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Aims and scope

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The Journal of Environmental Health Research is a peer reviewed journal published in three formats; Printed Full Journal, Printed Abstracts and On-Line Journal.

The Journal publishes original research papers, review articles, technical notes, professional evaluations and workshop/conference reports and short communications covering the diverse range of topics that impinge on public and environmental health including: occupational health and safety, environmental protection, health promotion, housing and health, public health and epidemiology, environmental health education, food safety, environmental health management and policy, environmental health law and practice, sustainability and methodological issues arising from the design and conduct of studies.

A special category of paper – the 'first-author, first-paper' – is designed to help build capacity in environmental health publications by encouraging and assisting new authors to publish their work in peer-reviewed journals. Here the author will be given active assistance by the editors in making amendments to his or her manuscript before submission for peer review.

The Journal provides a communications link between the diverse research communities, practitioners and managers in the field of public and environmental health and aims to promote research and knowledge awareness of practice-based issues and to highlight the importance of continuing research in environmental health issues.

Editorial correspondence: Papers for publication, letters and comments on the content of the Journal and suggestions for book reviews should be sent to the editors by email, to m.vaganay@ulster.ac.uk.

Details regarding the preparation and submission of papers can be found at the back of this issue and at www.jehr-online.org.

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Editorial team

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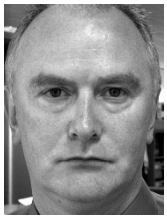
Miss Julie Barratt is Director of the Chartered Institute of Environmental Health, Wales. She graduated in environmental health in 1981 and has a wide range of practice experience in environmental health. She graduated in law in 1992 and qualified as a barrister in 1993. She is legal columnist for Environmental Health News.



Mr Martin Fitzpatrick is a practising environmental health professional with Dublin City Council currently specialising in environmental protection. He is an advisor, consultant and author to the World Health Organisation and the United Nations Development Programme and has advised on, and managed, environmental health projects in Europe, Indonesia, Latvia, Kazakhstan and Thailand. He was an advisor to the WHO preparatory meeting on the Third Ministerial Conference on Environment and Health, advisor to the Department of Health and Children in the Republic of Ireland and environmental health advisor to Concern International in Banda Aