In our data classification, we have discarded the following cases: (a) utterances whose *to/for*-PP denotes a locative or temporal meaning, as in (20) and (21), respectively, (b) passive idiomatic expressions such as those in (22), since they are fixed expressions that lack an active counterpart, (c) DOCs which are paraphrazable as one single verb, as in (23), where the constituent *give it a try* can be paraphrased as *to try* since they have a monotransitive nature, (d) *for*-PPs with a proxy meaning, i.e., those constructions where the preposition *for* is paraphrazable as *in exchange for*, such as those in (24) since the PP lacks a recipient connotation, (e) those constructions which do not show a canonical SVOO or SVO+*to/for*-PP order, as in example (25), which illustrates a non-canonical word order DOC whose wh-Od has undergone wh-movement for syntactic reasons; (f) ditransitive structures which subcategorize a *from*-PP, such as those in (26). These structures have been discarded since the PP denotes a source meaning, contrary to the recipient theta role patterns that (non)-dativizable prepositional constructions present in their PP.

(20) *SIM: he drives her to the car
(21) *MEL: who came for your birthday?
(22) *LEO: I am used to it
(23) *MEL: give it a try
(24) *MEL: you traded the plane for the little blue pistol
(25) *MEL: what did he tell you?
(26) *EMM: you learn that from mommy, don't you?

5 Results and Discussion

In order to analyze the relative order of acquisition between dativizable and non-dativizable constructions, we have taken into account three variables, as follows: (a) age of production, (b) language development as measured in terms of MLUw, and (c) the effect of adult input.

5.1 Age of onset of production

This section discusses three different analyses: (a) the age of onset production that the participants display regarding dativizable *to/for*-dative constructions as opposed to dativizable DOCs, (b) the results obtained concerning the first occurrence of non-dativizable structures, and (c) it offers comparative findings between structures that trigger dative shift (i.e., dativizable utterances) and non-dativizable constructions.

It should be highlighted that in order to determine the age of onset of production, we have taken into account the first clear use of dativizable and non-dativizable constructions.

5.1.1 Age of onset of production: dativizable constructions

As illustrated in Table 1, Simon and Leo start producing dativizable *to*-dative DOCs at around 2 years old. In particular, Simon begins to utter them at 2;03.26 as opposed to Leo, whose first constructions appear one month later, that is to say, at 2;05.00.

Examples in (27) show Simon's and Leo's first utterances, both produced with the verb *give*, the ditransitive verb par excellence. Notice also that the twins have not realized the head of the D(eterminer) P(hrase) encoded in the Od.

(27) a. *SIM: Give me tv (Simon, 2;03.26)
b. *LEO: Give me farmer (Leo, 2;05.00)

By focusing on the first occurrences of dativizable DOCs which trigger *for*-dative shift, Simon and Leo, and particularly the latter participant, present a divergent age of acquisition. They start producing dativizable *for*-dative DOCs at 2;11.06 and 3;03, respectively, with a four month difference between them. Examples (28a) and (28b) illustrate the twin's early production of prepositional ditransitives headed by the preposition *for*.

(28) a. *SIM: do you make me a cake? (Simon, 2;11.06)
b. *LEO: who brought me this? (Leo, 3;03.00)

Broadly speaking, the twins have shown a subsequent production of prepositional ditransitives in contrast with dativizable *to/for*-dative DOCs. Particularly, both children start uttering dativizable prepositional utterances at around the age of 3. Nevertheless, an exception has been found in Leo's early production of dativizable *for*-dative DOCs where, as shown in Table 1, his first occurrence correlates with his early production of prepositional ditransitives at around the age of 3. More specifically, and as shown in (29a) and (29b), Simon and Leo start uttering dativizable *for*-datives at 3;01.20 and 3;02.24, respectively, with a month difference between them. In turn, dativizable *to*-dative constructions, as illustrated in (30), were first produced at 3;02.12 by Simon and 3;01.06 by Leo, also showing a month's difference between the twins' output of those structures.

(29) a. *SIM: to make the honey just for me (Simon, 3;01.20)
b. *LEO: take it and the baby for you (Leo, 3;02.24)

(30) a. *SIM: we bought that to you (Simon, 3;02.12)
b. *LEO: give it to the zoo (Leo, 3;01.06)

When focusing on the age of early utterance of dativizable *to*-dative and dativizable *for*-dative prepositional constructions, our findings show that their age of onset production is at around the age of 3. Despite the fact that Simon and Leo start uttering dativizable prepositional structures at around the same age, they show different ages of onset for each construction. As illustrated in (30a), Simon's early dativizable *for*-dative prepositional ditransitives are initially uttered at 3;01.20, preceding his first production of dativizable *to*-dative prepositional constructions at 3;02.12, as illustrated in (31a). Alternatively, Leo shows a reverse order of acquisition between dativizable *to/for*-dative prepositional utterances since he starts producing dativizable *to*-dative prepositional constructions at 3;01.06, as exemplified in (31b), one month earlier than his early utterance of dativizable *for*-dative at 3;02.24, as can be seen in (30b). Thus, the production of dativizable *to/for*-datives goes hand in hand as syntactic analogous prepositional constituents, confirming our H3, as will be discussed in section 6.

(31) a. *SIM: do you make me a cake? (Simon, 2;11.06)
b. *SIM: give me tv (Simon, 2;05.00)

In particular, Simon's early production of dativizable *to/for*-dative DOCs, as illustrated in (29a), repeated as (31a) and (27a) repeated as (31b), respectively, has been observed at around the age of 2, one year earlier than his onset production of non-dativizable DOC at 3;05.12 (example in (32)). It is worth standing out that Simon's early production of dativizable *for*-dative DOCs is found one year later than the utterance of his early dativizable *to*-dative DOC, as discussed in section 5.1.2.

(32) *SIM: we call them blue (Simon, 3;05.12)

However, Leo's production of dativizable *for*-dative DOCs and dativizable *to*-dative DOCs shows a different time frame since he starts uttering the former constructions at 3;03 (as shown in (28b), repeated below as (33a), one year later than his first occurrence of to-dative DOC at 2;05 (as illustrated in (27b), repeated below as (33b)).

(33) a. *LEO: who brought me this? (Leo, 3;03)
b. *LEO: give me farmer (Leo, 2;05)

All in all, and as illustrated in Table 1, dativizable *to/for*-dative DOCs are the dativizable structures produced the earliest by the twins at the age of 2, preceded by the first occurrence of dativizable prepositional utterances, displayed at the age of 3.

Table 1: Age of onset production of dativizable constructions.

	Dativizable			
	DOC		**Prepositional ditransitive**	
	***to*-dative**	***for*-dative**	***to*-dative**	***for*-dative**
Simon	2;03.26	2;11.06	3;02.12	3;01.20
Leo	2;05.00	3;03.00	3;01.06	3;02.24

5.1.2 Age of onset of production: non-dativizable constructions

As illustrated in Table 2, non-dativizable constructions, regardless of their type, begin to be produced at the age of 3. Thus, the age of onset production of dativizable prepositional ditransitives correlates with the early production of non-dativizables. Nevertheless, for the twins non-dativizable prepositional ditransitives and non-dativizable DOCs do not begin to emerge in a parallel way. More specifically, Simon's linear order of the early production of non-dativizables is the following: he starts uttering non-dativizable prepositional *to*-dative constructions at 3;04.28, one month earlier than non-dativizable DOCs (i.e., at 3;05.12) and five months earlier than non-dativizable prepositional *for*-datives (i.e., at 3;09.13). On the other hand, Leo starts producing non-dativizable for-dative prepositional struc-

tures at 3;02.00, eight months earlier than his first non-dativizable to-dative construction (i.e., at 3;10.05). As displayed in Table 2, there has not been any utterance found concerning non-dativizable DOCs across Leo's corpus.

Table 2: Age of onset production of non-dativizable constructions

	Non-Dativizable		
	Prepositional ditransitive		**DOC**
	***to*-dative**	***for*-dative**	–
Simon	3;04.28	3;09.13	3;05.12
Leo	3;10.05	3;02.00	–

Despite the fact that dativizable prepositional ditransitives and non-dativizables start being uttered at around the age of 3 (compare Tables 1 and 2), Simon and Leo display subtle differences in their order of first production. As example (34) shows, dativizable *for*-dative utterances are the first prepositional ditransitives produced by Simon at 3;01.20, as opposed to Leo, who starts uttering dativizable *to*-dative constructions at 3;02.24, as illustrated in (35).

(34) *SIM: to make the honey just for me (dativizable *for*-dative; Simon, 3;01.20)

(35) *LEO: give it to the zoo (dativizable *to*-dative; Leo, 3;01.06)

Likewise, non-dativizable constructions reflect differences in the twins' order of first occurrence. Thus, as exemplified in (36), Simon begins to produce non-dativizable to-datives at 3;04.28, preceding the age of onset production of non-dativizable DOCs and non-dativizable for-datives, as can be seen in (32), repeated here as (37) and (38), respectively.

(36) *SIM: why is the cat saying that to the mice (non-dativizable *to*-dative; Simon, 3;04.28)

(37) *SIM: we call them blue (non-dativizable DOC; Simon 3;05.12)

(38) *SIM: I want one for myself (non-dativizable *for*-dative; Simon, 3;09.13)

On the contrary, as exemplified in (39), non-dativizable *for*-dative constructions start being uttered by Leo at 3;02.00, preceding the first production of non-dativizable *to*-datives at 3;10.05, as shown in (40).

(39) *LEO: thank you for my playdough (non-dativizable *for*-dative; Leo, 3;02.00)

(40) *LEO: you have something to the toad (non-dativizable *to*-dative; Leo, 3;10.05)

As shown in Table 2, non-dativizable DOCs are not reflected in Leo's data, hence comparative results cannot take place regarding these structures in the twins.

In view of the above-mentioned results, dativizable constructions (i.e. prepositional ditransitives and DOCs) are produced earlier than constructions which do not trigger dative shift (i.e. non-dativizables). More specifically, the relative order of early occurrence of both structures in Simon and Leo is illustrated in (41a) and (41b), respectively.

(41) a. Dativizable *to*-dative DOC > dativizable *for*-dative DOC > dativizable *for*-dative > dativizable *to*-dative > non-dativizable *to*-dative > non-dativizable DOC > non-dativizable *for*-dative

(Simon's relative order of first production of (non)-dativizable *to/for*-datives)

b. Dativizable *to*-dative DOC > dativizable *to*-dative > non-dativizable *for*-dative > dativizable *for*-dative > dativizable for-dative DOC > non-dativizable *to*-dative

(Leo's relative order of first production of (non)-dativizable *to/for*-datives)

These results evidence differences between the twins. On the one hand, Leo's early production of dativizable *for*-dative DOCs correlates with his first occurrences of dativizable *to/for*-datives and non-dativizable *to/for*-datives at around the age of 3. On the other hand, Simon displays a correlative age of early occurrence between dativizable *to/for*-datives and non-dativizable constructions (regardless of the type) at around the age of 3. One year earlier, Simon shows a correlation in his first utterances of the block of dativizable DOCs (i.e., dativizable *to/for*-dative and DOC), which differs from Leo's performance since he does not show a parallelism between the production of DOCs. In fact, as discussed in section 5.1.1, he begins to produce dativizable *to*-dative DOCs at 2;05.00, one year earlier than his first utterance of dativizable *for*-dative DOCs at 3;03.00. Furthermore, unlike Simon, we have not found evidence of Leo's production of non-dativizable DOCs; hence, we cannot offer comparative results between the participants regarding these types of utterances.

5.2 Language development

The Mean Length Utterance measured in words (MLUw) has also been used in this study, along with the chronological age, in order to address the twins' language development in correspondence with their production of (non)-dativizable constructions.

As depicted in Figure 1, Simon's first utterances start being developed with an MLU of 3 words, where he produces dativizable *to*-dative DOCs. In fact, as the MLUw of these constructions rises, the production of dativizable *to*-dative DOCs increases, being his most productive stage with MLUw of 4 and 5. Furthermore, although in a less prolific production, Simon also begins to utter dativizable *for*-dative DOCs and dativizable *to/for*-datives. Regarding Simon's linguistic development of non-dativizable constructions, our results show that even though the number of occurrences of non-dativizable *to/for*-datives are not significantly productive, Simon starts producing them with an MLUw of 4. Moreover, as illustrated in Figure 1, no non-dativizable constructions appear in Simon's production.

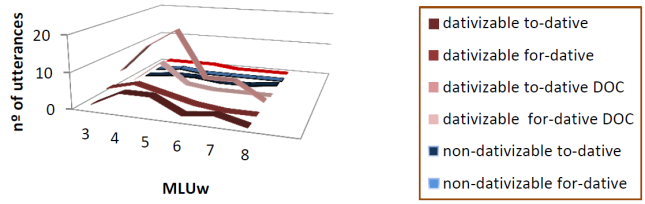


Figure 1: Simon's MLUw of (non)-dativizable constructions.

As shown in Figure 2, Leo starts developing dativizable *to*-dative DOCs which with an MLUw of 3, displaying a correlation with Simon's findings (see Figure 1). Unlike Simon, Leo begins to produce dativizable *for*-dative DOCs and dativizable *to*-datives with an MLUw of 4 and dativizable *for*-datives arise in Leo with an MLUw of 5.

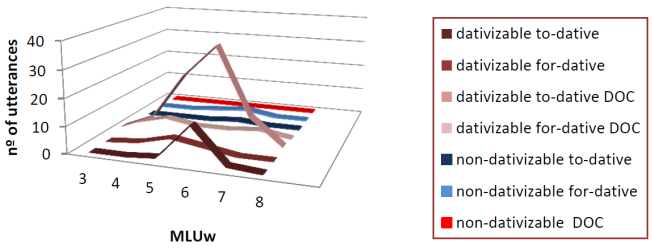


Figure 2: Leo's MLUw of (non)-dativizable constructions.

It must be also noted that Leo has productively uttered non-dativizable *to* and *for* constructions with an MLUw of 5 and 6, respectively. Nevertheless, the number of utterances of these two types of ditransitives is not highly productive.

Overall, and similar to the results found in Simon's production, there was a lack of occurrences regarding non-dativizable constructions in Leo's data.

Therefore, Simon and Leo correlate in the early production of dativizable *to*-dative DOCs with an MLUw of 3. Similarly, the twins show a concurrent lack of productivity in their data concerning non-dativizable DOCs. Despite these correlative results, the early occurrences of dativizable *for*-dative DOCs, dativizable *to/for*-datives, non-dativizable *to/for*-datives and non-dativizable DOCs have shown differences in the twins' production from the point of view of the MLUw (compare Figures 1 and 2).

Table 3 summarizes the linguistic development in both children taking into account the variables of the age of onset production and the MLUw.

As illustrated in Table 3, Simon and Leo show a correlation in the age of onset production of dativizable *to*-dative DOCs at 2;03 and 2;05 years old, respectively, and with an MLUw of 3. However, the age of onset differs in the twins' production of non-dativizable constructions. Simon starts uttering non-dativizable *to*-datives at 3;04.28 whereas Leo begins to produce non-dativizable *for*-datives at 3;02.00. Despite these differences in the age of first occurrence as well as the type of non-dativizable structures, the syntactic status of the object complement being produced is the same for both since, for both participants, a PP headed by a preposition *to* or *for* begins to emerge.

Table 3: Simon and Leo's language development of (non)-dativizable utterances

	Age of onset production		**MLUw**	
	Dativizable	**Non-dativizable**	**Dativizable**	**Non-dativizable**
Simon	*to*-dative DOC (2;03.26)	*to*-dative (3;04.28)	*to*-dative DOC (MLUw 3)	*to*-dative (MLUw 4)
Leo	*to*-dative DOC (2;05.00)	*for*-dative (3;02.00)	*to*-dative DOC (MLUw 3)	*for*-dative (MLUw 6)

Conversely, our results cannot correlate the twins' age of first production and their language development as far as the production of non-dativizable constructions is concerned. Simon starts producing non-dativizable *to*-dative utterances with an MLUw of 4, whereas Leo begins to utter non-dativizable *for*-datives with an MLUw of 6. Furthermore, this lack of correlation goes hand in hand with the type of non-dativizable structure produced the earliest. Thus, even though the syntactic status of the PP is shared in the first occurrences of non-dativizable constructions in the twins, the age of production and the MLUw differ between the participants.

Therefore, the MLUw only differs from the age of acquisition in the production of non-dativizable structures. Conversely, the first production of dativizable structures (i.e. dativizable *to*-dative DOCs) has displayed a correlation between the age of onset production and the MLUw.

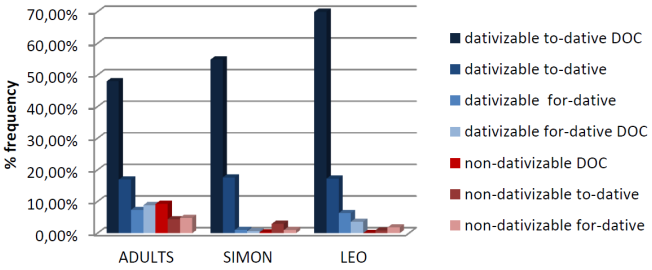


Figure 3: Children's (non)-dativizable utterances and their input.

5.3 The effect of input

The twin's input frequency could determine the twins' relative order in the production of (non)-dativizable constructions. In other words, child-directed speech could shape the children's output. A total of 1233 (non)-dativizable occurrences have been analyzed in the adults' speech as opposed to 102 and 110 (non)-dativizable utterances in Simon's and Leo's data, respectively.

As illustrated in Figure 3, the high input frequency shown in the production of dativizable *to*-dative DOCs by the adults (48.26% out of the total of (non)-dativizable utterances) is projected in the twins' high output of these structures (that is to say, 54.9% in Simon and 70.0% in Leo). Regarding Leo's production of dativizable *to*-dative DOCs, we observe that, compared to the adult input frequency, he shows a higher frequency in the production of these structures.

In turn, Simon and Leo also display a correlation between the relatively high adult input of dativizable *to*-dative constructions (i.e. 16.95%) that they receive and their relatively high output (i.e. 17.65% in Simon and 17.27% in Leo). Similarly, the low adult frequency that the twins receive regarding dativizable *for*-dative DOCs and dativizable *for*-datives goes hand in hand with the twins' low or null incidence of these structures. More specifically, 7.38% occurrences are observed in the adults' production of dativizable *for*-datives in correspondence to 1.05% and 6.36% in Simon's and Leo's output, respectively. Similarly, in correlation to the low adult input frequency of dativizable for-dative DOCs (i.e. 8.84%), Simon and Leo display 0.65% and 3.64% structures, respectively.

As far as the output of non-dativizable constructions is concerned, we can point out that low adult input frequency observed in these structures correlates with the children's low output. In fact, the low rate being observed in the adult input regarding non-dativizable *to*-datives (4.46%) and non-dativizable *for*-datives (4.87%) corresponds with the twins' low and null incidence in their output (i.e. Simon has displayed 2.94% and 0.98% occurrences of non-dativizable *to*-datives and non-dativizable *for*-datives, respectively, as opposed to Leo, who shows a rate of 0.98% occurrences for non-dativizable *to*-datives and a rate of 1.82% utterances of non-dativizable *for*-datives).

All in all, and taking into account the results obtained in Figure 3, adult input and children's output seem to be perfectly matched for this area of grammar.

6 Conclusion

This paper presents the differences that English dativizable and non-dativizable constructions display in the production of English/Spanish simultaneous bilingual children. Our results indicate that Simon and Leo show a tendency toward uttering dativizable *to*-dative DOCs at an earlier stage. In particular, Simon starts producing them at 2;03.26, two months earlier than Leo, who generates his early dativizable *to*-dative DOC at 2;05.00.

However, there are divergences in the twins' first occurrence of dativizable prepositional production in comparison with their early utterance of dativizable DOCs (both to and for dative DOCs) (see Table 1 in section 5.1.1). On the one hand, Simon starts uttering dativizable *to/for*-dative DOCs at around the age of 2, one year earlier than dativizable *to/for*-datives. Simon's findings confirm hypothesis 1 in that the later production of dativizable *to/for*-datives suggests that these structures could be transformationally derived from dativizable *to/for*-dative DOCs via a passive-like process (Aoun & Audrey Li, 1989; Snyder & Stromswold, 1997). On the other hand, Leo's production of dativizable *to/for*-datives arises earlier than dativizable *to*-dative DOCs. However, the delay in Leo's production of dativizable *for*-dative DOCs at 3;03.00 in relation to the first occurrence of dativizable *to*-dative DOCs at 2;05.00 and dativizable *to/for*-datives (at 3;01.06 and 3;02.24, respectively) illustrates contradictory results with Simon's data. In other words, Leo's results suggest that dativizable *to/for*-dative constructions are derived from dativizable *to*-dative DOCs via NP-movement, supporting hypothesis 1, as well. Nevertheless, Leo's correlation in the production of dativizable *to/for*-datives and dativizable *for*-dative DOCs at around the age of 3 infers that dativizable *for*-dative DOCs and dativizable *to/for*-datives are derived from dativizable *to*-dative DOCs via a passive-like process. Hence, due to the differences observed in the twins, further research is required to investigate the syntactic derivation of dativizable *for*-dative DOCs.

Furthermore, our findings have displayed a correlation in the first production of dativizable *to/for*-datives at the age of 3 (see Tables 1 and 2). Despite the fact that previous works have considered *to*-PPs and *for*-PPs as having an argument and an adjunct status respectively (M. C. Baker, 1997; Chomsky, 1955; Hudson, 1997; Larson, 1988, 1990), our results confirm our hypothesis 3 in that both PPs are regarded as a syntactic block of prepositional object constructions.

Taking into account the semantic status of dativizable utterances, Simon and Leo's later production of dativizable *to/for*-datives at around the age of 3 as opposed to their earlier utterance of dativizable *to*-dative DOCs at around the age of 2 confirms hypothesis 4 in that the delay in generating dativizable prepositional constructions may go hand in hand with the requirement of an additional constituent (mainly, the lexical item *to/for*) in order to meet semantic motion requirements (cause-GO). However, Leo's first occurrence of dativizable *for*-dative DOCs cannot support hypothesis 4, as opposed to Simon's data, because Leo starts uttering cause-HAVE structures headed by the preposition for (i.e., dativizable *for*-dative DOCs) at around the age of 3, showing a correlation with his early production of dativizable *to/for*-datives (or cause-GO structures). Hence, despite Leo's performance of dativizable *for*-dative DOCs, we can observe that, as shown in the results confirmed in hypothesis 1 and 4, the later production of dativizable *to/for*-datives could determine that they are syntactically derived structures from dativizable *to/for*-dative DOCs and along with their intrinsic semantic conditions.

Likewise, semantics plays a role in the first occurrence of dativizable and non-dativizable constructions that share a semantic analogy. We have observed in our results a correlation between the early production of dativizable and non-dativizable *to/for*-datives at around the age of 3, which confirms our hypothesis 5. Thus, we can claim that (non)-dativizable *to/for*-datives are considered as a semantic block, implying a cause-GO relation. On the other hand, the differences observed in the age of first production between (non)-dativizable *to/for*-dative DOCs suggests that non-dativizable DOCs cannot be treated as a semantic block as of constructions which denote a cause-HAVE relation. However, because of Leo's absence of evidence of non-dativizable DOCs in his production, it leads to inconclusive results regarding the semantic categorization of (non)-dativizable DOC structures.

Moreover, we cannot firmly assert that non-dativizable constructions are produced later than dativizable structures since the twins have displayed differences in their production. In particular, only Simon's data can confirm hypothesis 6 in that he starts uttering dativizables earlier than non-dativizables. However, Leo cannot support this hypothesis because the production of his first non-dativizable *for*-dative is found earlier than his first production of the two types of dativizable constructions, mainly, dativizable *for*-datives and dativizable *for*-dative DOCs.

Adult input plays a crucial role in the production of both dativizable and non-dativizable constructions. Thus, hypothesis 7 is confirmed. The twins' early production of dativizable *to*-dative DOCs is explained by the narrow correlation between the input found in child-directed speech and that in the twins' output. Similarly, Simon and Leo's low exposure to dativizable

to/for-datives, dativizable *for*-dative DOCs and non-dativizables reflects their low productivity in their output.

All in all, this study of the first occurrence of (non)-dativizable constructions suggests that dativizable *to*-dative DOCs are the dativizable utterances produced the earliest. They start being uttered by Simon and Leo at around the age 2 with an MLUw of 3. In turn, adult input also correlates with the variables of age of onset and language development; hence, the twins' high exposure to dativizable structures along with the twins' low input frequency of non-dativizable constructions can also explain their output.

The analysis of a broader selection of corpora (both English/Spanish bilingual and monolingual data) is, therefore, necessary so that more standing conclusions can be drawn. Moreover, constructions which are generated by the same syntactic process as dativizable constructions (e.g. passives and DOCs) need further research from the point of view of acquisition.

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Note Article

CM1106 STEMCHEM: Chemical Approaches to Targeting Drug Resistance Cancer Stem Cells

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Abstract. STEMCHEM is a COST action aiming to target causes of drug resistance in cancer stem cells. Cancer stem cells are cells which are believed to be responsible for the larger part of the regenerative capacity of cancers. They are also thought to be similar to adult stem cells in that they do not proliferate most of the time and are thus resistant to many kinds of chemotherapy. The action brings together labs around Europe in both biological and chemical fields to work together in this regard. Biologists targeting individual stem-cell related molecules as well as stem cell phenotypes (like the undifferentiated state), test chemicals from numerous labs for activity in high throughput screens, with the aim of identifying new drug targets. This COST action, like most others, offers opportunities for Malta, both in a general way and also particularly for a small country with small labs.

Keywords: Stem cells; Cancer; Differentiation; COST; drug resistance

COST (European Cooperation in Science and Technology) is one of the longest running European Scientific programmes, which allows for better coordination of nationally funded research at a European level.

Unlike the FP7/Horizon 2020 instruments which work in a top-down manner with calls being issued by the Commission as to what areas of research it is interested to fund, COST works in a bottom up or grass-roots approach. Any group of labs or interest groups from a few different countries can get together, start up a COST action, after it is vetted by the COST central administration in Brussels and then open it up to other partners to join in. This information is usually passed on the national contact point organisation (in Malta the MALTA COUNCIL FOR SCIENCE AND TECHNOLOGY), who informs interested potential participants on a regular basis of the newest set of COST actions to be set up.

For a small country like Malta, with small labs and minimal funding, where entry into the big consortia characteristic of the FP programmes has always been difficult (and I speak from experience here), COST allows the development of important scientific contacts, which may later lead to participation in such collaborations. It also allows increased scientific exposure of research done in our laboratories.

The University of Malta's Anatomy Department through a number of graduate students under Pierre Schembri-Wismayer's supervision has been working for some time on inducing differentiation of leukaemia cell lines using a variety of natural extracts, some of which have been published (Agius Anastasi, Cassar & Schembri-Wismayer, 2012) and others of which not yet due to the possibility of developing intellectual property for the University. The research uses the HL60 leukaemia cell line as an initial screen since it allows differentiation into both monocytic and granulocytic cell types.

When reviewing newer COST actions, Prof. Janet Mifsud, COST coordinator in Malta brought to our attention an action entitled "Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells" in December 2011. This seemed interesting so I, (Pierre Schembri-Wismayer) approached Prof. Mifsud about joining and was elected a member on the managerial board of the Action, representing Malta. I attended the

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kick-off meeting in Brussels, with the rest of the management committee since we got involved early on. The meetings of the management committee (which tend to be combined with workshops in this Action in order to allow more to get done at once) allow the regulation of the budget and of how the action is set to work.

Cancer stem cells (CSC) are a subpopulation of cells within tumours that exhibit enhanced tumor-initiating attributes and are a major contributing factor to current cancer therapy failure. The CSC phenotypic state comprises distinct molecular and functional differences that underpin resistance to current treatments and the unique ability spread and to seed new tumors throughout the body. This insight of this particular subpopulation of cells and its capability of repopulating tumours where most cells have been killed by conventional therapy, necessitates an entirely new approach to cancer drug development. This action aims to unite expertise in rational drug design and medicinal chemistry with biomedical investigators dedicated to understanding the mechanisms governing drug resistance in cancer stem cells. Thus it aims to develop effective methods for identifying novel compounds and drug candidates that target these drug-resistant cancer cells. One such way in fact would be to cause these cancer stem cells to differentiate into less stem-like cancer cells so that they can then be killed by more standard chemotherapy. The rationale and basis behind the action are reviewed in more detail in a recent publication from the consortium (Sotiropoulou, Christodoulou, Silvani, Herold-Mende & Passarella, 2014).

During the first meeting in Brussels we also chose a number of important positions such as the Chair (Prof. Daniele Passarella, a chemist from University of Milan, Italy who had initiated the action) and Vice Chair (Prof. Marija Balic, a medical doctor and biologist from Austria) as well as a number of important positions relating to specific instruments of the actions, (such as Dr Gabriela Almeida from Portugal who is in charge of short term scientific missions - STSMs - more about these later).

It was decided for example that members were divided into three working groups, one of chemists, one of biologists and one of pharmaceutical and medicinal chemists, including specialists in computational prediction of drug-target interactions. Our lab joined working group one, primarily for biologists. However, even within this group, there were numerous variations, which enriched the meetings since attending the various workshops not only allowed sharing of our own expertise but learning from others.

Amongst the biological experts were clinicians developing novel treatments for brain cancers, biologists developing *in vitro* models for various cancers, scientists developing *in vitro* systems of testing different well known stem-cell related candidate targets like Notch and Hedgehog, those screening for epigenetic modifiers of the stem cell phenotype and others like ourselves involved in phenotypic screening.

Chemists also hailed from different branches, including synthetic chemistry, producing steroids, retinoids and other potential drugs, pharmaceutical chemistry, *in silico* screening and development of chemical libraries.

These three groups worked within themselves, each setting up different workshops at the different group meetings. They also set up collaborative activities, such as the development of a chemical database of different kinds of agents for testing, from the chemists. However, it later became clear that the best work and publications would result from the development of cross-speciality collaborations where biologists tested new chemicals in their various test systems. In fact the three work-group system whilst still a functional grouping, became less significant as time went by. Numerous collaborations have been developed over the period of the COST action (although it is still ongoing) resulting in various publications (Madeira et al., 2014; Christodoulou et al., 2013; Majchrzak et al., 2013; Porcile et al., 2014).

Once the action was established, the first workshop was held in Milan in July 2012 and two members from our research group, Dr Pierre Schembri-Wismayer and Dr Krystle Blaire Theuma attended. The meeting was very well organised and showed many different approaches to tackling stem cells in Cancer, including both the targeting of specific stem cell-related molecules using chemical approaches and the wider search of natural or synthesised products for anti-stem cell activity using a particular biological model system.

One of the main points about such meetings is that apart from the consortium members themselves contributing, external speakers (usually experts in the field who can contribute a new view point or angle to ongoing research) are invited (and funded) to attend the different meetings.

We presented some research work, using natural extracts for leukaemia differentiation. An oral presentation presented by PSW was entitled "Insect cell-extract induces increased expression of differentiation markers in HL60 leukaemia cells" whilst Dr Theuma (also a managerial committee member in the STEMCHEM consortium) presented a poster entitled "Combination of DNA modifying agents and differentiation inducers can enhance differentiation in HL60 leukaemia cells", which was work she had done in collaboration with Ms Analisse Cassar (Fig. 1).

The advantage of this was that since we were working using phenotypic screening (looking for a biological effect which could lead to candidate compounds for drug development and since we (mostly A. Cassar) has de-

Figure 1: Dr Schembri-Wismayer and Dr Theuma presenting their poster and discussing with Prof. Navakauskiene.

veloped this phenotypic screen into a relatively high throughput system, many chemists in particular, were interested in talking to us to assess their compounds. The fact that the group's area of expertise is also the less commonly targeted mechanism of differentiation therapy rather than cytotoxicity also increased interest.

Basically, we spectrophotometrically assess a leukocyte differentiation-related enzymatic activity (NBTZ). This is then divided by a mitochondrial activity which acts as a surrogate for cell number giving us and indication of the average differentiation marker per cell (Fig. 2).

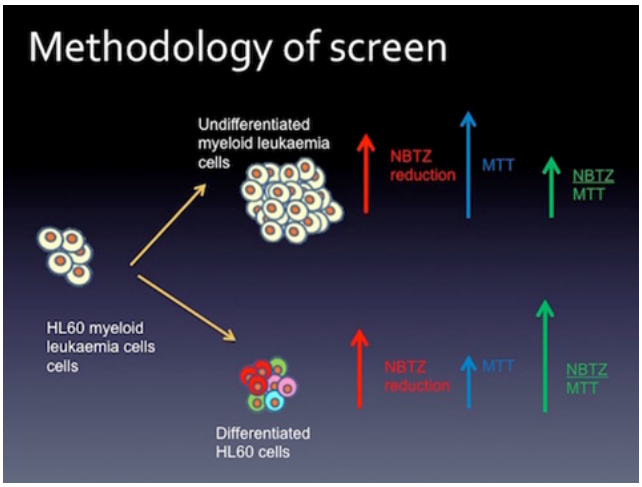

Figure 2: Schematic showing rationale behind screening tests.

At a second workshop meeting in Porto in February 2013, Analisse Cassar, another graduate student from the same lab presented her paper "Effects of Insect Conditioned Medium in Combination with Chromatin-Modifying Agents on the Terminal Differentiation of Leukaemia". She was given a lot of good feedback and I (PSW) received glowing reports of her very good presentation of very interesting work from other very authoritative STEMCHEM colleagues.

Analisse presented this work in the same session as a colleague form Lithuania, Ruta Navakauskiene from Vilnius University, who presented complimentary and similar work entitled "Effects of HDACI, HMTI and HMTI in combination with retinoic acid on granulocytic differentiation of human promyelocytic leukemia cells". Our lab and that of Prof. Navakauskiene have often found that we are working on similar areas so sharing our expertise and skills in STEMCHEM has been useful in turning us from primarily competitors to collaborators.

In fact, another post-graduate student in the lab, Mr Sherif Suleiman, supervised by Dr Jean Calleja Agius, the present head of the Anatomy department will hopefully be benefiting form a short term scientific mission to Prof. Navakauskiene's labs in Lithuania, where he will be learning how to test for different chromatin-modifying agents using molecular biology techniques not presently in common use in our own labs. We on the other hand have benefited from colleagues in Europe visiting our lab, from Greece (officially funded as an STSM) , Ireland and Serbia, through the STEMCHEM consortium.

This is in fact the aim of a short terms scientific mission. This is funded as part of the action and involved up to €300 for travel and €60–€90 as a daily allowance up to a maximum of €2500 for a period up to 3 months and in the case of early stage researchers (within 8 years of a PhD), even up to €3500 for longer periods.

I should at this point make a little note as to the various benefits of COST actions for our little nation. Many countries have two management committee members from two different labs. In our consortium, the management committee members are always funded in whatever conference or visit is organised, except when specific meetings for young scientists for example are organized when there is no management committee meeting . When possible, the Action also funds 1-2 participants from each other member (i.e. each lab or university involved in the action, since of course these may involve numerous labs from the same country). Since it is uncommon that too many researchers are involved in a particular area, in Malta and since one is allowed to have alternative management committee members, should one be able to attend, then it allows good funding locally for research staff, and even students to have the opportunity to present their work on a much wider stage.

In fact following the different talks by the group, collaborations initially started as contacts from individual labs who asked us to send certain chemicals for testing.

One of the earliest of these was Dr Danijel Kikelj who sent 12 chemicals for differentiation testing. Later Nadine Martinet (initially an invited speaker from INSERM in Nice in France and later a member of the consortium) asked all chemistry labs to contribute towards a large central chemical database which she administered and this was made available to all the various biology labs. As a result, we ended up screening over 600 chemicals (and still have more to work on) from numerous labs around Europe. Due to the large number of chemicals needing work-up and due to the limited hands on deck in terms of graduate students, more than 30 undergraduate students participated in this research, also offering undergraduate students (some of which are shown in Fig. 4) an opportunity to understand the research component of the University of Malta and contribute to international science. More than 80 very interesting hits (which cause leukaemia differentiation to different degrees) have been identified and many of these are being followed up.

The first 400 or so chemicals screened were presented in a fourth meeting in Budapest in March 2014 (no one from Malta attended the third meeting in Warsaw) and again this garnered a lot of attention with various labs interested in continuing collaborations. Some of the chemicals presented are indicated in Fig. 3 where different novel synthesised chemicals (named only by their catalogue number) are shown to be active in the screening method developed in our lab, again compared to positive and negative controls. The more active of these chemicals are then assessed by morphology – i.e. the effects of differentiation are assessed visually. Should this also be interesting, further testing can be done by means of flow cytometry (Fig. 5), locally to confirm the expression of differentiated chemistry markers. In fact this has resulted in students and post-docs from labs in Ireland and Serbia visiting our labs and more should be coming in the near future, from Greece and possibly elsewhere.

Figure 4: The students from the preclinical medical years who performed the bulk of the work in testing the first 400 chemicals on the STEMCHEM database.

Some of the chemicals from these labs have already shown activity in 2 or more assays and will be followed up for publication. The 80 or so hits from the first four hundred screen and all the new hits from the last 200 chemicals will also be assessed with morphology and flow cytometry and followed up accordingly with the

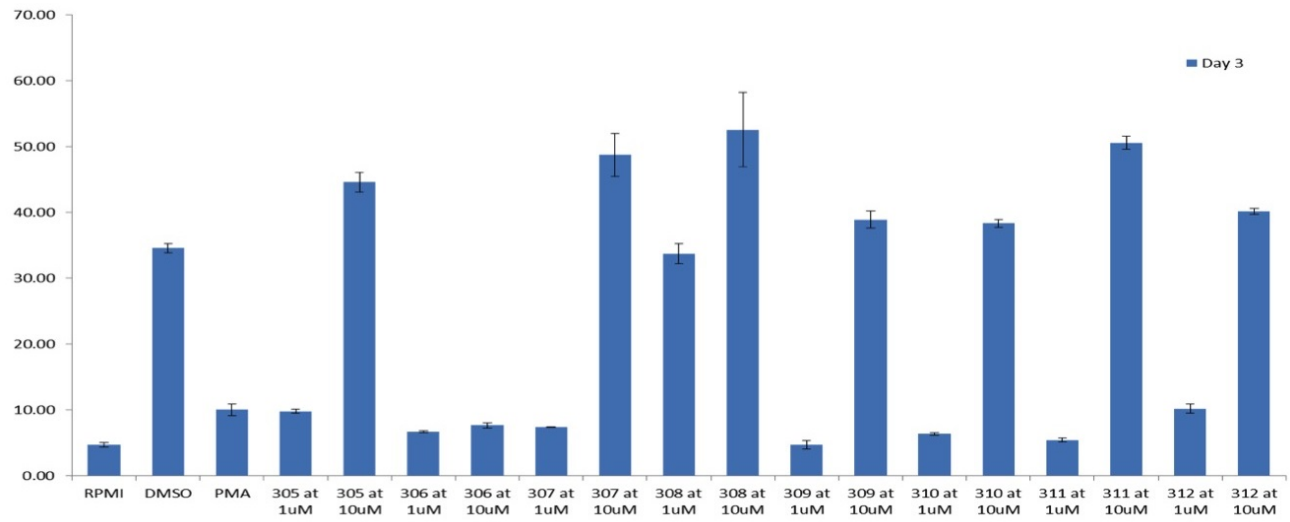

Figure 3: Some results out of the first 400 chemicals in the STEMCHEM chemical library, tested in the summer of 2013. Results show an index of differentiation created by dividing a leukocyte differentiation marker (NBTZ reduction function) by an indicator of cellular activity and thus of cell number (MTT activity). Results are an average of 3 replicates at each of two concentrations 1 and 10 mM. The first three columns show the results of negative control (normal culture medium) as well as two positive controls which however cannot be used in the clinic due to toxicity, PMA and DMSO.

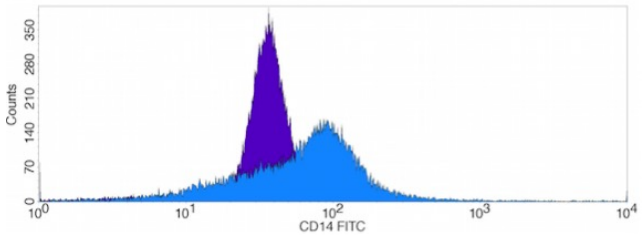

Figure 5: Changes in the expression of CD14 marker (monocytic differentiation) between untreated (violet) and treated (blue) HL60 populations.

chemists who produced them.

The common way that this is done is that the chemists make similar molecules to the hits and then these are once again screened to see which variations are even more active. The most active of these agents can then be tested on numerous leukaemia cell lines (we usually test 4 rather different myeloid leukaemias initially) and then can even be tested on primary leukaemia blasts from patients to see if the effect is also possible on patient cancers rather than the more artificial cell lines. All this information enhances the possibility of developing effective drugs for inducing differentiation, a rather novel kind of therapy for cancers, started a number of years ago with all-trans retinoic acid, which converted the normally fatal Acute promyelocytic leukaemia into a manageable disease where more than 90% of patients are cured nowadays.

Expanding this interesting branch of cancer therapy is one of the main interests of our lab at the Anatomy department and STEMCHEM has given us the capability to open up our expertise to collaborations all across Europe and to many more possible candidate drugs, apart from our own in-house derived extracts.

Another development in our own lab is the indication that many of these chemicals may also work on other solid tumours apart from leukaemias, especially the serious solid tumours of childhood. There is already evidence that all trans retinoic acid works to some extent on brain and bone tumours (Choschzick et al., 2014; Yang et al., 2012) but so do some of the extracts and drugs we are testing. In fact our preliminary work on osteosarcomas (bone tumours) was presented by another graduate student, Mr Sherif Suleiman (Fig. 6) at the last meeting of the Action in October in Tenerife where a good group of Action members enjoyed a meeting at one of Europe's more exotic destinations (Fig. 7).

Overall, COST actions offer extra funding for cash-strapped local research groups to travel, and train, and more importantly the opportunity to share data, discuss in a wider pan-European forum and set up collaborations which allow one to access larger sources of funding such as Horizon 2020 or Innovative Medicines

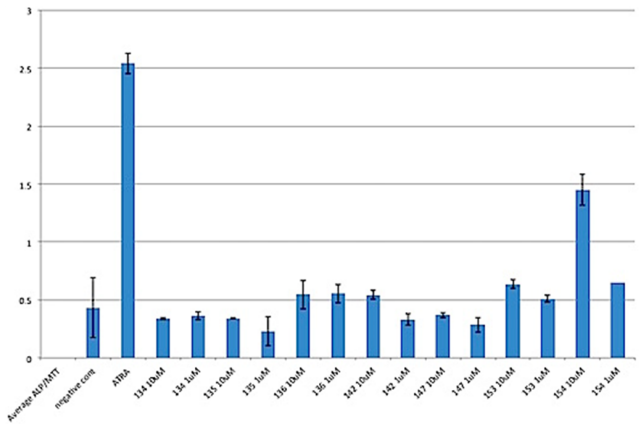

Figure 6: Changes in Alkaline phosphatase expression divided by indicator of total cell number assessing differentiation related changes in osteosarcoma.

Figure 7: The poster of the last action workshop meeting in Tenerife.

Initiative 2 funding. Importantly for our small country, they also provide an opportunity for students to travel and present their work. This was my first experience in COST but it will definitely not be my last.

Acknowledgments

The Authors would like to acknowledge the numerous laboratories who provided chemicals for testing to the STEMCHEM database and also to many other students (apart from those pictured), both Maltese and international who contributed to the screening of all of the chemicals.

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Review Article

Supramolecular Chemistry in Water: Self-Assembly of Multi-Component Fluorescent Molecular Logic Gates in Micelles

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Abstract. A recent strategy for developing supramolecular logic gates in water is based on combinations of molecules via self-assembly with surfactants, which eliminates the need for time-consuming synthesis. The self-assembly of surfactants and lumophores and receptors can result in interesting properties providing cooperative effects useful for molecular information processing and other potential applications such as drug delivery systems. This article highlights some of the recent advancements in supramolecular information processing using microheterogeneous media including micelles in aqueous solution.

Keywords: supramolecular chemistry in water, molecular recognition, chemosensors, micelles, molecular logic gates, biomedical diagnostics

1 Introduction

The objective of COST Action CM1005 is the development of supramolecular systems that work in water (Oshovsky, Reinhoudt & Verboom, 2007; Zayed, Nouvel, Rauwald & Scherman, 2010, http://supracheminwater.wordpress.com/). The COST Action is divided into three working groups (i) the molecular recognition of biologically and environmentally relevant species in water (ii) the selective control of reactions in water, and (iii) the self-assembly of organized structures in water that are stimuli responsive and can be used for programming functions in materials and devices. The Action aims to improve the understanding of multiple non-covalent weak bonds (hydrogen bonding, electrostatics, Van der Waals forces, pi-pi interactions etc.) that are collectively powerful interactions for selective recognition of chemical analytes and processes in water.

The majority of molecular receptors for recognition of physiologically important cations, anions and neutral analytes are not readily soluble in water (Magri & Mallia, 2013; Schneider, 2013). One working group within the COST Action is designing and synthesising novel intelligent molecules readily soluble in water, which is not always an easy task even for skilled organic chemists (Magri, 2012). A simple way to circumventing the issue of poor solubility of receptors in water is to incorporate them in micelles to form water-soluble nanoscale supramolecular devices (Pallavicini, Diaz-Fernandez & Pasotti, 2009). Micelles result from the spontaneous association of surfactants to form dynamic spherical conglomerates above the critical micelle concentration (cmc), and other shaped assemblies at higher concentrations, which are representative biomimetic models of biological membranes (Turro, Grätzel & Braun, 1980). In the case of ionic micelles, the micelle interface has an electrical double layer and a potential difference on the order of several hundred millivolts. The electric field can modulate the sensitivity of ion determination due to an amplifying effect on the local ion concentration, Moreover the receptor-micelle nanodevices often show enhanced binding properties as will be discussed.

This article highlights examples of supramolecular multicomponent systems with stimuli-responsive properties that perform molecular computation-based logic (de Silva, 2013; Szaciłowski, 2008). The examples illustrated are presented according to increasing complexity of the logic system (de Silva & Uchiyama, 2007). A common theme throughout is the use of micelle media, which introduces synergistic effects. Examples are included representative of fluorescent sensing devices for various kinds of chemical species as inputs including protons, cations and anions. Readers with a desire for background literature on fluorescent probes can view the cited references (Bissell, Bryan, de Silva & McCoy, 1994; Callan, De Silva & Magri, 2005; de Silva et al., 1997; Valeur & Berberan-Santos, 2012).

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2 Single-input Logic Gates

There are four possible single-input logic gates: PASS 0, PASS 1, YES and NOT. PASS 0 is the simplest of Boolean logic operations and appears trivial. Any molecule that is non-fluorescent remains so independent of the absence or presence of an input. PASS 1 is another trivial logic gate exemplified by a fluorophore that emits fluorescence on excitation independent of the absence or presence of an input. The design of 'fluorophore–spacer–receptor' and 'fluorophore–receptor' molecules allows for YES and NOT logic to be demonstrated. The standard molecular YES logic gate is based on the competition between photoinduced electron transfer (PET) and fluorescence yielding an *off-on* switching action of the fluorescence intensity ideally with no change in the wavelength (de Silva et al. 2009). A NOT gate, also referred to an inverter operates by *on-off* switching.

Akashi's team demonstrated a viable way for detecting barium by using an ether crown-based fluorophore **1** in aqueous solution (Nakahara, Kida, Nakatsuji & Akashi, 2004). The pyrene-functionalised monoaza-18-crown-6 ether derivative is a 'fluorophore-spacer-receptor' system with poor water solubility and Ba^{2+} binding properties in water. Addition of the non-charged detergent Triton X-100 above the cmc allows the chemosensor to position itself in the less polar micellar location, yielding a supramolecular assembly which results in binding of Ba^{2+} by the cryptand. The amino nitrogen atom is involved in the complexation of Ba^{2+}, which cancels the PET from the tertiary amine to the pyrene fluorophore with a high fluorescence output. Although the experiment is conducted at pH 10 due to the sensitivity of **1**, the strategy exemplifies a selective way of detecting barium by YES logic.

Bhattacharya and Gulyani are perhaps the first to develop the concept of multifunctional hydrophobic probe design (Bhattacharya & Gulyani, 2003). The method was demonstrated by detecting for Zn^{2+} in micelles and vesicles with 1-pyrenyl-methyl-*bis*(2-picolyl) amine **2**. In water the chemosensor aggregates as observed by an excimer emission about 500 nm. In micelles, however, aggregation of the probe molecule **2** is prevented such that no excimer emission is observed, while the monomer emission in the presence of Zn^{2+} at 400 nm is substantially enhanced. Large fluorescence enhancements were observed in polyoxyethylene (20) sorbitol monolaurate (Tween 20) micelles and in dipalmitoyl phosphatidylcholine vesicles.

A salophen-UO_2 complex has been demonstrated to exhibit a remarkable increase in binding of fluoride in CTAB micelles (Cametti, Dalla Cort & Bartik, 2008). In water alone, the salophen-UO_2 complex is not soluble. UV-visible titration studies of **3** in 50 mM CTAB

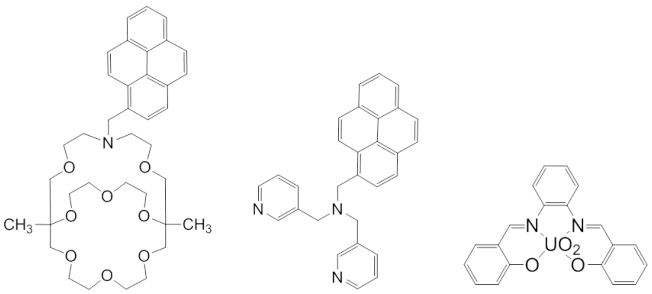


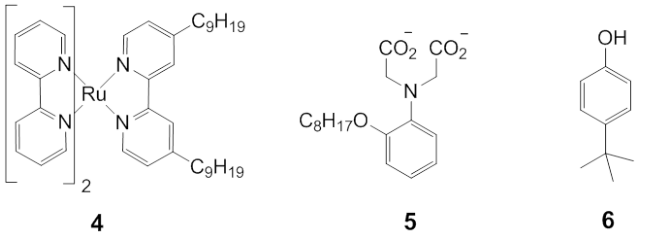
Figure 1: Chemosensors for detection Ba^{2+} **1** and Zn^{2+} **2** and F^- **3** with the assistance of micelles.

were consistent with a 1:1 binding isotherm with fluoride with a binding constant of 10 800 M^{-1}. Sulfate, acetate and phosphate also bind, but one to two orders of magnitude lower. An understanding of the spatial orientation of the salophen-UO_2 receptor **3** in the micelle was determined by NMR paramagnetic relaxation enhancement (PRE) and Nuclear Overhauser Effect (NOE) measurements (Keymeulen, De Bernardin, Dalla Cort & Bartik, 2013). It was discovered that **3** preferentially locates near the micelle interface orientated with the oxygen-linked aromatic rings facing the bulk aqueous solution and the nitrogen-linked phenyl ring backed into the hydrophobic core. The techniques presented by the collaboration of Dalla Cort and Bartik bring a fresh perspective with respect to shedding light on the location and spatial orientation of probes in miceller media. PRE and NOE experiments could be used to complement fluorescent mapping studies near micellar membranes (Bissell et al., 1994; Uchiyama, Iwai & de Silva, 2008).

By self-assembly of a lumophore and receptors with micelles, logic gates can be constructed in a 'plug and play' fashion (de Silva, Dobbin, Vance & Wannalerse, 2009). Triton X-100 is used to solubilize a hydrophobic tris(2,2'-bipyridyl)Ru(II) complex **4**, a lumophore with both a long excitation state lifetime of 200 ns and a long emission wavelength about 625 nm. The elemental PASS 0 and PASS 1 logic gates were mentioned as the micelle alone and the micelle containing **4**. YES logic is demonstrated using a 2-nitrophenyl-*n*-octyl ether receptor **5**, which is emissive on protonation of the aromatic amine at pH 2. Ligand **5** also binds Ca^{2+} at pH of 8 with YES logic behavior by a five-fold emission enhancement. This approach of using separate components for the lumophore and receptors allows for the configuration of new modules enabling new functions in the supramolecular ensemble. To reiterate, the microheterogeneous media is an essential component for enhanced luminescence to be observed.

Table 1: General truth tables for seven two-input logic gates.

$Input_1$	$Input_2$	AND	NAND	OR	NOR	XOR	XNOR	INH
0	0	0	1	0	1	0	1	0
0	1	0	1	1	0	1	0	0
1	0	0	1	1	0	1	0	1
1	1	1	0	1	0	0	1	0



4 **5** **6**

Figure 2: Components **4**–**6** for a supramolecular 'plug and play' logic device.

3 Two-Input Logic Gates

Double input logic gates were also demonstrated in Triton X-100 surfactant with **4** and **5** (de Silva et al., 2009). For referral, Table 1 summarises the two-input logic for seven types of logic gates. When both H^+ and Ca^{2+} are present as inputs, the assembly is an OR logic gate as the presence of either input or both provides a fluorescence output. AND logic is observed when *para-tert*-butylphenol **6** is added as a new module to the assembly. In basic solution, **6** is deprotonated to the negatively charged phenolate, which acts as an electron donor to the excited ruthenium complex rendering the luminescence *off* at pH 12. However, on decreasing the pH to 8, a six-fold luminescence improvement is observed at 625 nm.

A stimuli-responsive polymeric micelle was demonstrated by the Wang group (Wei, Guo & Wang, 2011) as a novel strategy for developing an intelligent drug delivery system (Alvarez-Lorenzo, Bromberg & Concheiro, 2009). Certain tumor cells are known to have characteristically high reductive environments and high proton concentrations. The Wang team developed polymeric crosslinked micelles with Andriamycin **7** conjugated to the micelles. The pH and reduction conditions are the key stimuli-based parameters for an AND logic result. The drug is initially doubly trapped in the micellar system by hydrazone and disulfide bonds. Drug release is achieved at pH 4 and in the presence of 15 mM of the redox agent, dithiothreitol. Addition of acid cleaves the hydrazone bonds while dithiothreitol cleaves the disulfide bonds. When both input chemicals are present, **6** is severed from the polymers, which disperse as smaller fragments. Liberation of the drug by both stimuli allows for selective release of the drug at the target tumor cells.

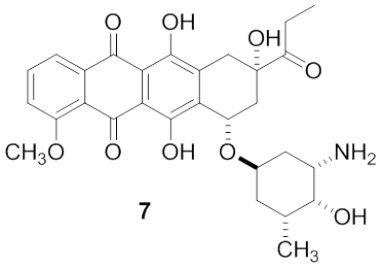


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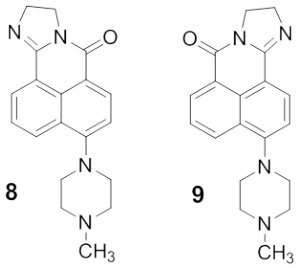
Figure 3: The molecular structure of the anti-cancer drug Adriamycin **7**.



8 **9**

Figure 4: The molecular structures of the naphthalimide regioisomers **8** and **9**.

The team of Qian demonstrates up to ten logic functions with the reconfigurable molecules **8** and **9** in water, and extends the use of sodium dodecyl sulphate (SDS) surfactant as an additional input using both absorbance and fluorescence outputs (Qian, Qian, Xu & Zhang, 2008). Six two-input gates are configurable for AND, NAND, OR, NOR, XNOR, INHIBIT logic in additional to the four one-input gates. The versatility of these naphthalimide-based molecules for logic applications is due to the two accessible sites of protonation according to a 'receptor$_1$-fluorophore-spacer-receptor$_2$' design (Zammit, Pappova, Zammit, Gabarretta & Magri, 2015). Though regioisomers, the fluorescence quantum yields of **8** and **9** are significantly different at 0.218 and 0.055 in water; however, implemented as logic devices the characteristics are similar. Addition of anionic SDS (low 0, high 8.2 mM) and hydroxide (pOH of 7 and 4) provides INH and XOR using the absorbance

at 425 nm and negative logic convention for the fluorescence output to form the basis of a half-subtractor. Dual protonation of both compounds provides pathways that change the absorbance and emission spectra, as well as the interaction of SDS below and above the cmc.

The theme of naphthalimides and SDS surfactant and protons is continued with the addition of using the inorganic salt Na_2SO_4 as an alternative input (Qian et al., 2008). With inputs SDS and Na_2SO_4, OR and AND logic are exhibited for **10** and **11**, respectively. XOR and INH logic gates can also be interpreted from the output **11** due to the additional PET pathway from the tertiary amine. Exploiting both the PET and ICT push-pull channels, the authors share their interpretation of a half-adder and half-subtractor functions (Pischel, 2007).

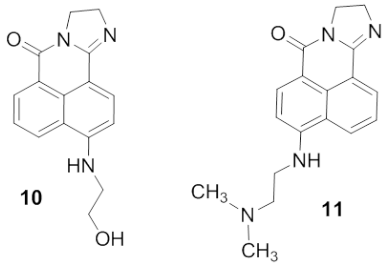


Figure 5: Examples of a two-input OR gate **10** and AND gate **11** with SDS and Na_2SO_4 as inputs.

The team of Uchiyama and de Silva demonstrated the first example of dual-input molecular computation within a small defined nanospace of 3 nm volume (Uchiyama, McClean, Iwai & de Silva, 2005). A lipophilic molecule **12** consisting of a benzo-5-crown-15 ether, an anthracene fluorophore, a tertiary amine and an octyl hydrocarbon chain was used as a probe of the micelle environments. The molecular device contains two classic electron donors used in PET systems. Self-assembly of the molecular probe in cationic and neutral micelles of cetyltrimethylammonium chloride (CTAC), octyl β-D glucopyranoside and Triton X-100 yielded no observable fluorescence response. However, in tetramethylammonium dodecyl sulfate (TMADS) micelles the molecular probe exhibits a ten-fold fluorescent enhancement in the presence of H^+ and Na^+ at elevated concentrations. The reason is the binding constant of benzo-5-crown-15 ether, at only $\log K$ of -0.3 in water, increases by two order of magnitudes ($\log K = 1.9$) due to the local concentration of Na^+ at the micelle interface. At pH 3 and 0.4 M sodium ions, the H^+ and Na^+ input concentrations are high resulting in a substantial fluorescent output due to the sodium ions binding to the benzo-5-crown ether and the protons to the tertiary amine, which in both cases, prevents PET to the anthracene fluorophore reminiscent to a AND logic gate. The strategy illustrates the ability to sense within a nanometer radius (a dimension where silicon-based electronic devices cannot approach) and opens up the possibility of molecular computation in other microheterogeneous (i.e. liposomes and vesicles) and biological systems.

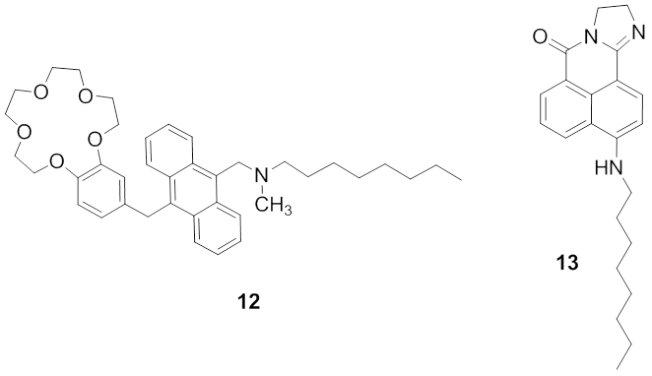


Figure 6: Hydrophobic molecular probes **12** and **13** for information retrieval in nanospaces.

4 Three-input Logic Gates

The first reported example of the potential cross-fertilization between Boolean algebra and biomedical sensing was reported for a three-input AND 'lab-on-a-molecule' based on a competition between PET and fluorescence (Magri, Brown, McClean & de Silva, 2006). In this instance, three receptors are incorporated within a single molecule: a benzo-15-crown-5 ether for Na^+, a tertiary amine for H^+, and a phenyliminodiacetate for Zn^{2+}. The modular covalent arrangement of the receptors, spacers and fluorophore facilitates the cooperative sensing algorithm (Magri & de Silva, 2010). Consideration of **12** with the micelle as one of the inputs illustrates a supramolecular system as an example of a 3-input AND logic gate with the inputs Na^+, pH and TMADS.

Three-input IMPLICATION logic is demonstrated using a naphthalimide probe with an octyl hydrocarbon chain **13** (Qian, Xu, Zhang & Qian, 2011). This type of logic is similar to an IF-THEN operation. However, in this example the fluorescence is modulated by the inputs SDS, CTAC and temperature. The molecule is fluorescent in water with a quantum yield of 0.135. Addition of SDS just below the cmc concentration $\sim$ 8.0 mM quenches the fluorescence. Subsequent addition of 100 µM cetyltrimethylammonium bromide (CTAB) causes a 25-fold fluorescence enhancement. The rational for these observations is that the opposite charges of SDS and CTAB in addition to the hydrophobic alkyl chains common to both surfactants results in aggregation between the two micelles, and consequently, liberation of the fluorescence probe. An increase in temperature enhances the fluorescence by making the probe molecule more soluble. The net outcome is that in the

Table 2: Truth table for the supramolecular three-input AND logic gate **12**.

$Input_1$	$Input_2$	$Input_3$	Output
Na^+ [a]	H^+ [b]	TMADS [c]	Fluorescence
0 (low)	0 (low)	0 (low)	0 (low)
0 (low)	0 (high)	1 (low)	0 (low)
0 (low)	1 (low)	0 (high)	0 (low)
0 (low)	1 (high)	1 (high)	0 (low)
1 (high)	0 (low)	0 (low)	0 (low)
1 (high)	0 (low)	1 (high)	0 (low)
1 (high)	1 (high)	0 (low)	0 (low)
1 (high)	1 (high)	1 (high)	1 (high)

[a]High input level of 0.4 M of NaCl. Low input level maintained with no added NaCl. [b]High input level 10^{-3} M acid. Low input level 10^{-11} M acid. [c]High input level of 20 mM TMADS. Low input level no TMADS.

Table 3: Truth table for the supramolecular three-input IMPLICATION logic gate **13**.

$Input_1$	$Input_2$	$Input_3$	Output
SDS [a]	CTAB [b]	T [c]	Fluorescence
0 (low)	0 (low)	0 (low)	1 (high)
0 (low)	0 (high)	1 (low)	1 (high)
0 (low)	1 (low)	0 (high)	1 (high)
0 (low)	1 (high)	1 (high)	1 (high)
1 (high)	0 (low)	0 (low)	0 (low)
1 (high)	0 (low)	1 (high)	1 (high)
1 (high)	1 (high)	0 (low)	1 (high)
1 (high)	1 (high)	1 (high)	1 (high)

[a]High input level of 10 µM of SDS. Low input level maintained with no added SDS. [b]High input level 20 µM CTAB. Low input level with no CTAB added. [c]High input level at 75 °C and low input level at 25 °C.

presence of SDS alone, the fluorescence (and absorbance) is low, while in the other seven combination of SDS, CTAB and temperature, the fluorescence in high (Table 3). We recently reported a colorimetric and fluorimetric inverted enabled OR logic array with CTAC, Triton X-100 and hydroxide as inputs using a rhodamine B probe derivatised with a hexane chain (Caruana, Camilleri Fava & Magri, 2015).

5 Multi-level Logic

In the previous section supramolecular systems were illustrated that detect for three input conditions and with only two output results of either a low fluorescence or a high fluorescence. Now we demonstrate systems with three output levels. Traditionally, the design principle for these systems is based on 'fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$' and 'receptor$_1$-spacer$_2$-fluorophore-spacer$_2$-receptor$_2$' strategies to develop *off-on-off* ternary systems (de Silva, Gunaratne & McCoy, 1996). At a low input level the switch is *off*, at a medium input level the switch is *on*, and at a high input level the switch is *off* again (Pais et al., 2013). The regulation of analytes in living things is governed by ternary logic. Too little or too much of analyte results in illness, and in extreme situation even death. Thus, good health requires the right balance of each analyte within a specific concentration range (Burtis & Ashwood, 2001).

Off-on-off systems were first demonstrated with **14** based on a fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$' design consisting of an anthracene fluorophore, a tertiary amine and pyridine as the receptors (de Silva et al., 1996). Pallavicini also demonstrates an easy-to-assemble approach with no synthetic effort in a 'plug and plug' fashion (Pallavicini et al., 2009). In the analogous supramolecular version, the fluorophore is pyrene **15** and the two receptors are the lipophilic bases *N*,*N*-dimethyl-*N*-dodecylamine **16** and 2-dodecylpyridine **17**. Assembled in Triton X-100 as the surfactant, and anionic SDS as the co-surfactant (at various concentrations), the multicomponent system is a tuneable *off-on-off* micellar sensor device with the capability of shifting the *on* window along the pH axis with the curve apex ranging between pH 5 to 10. In another *off-on-off* example from the Pallavicini group, the polyaspartamide based co-polymer, PHEA-PEG$_{5000}$C$_{16}$ is used as the surfactant and SDS and CTAC as the co-surfactants (Diaz-Fernandez et al., 2010).

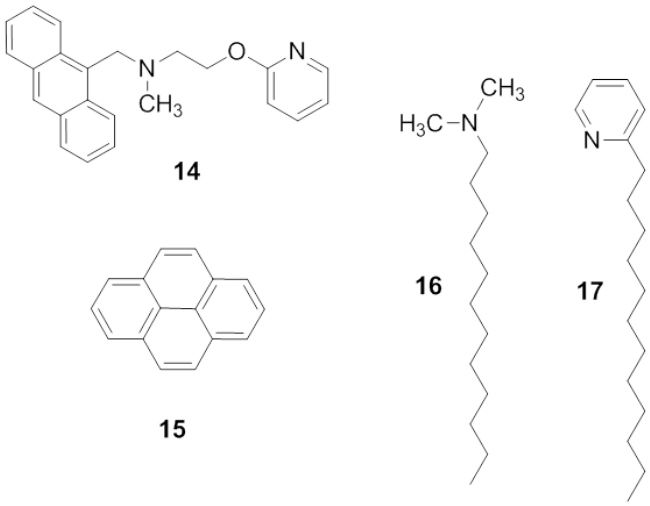


Figure 7: An *off-on-off* molecular device **14** and the components of a supramolecular device consisting of pyrene **15**, *N*,*N*-dimethyl-*N*-dodecylamine **16** and 2-dodecylpyridine **17**.

Das reported pH dependent fluorescence switching of salicylideneaniline in micelles according to *on-off*, *off-on*, and *off-on-off*. pH profiles (Das & Dutta, 2014). Salicylideneaniline **18** behaves as an *off-on* switch in 1:1 acetonitrile/H_2O and 3% negatively charged SDS aqueous solution. At pH 6 or lower, the fluorescence is *off* while at pH 10 the fluorescent is *on*. However, in CTAB and Triton X-100 ternary *off-on-off* behaviour is admirably observed with the fluorescence turning *off* pH 10. In CTAB a distinct *on* pH window is observed between pH 7–11. The differing chemistry is attributed to the equilibrium between the keto (fluorescent) form **18** and the enol form (non-fluorescent) **19** by acid and base catalysis.

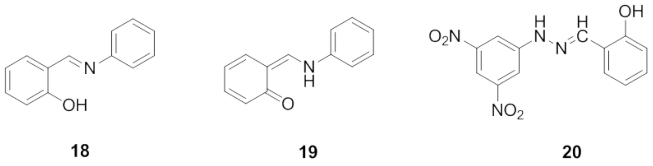


Figure 8: Examples of hydrazones **18** and **20** with *off-on-off* behaviour in micelles.

In another recent study, Goswami and Das also report the dinitrophenolhydrazone derivative **20** in 1:1 CH_3OH:H_2O, SDS, CTAB and Triton X-100 (Goswami & Das, 2011). In 1:1 CH_3OH:H_2O, a broad pH window from 5 to 12 is observed with *off-on-off* behaviour, while with CTAB a much narrow pH window is observed between 4 and 7. In SDS a *low-medium-high* response is observed on increasing pH. The sites of protonation are thought to be the phenol and the secondary amine. At pH 12 fluorescence quenching results from the phenolate to the 2,4-dinitrophenyl moiety. Below pH 6, fluorescence quenching is postulated to result from protonation of the dinitroanilic nitrogen, which lowers the oxidation further allowing for excited electron transfer from the phenol.

Pallavicini have both independently demonstrated *on-off-on* pH window sensing molecular devices (Denat, Diaz-Fernandez, Pasotti, Sok & Pallavicini, 2010). A multicomponent approach consisting of Coumarin 343 **21**, Cu^{2+} ions and *N*-dodecylated trimethylcyclen **22** are self-assembled in Triton X-100 micelles. At low pH the two organic components do not interact, and the fluorescence from 21 is high. At intermediate pH, 21 is deprotonated and coordinates to Cu^{2+} ions resulting in fluorescence quenching. At high pH, the carboxy end of **21** is displaced from Cu^{2+} by the formation of complex with hydroxide reviving the fluorescence. In both examples, the *off* window is between pH 6 and 8, which is the physiological pH sweet spot of 6.8 to 7.4. The lipophilicity of nonsteroidal anti-inflammatory drugs (NSAIDs) is also measurable by expressing an *off-on* fluorescent signal correlating the fluorescence increase with the logarithmic water/octanol partition coefficient ($\log P$).

The emissive and absorptive properties of **13** and other related members of the naphthalimide-based fluorescence sensors were investigated as chromogenic and fluorogenic sensors for anionic surfactants (Qian, Qian & Xu, 2009). The probe **13** is an *on-off-on* fluorescence sensor for SDS. Interpretation of the spectroscopic output provided for multiple output readouts at 430 nm by UV-visible absorption and 525 nm by fluorescence spectroscopy with SDS, CTAB and Triton X-100 allowing for a sensor array, which also discriminates SDS at different concentration ranges. The octyl hydrocarbon chain was found to be an important parameter as other model probes with butyl and dodeceyl hydrocarbon chains exhibited inferior emission switching properties.

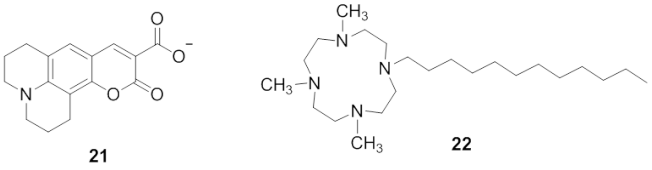


Figure 9: The components Coumarin 343 **21** and *N*-dodecylated trimethylcyclen **22** as part of an *on-off-on* supramolecular nanodevice for gauging drug lipophilicity.

6 Conclusion

The self-assembly of surfactants provides an alternative strategy for information processing applications at the molecular level. Supramolecular systems can be designed that respond to various chemical inputs such as cations, anions, pH as well as physiochemical parameters. Advantageously, supramolecular assemblies require minimal synthetic effort. Most of the one- and two- inputs logic gates have been demonstrated in micellar media as well as examples of supramolecular systems that exhibit *off-on-off* and *on-off-on* profiles within narrow pH windows and *low-medium-high* ternary pH profiles on sequential addition of proton inputs. Applications in drug delivery and smart materials are just a sliver of potential uses.

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Research Article

Accessibility as an indicator of transport equity. The case of public transport infrastructure in Malta, and its impact on the elderly

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Abstract. The concept of equity is essential in transport because inequities lead to the formation of transport-disadvantaged groups, such as the elderly, disabled and low-income people. This paper focuses on the elderly. Due to age-related circumstances, several elderly persons have to surrender on driving, consequently they become highly dependent on public transport. Hence, accessible public transport is crucial to provide them with the necessary mobility. This research considers accessibility as a key indicator for transport equity, since the latter primarily deals with the provision of equal access to opportunities. The study focuses on the case of Malta's public transport system, which is composed of the bus service. The uniqueness of the Maltese case is that transport policy is fragmented, and is not focused on equity. This paper looks at three aspects of accessibility related to road infrastructure, public transport infrastructure, and the bus fleet. The first aspect refers to accessibility at the macro scale, for instance, pavements may not be solely designed to cater for the bus service, but they are an integrative part of it. The meso scale refers to accessibility of infrastructure in physical and cyber form, such as access to and on bus stops and access to online travel information. The bus fleet refers to the micro scale of accessibility, which may include boarding and alighting the vehicle, and access on the vehicle. The research approach involves a review of existing Maltese public transport policy, with specific focus on whether accessibility for the elderly is considered in the context of the afore-mentioned scales. It is envisaged that the minimal or non-existent policy on accessibility in public transport that focuses on elderly, makes this population segment at a double disadvantage. The research concludes with implications for policy related to public transport accessibility in a Maltese ageing society.

Keywords: transport equity, accessibility, public transport infrastructure, elderly people, transport policies for elderly, Malta

1 Introduction

Accessibility refers to the ability of reaching goods, services, and destinations. It is linked with mobility, which provides the opportunity for people to move from an origin to a destination (Litman, 2016). Hence, accessibility and mobility are two interdependent concepts that encourage independent living (Suen & Mitchell, 2000).

Accessibility is a necessity for people to reach their destinations, whether they are daily commuters or not. A non-commuting group is the elderly population. Due to age-related circumstances, several elderly persons have to surrender on driving, consequently they become highly dependent on public transport (Whelan, Langford, Oxley, Koppel & Charlton, 2006). This is one reason why elderly are one of the transport disadvantaged groups in society (Wixey, Jones, Lucas & Aldridge, 2005). In fact, older people use public transport more than younger generations (Goodwin & Lyons, 2010).

Hence, equity is essential in public transport because it ensures that the population segments that are at a disadvantage are provided with the same opportunities as other population segments. In fact, the concept of transport equity is built upon connecting citizens to key activity destinations by means of public and private transport infrastructure (Di Ciommo & Lucas, 2014). Consequently, it is necessary to include the assessment

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of equity as part of the monitoring programme of a bus service. Two important factors that are used to gauge equity are accessibility and mobility (Litman, 2016).

This research focuses in particular on public transport accessibility as a key indicator for transport equity. It seeks to identify the availability of transport policy on different levels of the bus system's infrastructural accessibility. The first level refers to accessibility at the macro scale, for instance, pavements may not be solely designed to cater for the bus service, but they are an integrative part of it. The meso scale refers to accessibility of infrastructure in physical and cyber form, such as access to and on bus stops and access to online travel information. The bus fleet refers to the micro scale of accessibility, which may include boarding and alighting the vehicle, and access on the vehicle.

The case study is Malta's public transport system, namely the bus service. Hence, the premise for this research is that an accessible bus service is crucial to provide the elderly with the necessary mobility that retains their independence.

The Maltese case provides the opportunity to explore a fragmented transport policy in which equity is not at the top of the policy agenda. This makes the elderly segment at a double disadvantage. The paper concludes with implications for policy related to public transport accessibility in a Maltese ageing society.

Malta has several geo-demographic characteristics that make it a good candidate to have high public transport patronage. However, it is not the case as the modal split is 75 per cent car users and 15 per cent bus users (Transport Malta, 2010). Such factors include a population of 0.4 of a million residing on a land area of only 316 km^2, one of the highest population densities in the EU (1,317 persons per km^2). Moreover, with particular relevance to this paper, Malta has an ageing population that is increasing at a fast rate. For the first time in history the 65+ age group in Malta is exceeding the 0–14 age group (1901: 0–14 age group - 34.1%, 65+ age group - 5.4%; 2012: 0–14 age group 14.5%, 65+ age group - 17.2%) (National Commission for Active Ageing, 2013). Fig. 1 illustrates the distribution of the elderly population in Malta in 2011. The Northern Harbour District, followed by the Southern Harbour District has the highest elderly population. Malta's conurbation is found in these districts.

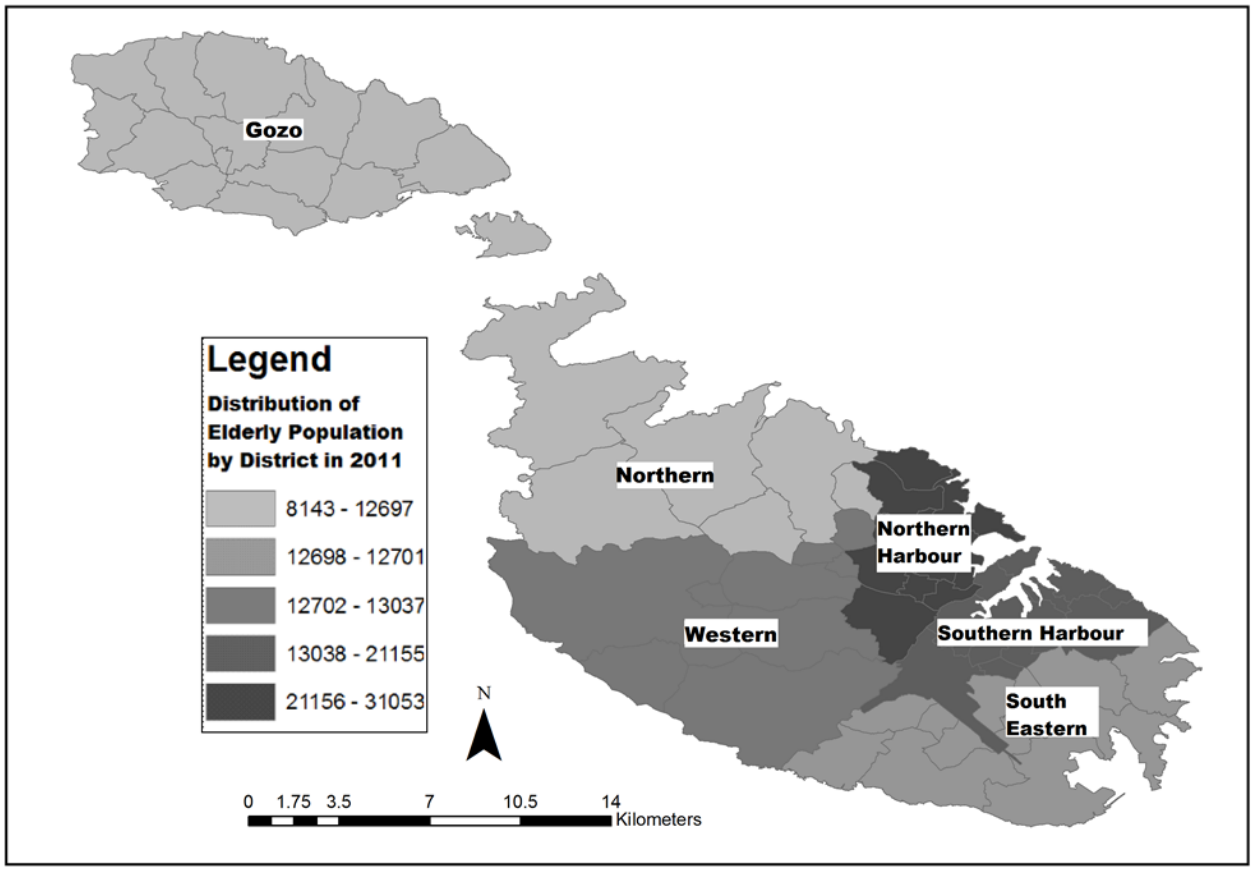

Figure 1: Map of Malta indicating the distribution of the Elderly Population by District in 2011 (Adapted from National Statistics Office, 2012a).

The paper is organised in five sections. Following this introduction, the second section provides a background literature review on transport equity in the context of elderly people as a socially excluded group, and referring in particular to work on accessibility. Section three provides an overview of the case study, the bus service in Malta. Section four explores the evaluation of Maltese transport policy in the context of elderly people and accessibility related to bus use. The fifth section provides the discussion and conclusion in view of land transport policy in Malta that relates to evaluation discussed in section four.

2 Literature Review

The concept of equity in transport research is relatively new (Trinder, Hay, Dignan, Else & Skorupski, 1991; Banister, 2000; Lucas, 2006; Martens, 2006; Mavoa, Witten, McCreanor & O'Sullivan, 2012). It has been classified into two dimensions. The first one is 'horizontal equity', which refers to an equal distribution of resources between individuals; and the second one is 'vertical equity', whereby resources are distributed according to similar abilities and needs (Litman, 2016; COST, 2012; Martens, Golub & Robinson, 2012). This study refers to 'vertical equity', because it focuses on elderly people who are a segment of the population with the same capabilities and requirements.

2.1 Transport Equity

Transport equity is considered as a way of providing social justice (Martens, 2006), and where transport equity is missing in terms of transport distribution, social exclusion takes place (Lucas, 2012). Factors that are used to gauge equity in public transport are system reliability, environmental impact (Bocarejo S. & Oviedo H., 2012), and accessibility, which affects the opportunities and capabilities of individuals to use the bus (Litman, 2016). This research focuses solely on accessibility because it is an important factor for elderly persons, since an inaccessible bus service impairs their mobility (Hanson & Giuliano, 2004).

2.2 The elderly as a socially excluded group

Older people who use public transport can become socially excluded due to limitations regarding the choice of other modes. Restricting factors include: age, income or lack of access to private transport (Beimborn, Greenwald & Jin, 2003).

Opportunities for various demographic groups are often reduced due to a reduction in accessibility, affordability and availability of transport (Church, Frost & Sullivan, 2000; Sen, 2000; Wixey et al., 2005). Elderly women tend to suffer more than men as they are more likely to spend more time relying on public transport after retirement (Foley, Heimovitz, Guralnik & Brock, 2002; Stutts, Wilkins, Reinfurt, Rodgman & Van Heusen-Causey, 2001).

2.3 Elderly and public transport accessibility

Social injustice is experienced when, for example, elderly people suffer from difficulties in mobility and feel insecure while waiting for the bus (Dunbar, Holland & Maylor, 2004). Such problems lead to inaccessibility, which hinders the quality of life of elderly people (Peel, Westmoreland & Steinberg, 2002; Hess, 2009; Frye, 2012). This can lead to social isolation, depression, and general health deterioration (Marottoli et al., 1997; Victor, Scambler, Bowling & Bond, 2005; Siren & Hakamies-Blomqvist, 2009). Hence, in an ageing country like Malta, it is important to consider and plan for equity in the public transport system, and gauge equity through factors such as transport accessibility.

Accessibility can be measured using different factors. The first factor is infrastructure accessibility. Pedestrians require accessible walkways, suitable traffic signals and street crossings (Suen & Mitchell, 2000). Older persons appreciate an accessible walking environment with pedestrian crossings and signs, much more than younger adults. They are more cautious and try to avoid crossing roads without pedestrian facilities (Bernhoft & Carstensen, 2008).

Additionally, accessibility comprises the ability to move from one bus stop to another, within a specific timeframe, particularly if a person is interchanging from one mode to another. In fact, the distance to bus stop, waiting time and ease of transfers are major factors that attract elderly persons to use public transport (Wardman, 2001). Since people in public transport services often cite the elderly population as one of the major rider segments (Carr, 2003) it is important that such infrastructure is suitable to accommodate the elderly.

Another key concept is knowledge. Bus users must be well-informed about the service before scheduling a trip, such as knowing the location of the bus stop and travel times (Beimborn et al., 2003). Information can attract more people to use public transport (Beirão & Sarsfield Cabral, 2007). A study carried out in Luqa, Malta, identified that lack of information was one of the factors that hindered elderly persons from using the bus service (Mifsud, 2013).

Moreover, people should find it easy to board on and off the vehicle (Beimborn et al., 2003); particularly old persons who generally suffer from health problems such as, arthritis, rheumatism and cardiac conditions (Smith, 2001). In Nigeria, 46 per cent of the transport constraints for elderly were related to boarding problems and inappropriate vehicle conditions (Olawole & Aloba, 2014), such as absence of low floor buses (Wixey et al., 2005). The feelings of resentment from other passengers if old persons take too long to access the vehicle are an-

other common problem that elderly people face when using public transport (Wixey et al., 2005).

2.4 Polices on Transport Equity

Different countries have adopted various policies and programmes related to elderly mobility and accessibility. In the United States equity in public transport provision is required by the legislation SAFETEA[1] (Delbosc & Currie, 2011).

The ECMT[2] has identified these main policy areas: ensure an accessible mobility environment and legislative reforms that address elderly transport issues, such as improving accessibility to public transport (European Conference of Ministers of Transport Council of Ministers, 2003), and monitoring the progress of accessibility policies (European Conference of Ministers of Transport Council of Ministers, 2006). Moreover, the European Commission (2011) acknowledges the difficulties that elderly persons encounter in their walking environment, and highlights the need to improve the accessibility of transport infrastructure for elderly and disabled passengers.

The current generation of elderly people is healthier than prior ones, and they have a more mobile lifestyle. However, there is still need to focus policies on the ageing population. Such policies are often lacking, as often only short-term goals of transport are considered. In Ontario (Canada), for instance, the ageing population is not even considered in transport policies on long-term basis (Mercado, Páez & Newbold, 2010). This is due to political and economic bias. Priority is given to economic and environmental issues, leaving the ageing population perspective behind. Additionally, most transport policies for elderly people are just related to private cars (example, screening drivers to analyse whether they should stop driving) (Mercado, Paéz, Scott, Newbold & Kanaroglou, 2007).

Furthermore, most of the current policies related to elderly in the transport environment are just concerned with disability aspects. In Ontario, the AODA[3] published in 2005, aims that by 2025 the province's infrastructure is accessible to elderly with impairments. Transport policies should go beyond limiting the elderly within the policy framework of disabled persons (Mercado et al., 2010). In fact, developed countries, which take primarily into consideration the needs of the elderly population, such as Japan serve the general public better. However, when referring to a public transport service one needs to consider the particular context and necessities of the country (Mercado et al., 2007).

In 2013, Malta launched *The National Strategic Policy for Active Ageing* 2014–2020 (National Commission for Active Ageing, 2013). This shows how lack of access often leads to social exclusion. Unfortunately, although the policy tackles independent living amongst the elderly, the transport dimension is not given detailed consideration. Hence, although Malta has a projected increase in the elderly population, it is clear that more national plans dealing with this population segment's transport necessities are lacking. Therefore, in 2012 the University of Malta joined the Transport Equity Analysis: assessment and integration of equity criteria in transportation planning (TEA) Cost Action №1209 to acquire an understanding on the equity implications of transport policies. This is a positive step in the interest of increasing awareness about equity in transport policy.

2.4.1 The need for stakeholder involvement

There is the need for integrating various stakeholders in order to have a more inclusive approach in transport policy (Smith, 2001; Mercado et al., 2010). For instance, an accessible walkable environment is both a transport and a health concern. Therefore, links between health and transport institutions should be accentuated.

This means that transport policies should take into consideration a holistic approach of the older persons' necessities that comprise their lifestyles, health, and physical abilities. They should support an integrated mobility approach. If all the laws are in place and the concerned institutions are interrelated, they can contribute to secure consistency in policy directions and trigger accessibility innovations. A comprehensive literature review has recently been finalised as part of one of the milestones of the TEA Cost Action (Bastiaanssen, Lucas & Martens, 2014) that refers to the inclusion of accessibility in equity appraisal. Reference to this work can lead to new ideas on how to evaluate and improve equity in the field of transport.

3 The Case Study of the Bus Service in Malta

The Malta bus service has gone through radical changes in the past three years. Table 1 shows the timeline of the bus service until January 2014.[4] Following nationalisation of the bus service, the Maltese government has issued an expression of interest to find a new operator.

Although the bus service reform failed in achieving modal shift, it led to the improvement of some bus service quality characteristics. Such achievements were acquired through the onerous service level agreement that was included in the contract.

[1] Safe, Accountable, Flexible, Efficient Transportation Equity Act: A Legacy for Users

[2] European Conference of Ministers of Transport

[3] Accessibility for Ontarians Disability Act

[4] This research was carried out in 2014.

Table 1: Timeline of events related to the bus service in Malta.

Month/July	Description
2008	A policy document entitled "Public Transport in Malta: A vision for Public Transport which fulfils public interest in the context of environmental sustainability" (Ministry of Infrastructure Transport and Communications, 2008), paved the way for the bus service reform.
Pre-July 2011	Bus services provided by the Public Transport Association, comprised by 400 bus owners/drivers, operated under the form of a monopoly.
July 2011	Commencement of the bus service reform.
December 2013	"Arriva Malta" bowed out of the country.
January 2014	Bus service nationalised.

3.1 Bus Service Quality Characteristics

An increase in bus patronage means that customers are satisfied (Eboli & Mazzulla, 2007). The bus service quality characteristics that improved after the reform were comfort, fare, and customer care (Attard, 2013). Infrastructural changes included low kerbsides in some areas where there are main bus stops, tactile surfaces, and low floor buses. These improvements were important milestones that made the bus service better, at least for those users who have no other mode of transport available. The factors that need immediate improvement are punctuality related issues (Attard, 2013).

3.2 Effects of the Malta bus service on the elderly

An increase in longevity allows the elderly generation to have a more mobile lifestyle (Alsnih & Hensher, 2003; Banister & Bowling, 2004). In a decade (between 2001 and 2011), Malta witnessed an increase of 19,279 driving licence holders for people aged 60 years and more (National Statistics Office, 2012b).

Yet, the elderly population still represents the highest number of bus users. When compared to other age groups, a minimal distinction between males and females using the bus service is found in older people (Transport Malta, 2010). Since 2011, The Public Transport Customer Satisfaction Survey (Institute for Climate Change and Sustainable Development, 2013) shows that the majority of the frequent elderly bus users are actually non-car owners; hence, as seen in other cases, such as Portland, Oregon, they are potential captive bus users (Beimborn et al., 2003).

Elderly bus users appreciate customer care assistance, fare structure (value for money), and comfort (Institute for Climate Change and Sustainable Development, 2013; Mifsud, 2013). Users also rated positively accessibility in terms of low-floor buses (Mifsud, 2013), which is convenient for elderly persons as seen in cases around Europe and North America (Suen & Mitchell, 2000).

In Malta, negative factors include unreliability, inaccessible and out-dated travel information, lack of safety, fear to travel alone, low frequency of services, and inappropriate bus driver travel behaviour (Institute for Climate Change and Sustainable Development, 2013). Despite the fact that the elderly travel mostly for medical issues and errands, the temporal accessibility to reach Malta's general hospital is still not sufficient, as all the desired time budgets of the elderly are exceeded (Mifsud, 2013).

The time ratio between bus use and car use is significant when considering the locations where elderly people reside and their travel destination. In a study in Madrid, it has been identified that in the congested section of the M40 the travel time ratio of public transport and car is on average 1.62 (Di Ciommo & Lucas, 2014). Thus, the long journeys associated with bus use contribute more to social exclusion, in this case when elderly find it more difficult because of time issues to reach their destinations.

Moreover, elderly persons are well concerned about the inappropriate distribution of bus stops, which does not cater for their needs. Inaccessibility to bus stops is also expressed through difficulties in crossing roads that have high traffic volumes.

4 Maltese Transport Policy

The Structure Plan of the Maltese Islands (Buchanan, 1990) is one of the earliest policy documents that looks holistically at land-use planning policies, including land transport (Buchanan, 1990, Section 14). The policies refer particularly to the land transport matters listed in Table 2.

Table 2: Land Transport Policies referred to in the Structure Plan (Buchanan, 1990)

Land Transport Policies referred to in the Structure Plan (1990)
Development and maintenance of a hierarchical network of roads
Traffic and environmental management
Public transport
Legal and educational measures

Over the past twenty-four years, implementation of these policies was restricted since the aim of this document was to provide a larger planning vision for Malta. Additionally, organisational fragmentation contributes to a disjointed transport policy (Attard, 2005) that is divided between the Planning Authority (PA), Transport Malta (TM) and the Ministry of Transport. Hence, an update of the Structure Plan was long overdue.

The SPED[5] has been issued for public consultation in March 2014 (Malta Environment and Planning Authority, 2014), and is the follow up to the Structure Plan (Buchanan, 1990). The issues related to transport are reported as key issues under the section Travel Patterns (Malta Environment and Planning Authority, 2014, p. 13). They echo the same problems discussed in the original Structure Plan and refer to the white paper that triggered the bus service reform (Ministry of Infrastructure Transport and Communications, 2008).

In fact, the white paper (Ministry of Infrastructure Transport and Communications, 2008) is the only policy document that is directly related to public transport in Malta. Additionally, TM had issued the Accessible Public Transport Infrastructure Policy, Design Guide (Transport Malta, 2009).

The following section discusses these three policy documents in terms of the three different scales of accessibility discussed in Section 1: the macro scale, meso scale and the micro scale. The initial observation when looking at the three policy documents is the absence to the reference of social equity when discussing land transport policy.

4.1 A Methodological Approach: The Three Scales of Accessibility

The structure plan refers to the configuration of the road network as the major criterion that affects the accessibility level (Buchanan, 1990). It focuses on the arterial and distributor road network that forms the main roads in Malta, and link the urban and rural areas.

Apart from this focus, the structure plan mentions the elements listed in Table 2. With the exception of the improvement of public transport, the other elements influence indirectly the bus system since it operates on this road network.

Hence, the policies that reflect these elements affect the bus service and its accessibility. Table 3 shows the policies related to both the macro scale and the meso scale. The macro scale is the shared infrastructure between the public transport system and the road network, as found in the Structure Plan of the Maltese Islands (Buchanan, 1990).

The meso scale refers to infrastructure, both physical and online, that is directly related to the bus system. The physical form is referred to in the Structure Plan (Buchanan, 1990) and in the Accessible Infrastructure for Public Transport Policy Design Guide (Transport Malta, 2009). The cyber form is mentioned briefly in the white paper (Ministry of Infrastructure Transport and Communications, 2008), stating that information technology should be applied at all levels, and give more facilities and information to the public. Although this provides more accessibility, it fails to address equity, and direct access to elderly people. In a world where elderly people are becoming more capable of using technology that aids in increasing accessibility (Mikkonen, Vayrynen, Ikonen & Heikkila, 2002), this concept is even more important to integrate in a transport system that provides services to the elderly. The importance of this is related to the possibility of increasing opportunities and abilities to elderly persons (Geurs & van Wee, 2004).

Table 3 also shows the inclusion of road transport policy that is at the meso scale. All the references made to the meso scale are generic policies that fail to address accessibility for elderly persons. The main barriers related to developing further such transport policies are linked to lack of proper and accurate information; there is a deficiency in transfer of knowledge that is related to a small number of transport professionals in Malta. Another issue arises from the two-party political situation, so politicians do not embrace projects that impose a cost on the population, because the projects may influence whether they are elected in the next legislation. Another issue arises from the lack of infrastructural and professional investment in public transport operations (Attard, 2005).

Micro scale accessibility refers to the ability to move easily when boarding and alighting the bus and on the vehicle itself. The policy documents mentioned in Table 3 do not mention in detail the requirements for an accessible service. However, the service level agreement signed in the contract (Transport Malta, 2009) specifically required low floor buses that are easily accessible by vulnerable groups of society, such as elderly persons.

5 Discussion and Conclusion

This research shows that in Malta land transport policy is limited (Attard, 2005) and public transport policy is even more restrained. Some examples include deficiencies in waiting time conditions and interchanging facilities that increase accessibility to elderly persons. Moreover, policy is fragmented between different institutions within government, namely PA (Buchanan, 1990), TM (Transport Malta, 2009) and the Ministry of Transport (Ministry of Infrastructure Transport and Communications, 2008). This fragmentation leads to a lack of detail in land transport policy that focuses particularly on public transport and on the availability of

[5]Strategic Plan for Environment and Development

Table 3: Policy Documents that address the Macro and Meso Scale of Public Transport in Malta.

Scales				
	Macro		**Meso**	
Policy Document	**Policy**	**Comment**	**Policy**	**Comment**
Structure Plan of the Maltese Islands	**TRA3:** During the time that urban development takes place, developers are subject to fund necessary remedial road works.	There is no specification to accessibility or equity, and such works tend to be temporary, which might imply that provision of necessities that cater for vulnerable groups of society might not be implemented.	**TEM7:** Bus priority lanes and other priority measures in locations where they are feasible, and where the time and cost savings to the bus operators and passengers exceed the equivalent delays to other road traffic.	All these policies are generic and address accessibility without going into detail of usage for elderly persons, hence omitting the concept of equity particularly for vulnerable groups.
	RDS7: The extension of pedestrian priority and access only restrictions in UCAs (Urban Conservation Areas), including areas suffering from the environmental impact of traffic.	Elderly people require pedestrian priority, because they have to walk to reach the nearest bus stop. Moreover, UCAs are the core of urban areas, where generally elderly people live. This policy focuses on the benefit of the environment but fails to address the element of equity.	**PTR2:** Appropriate bus fleet for the narrow road types. **PTR3:** Studies that minimise interchange. **PTR5:** Efficient intermodal interchanges. **PTR7:** Smaller bus terminus in Valletta, the Capital City and main hub of the bus service.	
	TEM1: Design of traffic management will conform to agreed standards for road design and construction.	The design guide for public transport infrastructure was only prepared nineteen years later. During these years, as still happens with road infrastructure, the designs are known and adapted by road engineers, and knowledge is transferred from one person to the other. This leads to the possibility of omitting equity measures that could improve, amongst other factors, accessibility for the elderly.	**PTR8:** Better accessibility during and after operations of major developments. **PTR9:** Improved waiting conditions with reliable passenger information on shelters at bus stops.	
Public Transport in Malta: A Vision for Public Transport which fulfils public interest in the context of environmental sustainability	N/A	N/A	Addressed inter-modal accessibility, new bus fleet, express and regular bus service, a service that caters for peripheral destinations, and night services, discount schemes for frequent users, and provision of information technology services.	These factors were mentioned briefly and there were no policies that referred particularly to each point, and even more so that addressed equity to vulnerable groups.
Accessible Public Transport Infrastructure Policy, Design Guide	N/A	N/A	Details specifications and measurements for the design of bus stop signs and information signs, bus stop and bus shelter designs, including kerb dimensions and bus priority dimensions. It follows the framework of national accessibility guidelines for disabled persons	It primarily focuses on infrastructure design it does not refer directly to usage of the infrastructure by elderly persons.

public transport to elderly people.

In Malta it is necessary to focus more on land transport policy making. There is the need of having an integrated approach to the formulation of land transport policy. Policy should directly address equity and vulnerable groups in society, including elderly persons. This can be done by providing additional policy documents and guidelines to the existing documents. This measure would allow more focus that is direct on equity issues, such as long walking distances to bus stops, which could be identified by using time ratios (Di Ciommo & Lucas, 2014).

However, the SPED (Malta Environment and Planning Authority, 2014) does not seem to address these issues. It builds upon the Structure Plan (Buchanan, 1990) and refers to the public transport policy document (Ministry of Infrastructure Transport and Communications, 2008). The objectives for transport and public transport reproduce the objectives of these two documents, and there is limited direct addressing to accessibility in general and for the elderly.

Meanwhile, TM is in the process of designing the National Transport Strategy and Transport Master Plan. This process is still in its early stages; TM is proposing that an SEA is undertaken as part of the development of the master plan (Transport Malta, 2014). This will allow for the evaluation of policy within the transport framework. Consequently, it is essential that at this stage the relevant stakeholders meet to discuss the needs for improving accessibility to elderly persons and address transport equity issues.

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Note Article

Reviewing the review process: New Frontiers of Peer Review

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Abstract. This news article introduces a new COST Action entitled PEERE (TD1306), which stands for New Frontiers of Peer Review (PEERE). PEERE is a trans-domain proposal which brings together researchers from various different disciplines and science stakeholders for the purpose of reviewing the process of peer review. PEERE officially began in May 2014 and will end in May 2018. Thirty-one countries, including Malta, are currently participating in the Action. In order to set the context in which this COST Action was initiated, we first look very briefly at the history of the process of peer review and various models of peer review currently in use. We then share what this COST Action hopes to achieve.

1 Introduction

As researchers, we are no doubt all too familiar with the feelings of euphoria associated with having a paper accepted for publication in a peer-reviewed journal, especially if the journal in question happens to be the top journal in our academic field, which is likely to be a journal with a high "impact factor"[1]. Sadly, probably even the best among us would also have experienced the sting of having a paper rejected, although these feelings can be somewhat mitigated if we feel that the paper has undergone an impartial peer-review process, and we are provided with good review comments that can help us to improve the paper for submission to the next journal on our list.

Of course, this is from our own viewpoint, as researchers. As researchers, what we sometimes forget to dwell on is the important "gatekeeper" function peer review can play (deciding which information "deserves" to be disseminated). Wrong or misleading information can have a huge impact on the daily life of people, from medical treatments to recovering from the economic crisis. Therefore, the correct functioning of the peer review process is in the interest of science and of society as a whole.

Although the need for some form of peer review (either pre- or post-publication) is acknowledged by most researchers, the system of peer review is far from perfect and there have been numerous high-profile cases of fraudulent publications that have passed the peer review process (Martin, 2012; Storbeck, 2011, July 7).

[1] The "Impact Factor" is probably the most commonly accepted, if controversial, way of rating the quality of academic journals. It is a quantitative tool for ranking, evaluating, categorizing, and comparing journals. It is a measure of the frequency with which the "average article" in a journal has been cited in a particular year or period. The annual Journal Citation Report impact factor is a ratio between citations and recent citable items published. Thus, the impact factor of a journal is calculated by dividing the number of current year citations to the source items published in that journal during the previous two years. An Impact Factor of 1.0 means that, on average, the articles published one or two years ago have been cited one time. An Impact Factor of 2.5 means that, on average, the articles published one or two years ago have been cited two and a half times. http://admin-apps.webofknowledge.com/JCR/help/h_impfact.htm

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Problems are frequently attributed to the social and subjective dimensions of the process (e.g. bias and conflict of interest; Lipworth, Kerridge, Carter & Little, 2011). Other common criticisms levied against the peer review process include unacceptable delays in publication, expense, inconsistencies, fraud/plagiarism, nepotism, and counter to innovation – and the list goes on!

2 What exactly is scientific peer review?

Peer review or refereeing is the process of subjecting an author's work, research, or ideas to the scrutiny of experts (peers) in the same field, traditionally, before the work is published in a journal.

In its most basic form, peer review is the evaluation of an author's manuscript by identified reviewers, who make recommendations to the journal's editor as to whether or not a manuscript should be accepted as is, revised prior to publication or rejected, based on the quality, originality and importance of the manuscript (Sense about Science, 2009). Peer review is one significant method by which research grants are allocated, papers published, academics promoted, and Nobel and other major prizes won (Smith, 2006).

Peer review concerns all of us. As aptly stated by The Publishers Association in response to the UK House of Commons Science and Technology Committee consultation on Peer review in scientific publications conducted in 2010–2012, peer review is "a duty and a skill, performed by researchers, for researchers. It is a system that has been developed by the academic community, for the academic community over centuries and it is established practice that professional scientists are prepared to engage in peer review as a service to the community at large and as a contribution to the progress of science" (The National Archives of the UK, 2011).

The first recorded use of peer review is ascribed to Ishaq bin Ali Al Rahwi (AD 854–931). In his book, Ethics of the Physician, Al Rahwi apparently encouraged doctors to keep contemporaneous notes on their patients, later to be reviewed by a jury of fellow physicians. Journal peer review followed much later, when Henry Oldenburg, editor of Philosophical Transactions of the Royal Society, adopted peer review in the seventeenth century (The National Archives of the UK, 2011). Since then, peer review has played an increasingly important role in scientific publishing: in 2008, 1.3 million learned articles were published in peer-reviewed journals. Peer review is now fundamental to the integration of new research findings into established knowledge, enabling other researchers to analyse or use findings and, in turn, society at large to access and interpret research claims (Sense about Science, 2009).

Several different types of peer review process are now available (see Box 1). It is an evolving process, with continuous attempts being made to find better, more effective models of peer review. The peer review process has also been complicated by the increasing use of institutional repositories, self-archiving, data sharing, social media, and other tools. However, the underlying assumption in all situations is that, since peer review is based on human labour and judgement, it is unlikely that a perfect system can ever be found.

3 Why PEERE?

This COST Action aims to improve the peer review process, potentially increasing the credibility of science in Europe in an era of increasing scandals and public concern. The main objectives of the Action are given in Box 2.

In order to achieve these objectives, three working groups (WG) have been created. They will be working in the following areas:

- WG1: Theory, analysis and models of peer review (Analysing peer review by integrating qualitative and quantitative research and incorporating advanced computational and experimental investigation; Testing implications of different peer review models).
- WG2: Data sharing and testing (Establishing standards and appropriate Information and Communications Technology (ICT) applications to treat, manage and share data on peer review between stakeholders; Providing guidelines and protocols for data sharing; Developing quality and efficiency indicators and monitoring measures to evaluate the potential impact of new models).
- WG3: Research and implementation agenda (Defining and monitoring challenges and prospects for an evidence-based evolution of peer review; Leveraging existing resources and identifying new opportunities for collaboration and research).

4 Opportunities for Malta

As this is a new COST Action, Malta currently has only one representative on the Management Committee. The Action currently includes researchers from diverse disciplines such as computational sociology, economics, basic sciences, etc. Some members have experience as journal editors. Important stakeholders such as the publishers Elsevier, Springer and Wiley, and partners from the US, Canada and Brazil are also included. Whatever your research background, you may have ideas that can help to improve the process of peer review, which is (arguably) the cornerstone of science! If you are interested in joining this Action, please contact Prof. Janet Mifsud (janet.mifsud@um.edu.mt), COST Malta Country National Contact. More information on the Action is

available at: http://www.cost.eu/COST_Actions/tdp/TD1306 and from http://peere.org/.

Box 1: Types of peer review

"Single blind" peer review: The author's name and institution is known to the reviewer, but not vice versa. This is the most common form of peer review, especially in the sciences.

"Double blind" peer review: This system is fully anonymised i.e. the authors are unaware of the identity of the reviewers, and vice versa. This is more common in the social sciences.

"Open" peer review: In which the authors' and reviewers' names are revealed to each other. This is not too common, but is used in some biomedical journals, such as BioMedCentral journals and the British Medical Journal (BMJ).

Post-peer review or post-publication peer review: Different models can be found under this title, for example, Review by formally invited reviewers, after publication of the un-reviewed article; Review by volunteer reviewers, after publication of the un-reviewed article; and Comments on blogs or third party sites, independent of any formal peer review that may have already occurred on the article. Post-publication peer review can be named or anonymous, and reviews can in some cases be written by uninvited reviewers who may not necessarily be literal "peers" in the field (Amsen, 2014).

Cascading peer review (or cascading reviews between linked journals): This is a system whereby a publishing house redirects rejected manuscripts to related journals that have lower rejection rates, in the same field. Advantages to the publisher are reduced cost and higher efficiency, while the advantages to the author is faster publishing (Davis, 2010).

Pre-print servers such as the arXiv repository of electronic preprints (http://arxiv.org/), where the e-prints are commented on by the community, and can later be submitted to a journal and published. Some of the benefits of the arXiv system are that it "allows the scientists to publish research quickly and get informal feedback and identify any weaknesses. This is then followed by formal peer review in a journal" (The National Archives of the UK, 2011).

Box 2: Objectives of PEERE

The main objective of the Action is to improve efficiency, transparency and accountability of peer review through a trans-disciplinary, cross-sectorial collaboration. This is will be achieved through:

- analysing peer review by integrating qualitative and quantitative research and incorporating advanced computational and experimental investigation;
- testing implications of different peer review models (e.g., open vs. anonymous, pre vs. post publication) and different scientific publishing systems (e.g., open vs. subscription based publication systems) for the rigour and quality of peer review;
- discussing present reward structures, rules and measures and exploring new solutions to improve collaboration in all stages of the peer review process; and
- developing a coherent peer review framework (e.g. principles, guidelines, indicators and monitoring activities) for stakeholders that truly represents the complexity of research in various fields.

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Research Communication

A culture collection of Maltese microorganisms for application in biotechnology, biomedicine and industry

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1 Introduction

Over the years, very few studies have been conducted on microorganisms growing in the Maltese islands, and these have rarely resulted in the description of new genera, species or serovars. Two important exceptions are the studies on *Brucella melitensis*, by Sir Temi Zammit in 1905 (Wyatt, 2005) and a new serovar of *Salmonella* from Gozo (Vella & Cuschieri, 1995).

Ten years ago, sampling of microorganisms growing as biofilms on different substrates around the Maltese islands was initiated. The microorganisms consisted mainly of chemoorganotrophic bacteria, cyanobacteria and microalgae. Today the culture collection of Maltese microorganisms contains over a hundred new microbial strains that are new to science and which include freshwater, marine, soil and subaerophytic microorganisms.

The aim of the research is twofold. Firstly, it is important to characterise the Maltese microbial strains and describe new taxa as required. Secondly, the extraction of important metabolites for application in biotechnology, biomedicine and industry.

2 Materials and Methods

Sampling of microbial biofilms was carried out using non-invasive sampling techniques (Zammit, De Leo, Albertano & Urzì, 2008; Zammit, Psaila & Albertano, 2008; Zammit, De Leo, Urzì & Albertano, 2009). Isolation of microorganisms from biofilms was undertaken (Zammit, Billi, Shubert, Kaštovský & Albertano, 2011). Observations by light, confocal, scanning and transmission electron microscopy were carried out according to published methodologies (Zammit, Billi & Albertano, 2012).

Genetic studies involved the sequencing and subsequent analysis of 16S rRNA and ITS genes of cyanobacteria (Zammit et al., 2012) and chemoorganotrophic bacteria (De Leo, Iero, Zammit & Urzì, 2012) and also the 18S rRNA genes of microalgae (Zammit, Billi, Shubert et al., 2011).

3 Results and Discussion

Microbial strains in the culture collection of Maltese microorganisms are grown in a variety of nutrient media and under specific conditions of light and temperature (Zammit, Billi, Shubert et al., 2011).

Of these, the cyanobacterial strains belong to new species of the well-known genera *Leptolyngbya*, *Pseudanabaena*, *Nostoc*, *Fischerella*, *Chroococcidiopsis*, *Asterocapsa* and *Gloeocapsa*. A number of other strains belong to new genera that are currently being described. A large number of cyanobacterial strains possess a simple morphology and are considered to be members of cryptic species (Osorio-Santos et al., 2014). Some are slow growing in culture and are difficult to isolate from other microbial strains, since they are likely to share a symbiotic relationship (Fig. 1).

To date, a number of new strains of cyanobacteria from the collection have been characterised (Zammit, Kaštovský & Albertano, 2010), new genera and species have been described (Zammit et al., 2012), while the taxonomic position of others is currently being elucidated. Understanding the ecology and evolutionary history of new microorganisms is one key to realizing their potential to produce new biomolecules.

Important milestones have included the description of the new genus *Oculatella* (Zammit et al., 2012), with strains of the type species *O. subterranea*, isolated from both Maltese and Italian hypogea. This genus has since been confirmed by the description of seven other species isolated from a variety of habitats including arid to semi-arid soils, a temperate lake and sea caves (Osorio-Santos et al., 2014).

O. subterranea is a filamentous cyanobacterium that

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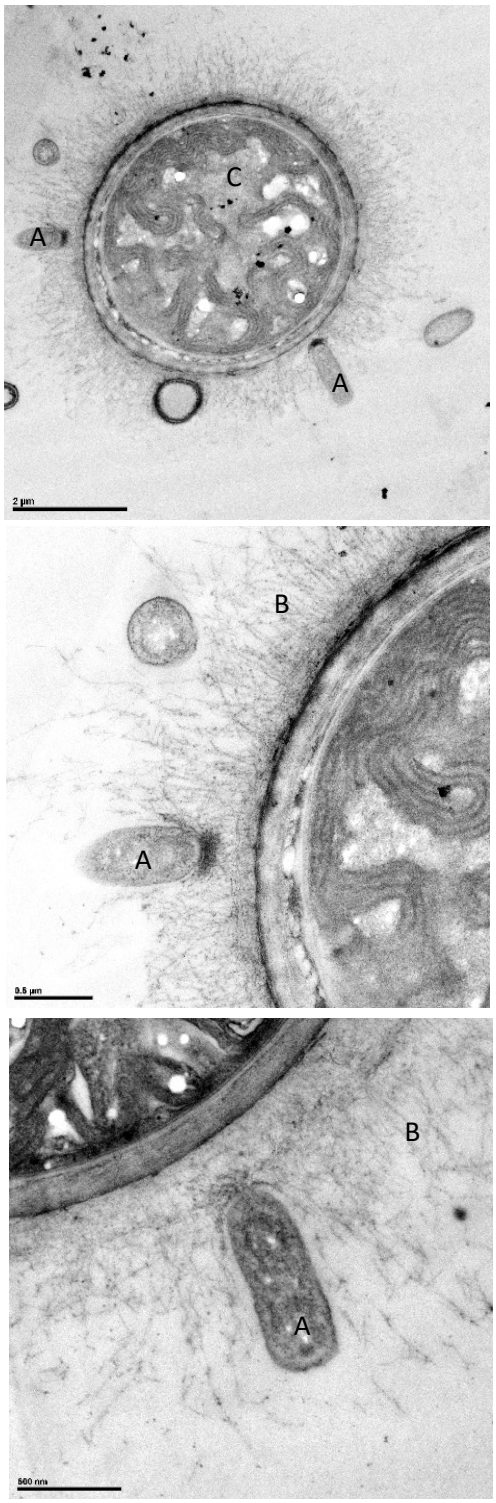

Figure 1: Transmission electron micrographs elucidate intimate symbiotic relationships. Here rod-shaped chemoorganotrophic bacterial cells (A) are seen adhering to and feeding on the capsular exopolysaccharide layer (B) produced by a larger cyanobacterial cell (C).

is characterised by fine trichomes 1–3 µm wide. These appear purple-red due to a predominance of phycoerythrin over phycocyanin inside the cells. The trichomes exhibit phototaxis and are able to glide along an extracellular sheath due to the presence of a photosensitive apical cell containing a rhodopsin-like pigment (Zammit et al., 2012).

Cytochemical stains used in transmission electron microscopy allowed detection of the presence of two different layers of carboxylic groups within the sheath. The composition of exopolysaccharides of *O. subterranea* were found to be composed of the following monosaccharides; the acidic galacturonic and glucuronic acids, and the neutral sugars; galactose, glucose, mannose, xylose, fructose and rhamnose (Bellezza, Paradossi, De Philippis & Albertano, 2003).

Apart from the important role they play in bacterial biochemistry, and the formation of a thick microbial matrix that facilitates adhesion and prevents desiccation and exposure to UV, these polysaccharides may have chemical and physico-chemical properties of industrial interest. In fact, polysaccharides released into the culture medium are anionic due to the presence of uronic acids and other charged constituents, such as sulphate groups; these latter groups may confer antiviral properties (Bellezza et al., 2003).

Lipid profiles from *O. subterranea* extracted from a limestone cave in Greece have revealed the presence of palmitic (16:0), palmitoleic (16:1), linoleic (18:2), oleic (18:1) tridecylic (13:0) and myristic (14:0) acids, isomers of C15, C17, C20 and C22 as well as stearic (18:0) and linolenic (18:3) acids (Christodoulou, Meletiou-Christou, Parmakelis, Economou-Amilli & Pantazidou, 2015). The predominance of unsaturated fatty acids is likely related to the low temperature at which the cyanobacteria were grown.

To date, little has been done to compare the fatty acid and lipid profiles from various marine, freshwater and terrestrial habitats and further studies are required in order to understand the reasons leading to the production of specific fatty acids by specific cyanobacterial strains. So far, lipids and fatty acids extracted from cyanobacterial species and used as food supplements, are characterized by the high amount of essential fatty acids such as α- and γ-linolenic acid, and their C20-derivatives, arachidonic acid and eicosapentaeonic acid.

The interesting molecular biology and biochemistry of *O. subterranea* warrant further in depth study. In fact, whole genome sequencing, together with a reconstruction of biochemical processes, are both currently ongoing and will be published in the near future.

As regards the microalgae, strains of *Trentepohlia*, *Pseudococcomyxa* and *Diadesmis* have been isolated in culture. Many other strains are characterised by pro-

hibitive simple morphology. Genetic analysis, though, has facilitated the identification of new strains from the genus *Chlorella* (Zammit, Billi, Shubert et al., 2011) and also a recently described species, *Jenufa aeroterrestrica* (Prochazkova, Nemcova & Neustupa, 2015). *J. aeroterrestrica* strains grow on tree bark; to our knowledge this is the first time a member of this species has been isolated from a hypogean environment and successfully grown in culture.

Metabolites from a number of promising microalgal strains in the collection are currently being extracted. Our main aim is in the discovery of new bioactive substances for application in biomedicine. Other important applications of microalgal secondary metabolites are the cosmetics and aviation fuel industries. Maltese microalgae can also be used as important bioindicators for global climate variability and as important tools in forensic studies.

As with cyanobacteria, the molecules of interest include polyunsaturated fatty acids such as eicosapentaenoic acid and acrylic acid, which have anti-microbial and anti-inflammatory properties. Algal sterol extracts such as lathosterol, ergosterol, stigmasterol, 24-ethylcholesta-5,7,22-trienol, stigmasta-7,24(241)-dien-3ββ-ol, and cholesterols, have the ability to potentially lower plasma non-HDL cholesterol by increasing the excretion of total faecal sterols and decreasing cholesterol absorption and synthesis, reducing the risk of heart disease in the process. Sulfated polysaccharides have anti-inflammatory, anti-cancer and immunomodulating properties. Microalgal pigments such as lutein and astaxanthin have been shown to exhibit antioxidant, anti-inflammatory and anti-cancer activities.

Apart from the characterisation of new cyanobacterial and microalgal strains for biotechnology application, recent studies have involved the use of chemoorganotrophic bacteria in consolidating deteriorated local Globigerina limestone.

Past studies had shown that microbially induced calcite precipitation occurred naturally on ancient limestone surfaces (Zammit, Sánchez-Moral & Albertano, 2011). We exploited this phenomenon with the successful bioconsolidation of deteriorated limestone through the application of treatments enriched with *Bacillus subtilis*. The treated stone was subjected to various mechanical and physical tests in order to present a statistically robust data set to prove that the treatment was indeed effective. Uniform bioconsolidation occurred to a depth of 30 mm, an unprecedented result. Drilling resistance values for the treated limestone were similar or better than those obtained for freshly quarried stone blocks. We reported an eco-friendly bioconsolidative treatment that closely resembles the mineral composition of the limestone and that penetrates into the porous structure without affecting the limestone's natural properties. The treatment is of industrial relevance since it competes well with stone consolidants available commercially (Micallef, Vella, Sinagra & Zammit, 2016).

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I am indebted to a number of collaborators who have contributed to this fascinating research over the years: Professor Clara Urzì, Professor Patrizia Albertano, Professor Elliot Shubert, Dr Jan Kaštovský for many interesting discussions about newly discovered microorganisms, and Professor Alexander Felice.

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News Article

The first Malta Brain Awareness Week: An Interview with the Coordinator of the Malta Neuroscience Network

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[1] *Coordinator of the Malta Neuroscience Network Program, University of Malta*

Professor Di Giovanni, can you give us a brief overview of your career?

I am a neuroscientist. For more than 2 decades, my research has focused on understanding the pathophysiology of central monoaminergic systems in different neuropsychiatric disorders such as depression, schizophrenia, drug abuse, Parkinson's disease and epilepsy. My research group uses a combination of *in vivo* and *in vitro* electrophysiological techniques and behavior tests in rodents. My work has attracted funding from international bodies and national Maltese bodies such as the MCST.

My principal scientific achievements have firstly been in revealing the role of serotonin in the modulation of central dopaminergic function (see recent review by De Deurwaerdere & Di Giovanni, 2016) and more recently, the pivotal involvement of 5-HT2C receptors in absence epilepsy and temporal lobe epilepsy (see recent review by Di Giovanni & De Deurwaerdere, 2016) with important insights for the pathogenesis and therapy of both depression and epilepsy. Moreover, in collaboration with Prof. Crunelli I showed that the aberrant GABAA tonic inhibition is one of the pathological mechanisms of absence epilepsy (Cope et al., 2009).

I love what I do and I consider myself very lucky to be doing it. Obviously, I had to work very hard to arrive at this point. I decided to become a neuroscientist when I was 15 after reading "La macchina per pensare" by Piero Angela (Angela, 1983), the first and still the best Italian science communicator. His book changed my life. As a child, I fell in love with brain science and, after the Liceo Scientifico, I took a degree in Biology and another in Natural Science at the University of Palermo, Sicily. After this, I received my Ph.D. in Neuroscience from the University of Chieti working at the Consorzio Mario Negri Sud in Santa Maria Imbaro, Italy, in the laboratory of Neurophysiology led by Dr Ennio Esposito (who died suddenly of a heart attack, on October 23, 2011). Subsequently, I completed my post-doc at the Department of Pharmacology at Yale University, USA, one of the most prestigious universities in the world, under the supervision of Prof. Benjamin Bunney and Wei Xing Shi, pioneers in the study of the dopaminergic systems in normal and pathological conditions.

Successively, I was appointed Lecturer and then promoted to Senior Lecturer of Human Physiology at the Faculty of Medicine and Surgery, University of Palermo. In 2007 I went to Cardiff Wales, Uk, on sabbatical and then I moved to Malta where I was appointed as Associate Professor at the University of Malta. Since 2013, I have been a Professor of Human Physiology at the University of Malta.

I have published more than 110 peer-reviewed papers, edited 7 books and 6 special issues of various journals. As researchers, our work is continuously evaluated by our peers, both for publishing and receiving external funding. To evaluate the impact of a researcher's work, two bibliometric (or better "scientometric") indexes are commonly used, the H-index score and the total number of times the scientific publications have been cited by other researchers.

My H-index score is 32 and my number of citations is more than 3370 (as of May 2016, source: http://scholar.google.com/citations?user=pJFQfAkAAAAJ), positioning me among the most cited researchers in Malta in all disciplines (https://scholar.google.com/citations?hl=en&view_op=search_authors&mauthors=um.edu.mt). I hope to be able to contribute further to scientific development of Malta.

Apart from my intense research activities, I am actively involved in editorial work. I am the Editor-in-

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Chief of the prestigious book series "The Receptors" by Springer, USA (http://www.springer.com/series/7668) and serve as associate editor in neuroanatomy and neurochemistry for the Journal of Neuroscience Methods by Elsevier, Amsterdam, Netherlands and CNS Neuroscience and Therapeutics by Wiley, London. Locally, I am the Editor-in-Chief of Xjenza Online, the journal of the Malta Chamber of Scientists (http://www.mcs.org.mt/index.php/xjenza).

When was the Malta Neuroscience Network founded?

I remember very well the first moment that I thought about it! It was a hot afternoon in September 2014, I was at the University as usual until late and I was thinking about a documentary I had been involved in: *Jien Min Jien* (https://www.youtube.com/watch?v=ofpyAuy-aLo), produced by Studio7 for RIDT and Science in the City. This documentary was about research at the University of Malta for which I organized the part on neuroscience research and how this has grown in the last 10 years also in terms of articles published in peer-reviewed journals. I realized then that there were many people interested in neuroscience, but not enough coordination and collaboration among them. Malta is a small country, therefore collaboration among scientists is of pivotal importance for the scientific development of the nation, the university and the education of our students. I went to Richard Muscat's office and I said to him "Richard, what if we form a virtual neuroscience institute to bring together all of the neuroscience researchers in Malta?" I could see the enthusiasm on his face "Yes", he said, "but do not call it Institute, the structure is too heavy, call it something like a group." "What about a network?" I said. "Perfect," replied Richard. So, I started contacting everyone working on neuroscience, either directly or by email, and everybody was enthusiastic about this proposal to create a network of people, all with different neuroscience specialisms. An important person who gave me support and input in the creation of the MNN was Wilfred Kenely, the CEO of RIDT. In reality, we started even earlier than this, during a fundraising event to support my research on depression and epilepsy. We had started working together on an exciting project; to have Carmine Lauri play a concert for Brain Research on Easter Sunday which proved to be a great success (https://researchtrustmalta.wordpress.com/tag/carmine-lauri/). This was successively included in the activities of MNN and the first Malta Brain Awareness Week (BAW) and I have decided to direct this funding to the entire research community (soon there will be calls for grant applications in which, obviously, I will also participate). Following the peer review process, we will have the first 2 projects funded by MNN/RIDT. On Monday 20th April 2015, I was received by the Rector Prof. Juanito Camilleri with other members-to-be of the MNN: Prof. Richard Muscat, Dr Norbert Vella, Prof. Kenneth Camilleri, Prof. Alex Felice and Wilfred Kenely. The Rector followed my presentation on the creation of the network and on the existing similar networks around the world that promote research and training in the rapidly growing field of Neuroscience with great interest. Prof. Camilleri, firstly congratulated me and the other colleagues and then he suggested creating a new Programme that presents a light governance structure. In a few minutes we agreed that I would present the description of the new Neuroscience Programme proposal in time for the Senate meeting on the 7th of May 2015.

I did not waste time, and on the 21st of April we officially presented the new Malta Neuroscience Network. For this occasion, Prof. John Stein from Oxford University gave a talk to the general public at St James Cavalier in Valletta. Prof. Stein talked about modern techniques that help us understand the brain, the brain's plasticity and the genetics that affect day to day activities. Prof. Stein also talked about deep brain stimulation to identify tremors, dyskinesias (involuntary movements) and pain, as well as discussing how the cerebellum controls our motor functions (https://www.facebook.com/events/413741275467272/).

On the 8th of May 2015 the Senate appointed me as Coordinator of the Board of the MNN, up to 31 December 2016.

The next MNN event was on 15th May, when Dr Owen Falzon gave a Caffe Scientifique talk on Brain Linked Machine.

On the 29th of July 2015, the MNN presented a DUAL SEMINAR at the South Auditorium, Mater Dei Hospital. Msida. The two invited speakers were well-known international scholars.

Prof. Vincenzo Crunelli, from Cardiff University, UK, talked about "Thalamocortical rhythms in wake, sleep and epilepsy" and Prof. Robert Fern, from Plymouth University, UK, focused his talk on "Glia, white matter and disease". After the dual seminar, we had the first Malta Neuroscience Board Meeting in the Board Room, Medical School, MDH. From this moment we started working on the different activities of the MNN.

Thanks to Mr Malcolm Bonello of the Faculty of Media & Knowledge Sciences, who designed the logo of MNN (Fig. 1).

What is its function?

From the MNN statute...

"The Malta Neuroscience Network is under the aegis of our Faculty of Medicine and Surgery, joined with

Figure 1: Logo of Malta Neuroscience Network (MNN) designed by Mr Malcolm Bonello of the Faculty of Media & Knowledge Sciences.

the Faculties of Engineering, Health Sciences, Information & Communication Technology, Media & Knowledge Sciences, Science, Social Wellbeing and the Centres for Biomedical Cybernetics and for Molecular Medicine and Biobanking.

The aims of the Network are:

(a) To encourage and facilitate interdisciplinary research that brings together academic members from all the Faculties of the University of Malta with an interest in in the rapidly growing field of Neuroscience.
(b) To promote interdisciplinary dialogue among all the disciplines involved with Neuroscience.
(c) To foster research and training in neuroscience at the University of Malta.
(d) To hold regular meetings, seminars and conferences (MNN Conferences) in which to present research ideas, discuss work in progress and generally promote the sharing and dissemination of knowledge.
(e) To sponsor and coordinate seminars by leading neuroscientists from home and abroad.
(f) To create and maintain an electronic portal for the publication, discussion and dissemination of research.
(g) To offer study-units in Neuroscience that may be included in both undergraduate and postgraduate programmes.
(h) To collaborate with local and overseas centres/Universities, programmes and individuals with similar purpose and scope.
(i) To raise public awareness in Neuroscience, brain disorders and mental health and other related areas through public talks, evening courses, Annual Brain Awareness Week, Brain Research fundraising in conjunction with RIDT and different NGOs and scientific associations."

Apart from local neuroscientists, we have in our network Prof. Giacomo Rizzolatti (Fig. 2), who discovered mirror neurons and Prof. Vincenzo Crunelli form Cardiff University, a world renowned neuroscientist specializing in epilepsy. This thanks to our Rector Juanito Camilleri who put forward my request, supported by Prof Christian Scerri and Prof Richard Muscat, to make Prof. Rizzolatti and Prof. Crunelli Affiliate Professors at the Department of Physiology and Biochemistry.

Figure 2: Professor Giacomo Rizzolatti (left) and Professor Giuseppe Di Giovanni (right) in the laboratory of Cognitive Neuroscience, University of Malta (March 2016).

If you helped found the MNN, could you tell us what the inspiration behind it was?

The human brain is the most complex organ in the known universe. This complexity makes it the last and hardest frontier in medical research. Unravelling the brain's secrets could change the lives of millions of people of all ages, suffering from neurological and psychological conditions, lesions and addictions. Brain diseases can affect anyone. One in three Maltese people and about 1 billion people worldwide suffer from some form of condition or disease at some point in their lives. For example autism, multiple sclerosis, depression, and dementia are brain disorders that represent the most important challenges to public health in the 21st century. We need to develop new ways to cure these conditions rather than simply treat them. Discovering the reasons behind brain disorders necessitates the collaboration of many different scientific disciplines and clinicians. We now have a much deeper understanding of the brain's complexity that has greatly improved human health. We have also increased our understanding regarding the genetic basis of diseases like autism, schizophrenia, Parkinson's and Alzheimer's disease. These advances are promising but further steps are needed to allow researchers to translate these findings into treatments. Basic researchers need to work with clinicians to ensure that these new discoveries within the lab end up at the bed-

side. This is the only approach that will allow us to understand the brain and subsequently protect brain health and benefit patients, their families, and health workers. This is the aim of Malta Neuroscience Network. This collaboration is precisely what is needed right now.

Is it linked to any foreign Networks? If so, which and how?

One of our first accomplished achievements for the MNN was to be accepted as the 43rd member organization among the other European Neuroscience societies by FENS in January 2016 (http://www.fens.org/News-Activities/News/20161/01/Malta-Neuroscience-Network-as-new-member/). Our affiliation to FENS will be pivotal in the development of Neuroscience in Malta.

Another important collaboration is with the Mediterranean Neuroscience Society, made slightly easier by the fact that I am the treasurer of this Society (www.mnsociety.net/). The Mediterranean Neuroscience Society was created to support and help strengthen all initiatives that bring together Mediterranean neuroscientists. This has been achieved through schools and biannual meetings that have proved to be highly beneficial, not only for the scientific exchanges, but also in terms of training opportunities for students and young researchers. I am very happy that, after a successful 2015 meeting in Cagliari (Sardinia, Italy), the next meeting will take place in Malta in 2017 and MNN will be involved in its organization.

This year, you are also running a Brain Awareness Campaign. Why is that?

Brain Awareness Week (BAW) is the global campaign to increase public awareness of the progress and benefits of brain research. The global celebration, launched by The Dana Alliance for Brain Initiatives (http://www.dana.org/About/DABI/), presents an opportunity to bring attention to advances in brain science and advocate for science funding. Brain Awareness Week serves as a launching point for year-round Brain Awareness activities. Every March, BAW unites the efforts of partner organizations worldwide in a celebration of the brain for people of all ages. Activities are limited only by the organizers' imagination and include open days at neuroscience labs; exhibitions about the brain; lectures on brain-related topics; social media campaigns; displays at libraries and community centres; classroom workshops; and more.

The Malta Neuroscience Network launched the first Brain Awareness Week (March 14–18, 2016) in Malta (Fig. 3) and joined the global campaign with prominent neuroscientist Prof. Giacomo Rizzolatti, and Prof. Vincenzo Crunelli from Cardiff University. Moreover, local neuroscientists, patients and NGOs have participated. The events have been organized in conjunction with RIDT, the University of Malta, Caritas Malta Epilepsy Association, the Istituto Italiano di Cultura and the Malta Chamber of Scientists. The following shows the events we have organized in more detail.

Figure 3: Official logo of the 1st Malta Brain Awareness Week designed by Mr Malcolm Bonello of the Faculty of Media & Knowledge Sciences.

Brain Awareness Week Program

Monday 14 March
CINEXJENZA & MNN

Film:	The Diving Bell and the Butterfly
Speakers:	Dr Owen Falzon and Dr Josanne Aquilina
Venue:	ICT Auditorium, Faculty of ICT, University of Malta
Time:	18:30

Tuesday 15 March
Lab visits at University of Malta

Time:	10:00 – 13:00
Open Lecture:	The Anatomy and Function of the Brain
Speaker:	Dr Christian Zammit
Venue:	Anatomy Lecture Room (ALR), Faculty of Medicine & Surgery, University of Malta
Time:	17:30

Wednesday 16 March
Café Scientifique & MNN

Discussion:	Brain Oscillations in Epilepsy
Speaker:	Prof. Vincenzo Crunelli
Personal experience by:	Ms Trudy Kerr and Dr Anna Micallef
Venue:	Italian Cultural Institute, Valletta
Time:	19:00

Thursday 17 March
Lab visits at University of Malta
Time: 10:00 – 13:00
Autism Round-table: Malta/Italy experience
Venue: At the Medical School Boardroom, Medical School, Mater Dei Hospital
Time: 15:30 (Fig. 4)

Open Lecture: From mirror neurons to a mirror brain
Speaker: Prof. Giacomo Rizzolatti
Venue: South Auditorium, Mater Dei Hospital
Time: 17:30

Friday 18 March
Video conference: Controversies in ADHD
Transmitting live from ACAMH UK
Venue: The Illustrations Unit, Mater Dei Hospital
Time: 10:00 – 17:00

Figure 4: Professor Rizzolatti and Professor Di Giovanni at the "1st Autism Round-table: Malta/Italy experience" at the MDH.

The BAW picked with a fund-raising concert on Easter Sunday, 27 March, at St Publius Church Floriana at 19:30 by the internationally acclaimed violinist, Carmine Lauri and a chamber ensemble under the direction of Michael Laus that performed Vivald's Four Seasons. The concert was organized by the University of Malta's Research, Innovation and Development Fund with all proceeds going towards brain research at the University of Malta.

Was the Brain Awareness a success? Why?

It was a great success! There was a very good response from the general public. Considering that this was the first national campaign I was very satisfied. People are very keen to learn more about the mysteries of the brain. Moreover, 1 out of 4 families have a person with some brain disorders, so it is clearly not just an academic problem. Importantly, we also targeted the young generations and facilitated school visits to some laboratories at the University of Malta.

Department of Physiology and Biochemistry (Prof. Mario Valentino)
Introduction to the brain, understanding of normal brain function, videos and images of live brains (from mice), and effects of disease (Fig. 5).

Department of Communications Therapy (Prof. Helen Grech), Department of Cognitive Science (Prof. Ian Thornton & Prof. Noellie Brockdorff) (Fig. 6)
A brief introduction to cognitive science and experiments that are done to measure cognitive performance.

Department of Intelligent Computer Systems (Dr George Azzopardi)
Introduction to the visual system of the brain and how information about this can be used to build intelligent robots.

Department of Systems and Control Engineering & Center for Biomedical Cybernetics (Prof. Kenneth Camilleri, Dr Tracey Camilleri, Dr Owen Falzon)
An introduction to brain computer interface (BCI) systems, showcasing various EEG recording systems, videos of BCIs that have been implemented in our labs and performing an EEG recording of a volunteering student.

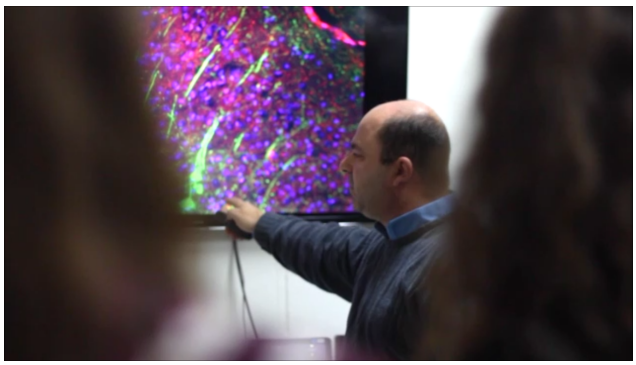

Figure 5: Prof. M. Valentino during a school visit to his laboratory at the University of Malta.

Why should people know more about the brain?

Brain illnesses are among the most important causes of death and disability worldwide. While 0.4 million people die from breast cancer every year, 1 million commit suicide. Add death due to drug abuse, Alzheimer's,

Figure 6: Professor Ian Thornton during a school visit to his laboratory at the University of Malta.

Parkinson's and other neurodegenerative disorders, and the numbers skyrocket. Yet, despite the high numbers, social awareness of brain research is low. Mental illness is still perceived as an indulgence, a sign of weakness or punishment. For patients, it carries powerful negative attributes in all of their social relations. The situation must be improved. Neuroscientists need to work together with the media and educators to raise awareness among the general population and politicians. Mental illnesses are just as important as other physical illnesses, only much more complex, due to the limited understanding of our brain (http://www.um.edu.mt/think/brain-awareness-research-and-facts/).

All people who work in the field of research know that science is needed to generate new knowledge. Science and technology have undergone continuous development over the past 400 years. As a result, our society today is highly technical and specialized. However, there is a parallel public ignorance of scientific knowledge and everything that has to do with scientific culture, especially in brain research. Brain awareness should be elevated to the centre of the focus of our society and politicians, particularly since our health starts, and ends, with brain health.

What other projects are incorporated within this Network?

After concentrating on increasing brain awareness, we next want to focus on facilitating the collaboration among us and start multidisciplinary research projects. We will keep working with RIDT to increase brain awareness and the fundraising campaign. In terms of brain awareness, I have stared a series of Feature Articles in THINK. In the next issue, I will talk about the use of marijuana in medical research and my research on this controversial drug (Di Giovanni, 2016a). This will be followed by other articles by other members of the MNN. I also published a divulgative article in the March 2016 issue of PINK magazine, regarding an ongoing research project in my laboratory entitled "the Paceville project", also known as "The Long-term Effects of Chronic Tobacco Use and Bingeing on Alcohol and Marijuana in Adolescence – a Gender Study". This project is the first to address the long-lasting and negative impacts of the abuse of these three substances together during the teenage years on mood, learning and memory in adults, with a focus on gender differences (Di Giovanni, 2016b). These articles were published in March in order to publicize the BWA and Carmine Lauri's concert.

Anything else you'd like to add?

We need to break research out of closed laboratories and truly collaborate. The future of brain health will expand exponentially when cognitive neuroscientists, medical doctors, molecular biologists, neuroengineers and other interdisciplinary team members come together to discover ways to promote brain performance in health, neurologic injury, psychiatric disturbance and brain disease. In Malta, we are behind the other European countries in this area. I know, it is hard to collaborate, to find the time to communicate science, to interact with colleagues and people, and to think about the community when you have your own personal problems, deadlines, lack of resources - both human and economical ones - but this is the only way to contribute to Malta's scientific development. I hope all my personal efforts and those of a few enlightened colleagues will not be wasted.

Collaboration is the fastest way to find real solutions that can change lives for all people – today.

For more information, join Malta Neuroscience on https://www.um.edu.mt/research/notices/joinmaltaneurosciencenetwork and follow https://www.facebook.com/uom.neuroscinet/ on Facebook and @neuroscienceUoM on Twitter.

The above questions were asked to Professor Giuseppe Di Giovanni by Mr Iggy Fenech and an edited version of these is posted on RIDT blog.

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Cover Picture Xjenza 1, 1 1996: The sponge *Cacospongia sp.* with the opistobranch, *Cratena peregrina* grazing on its surface. **Picture by:** Martin Vella.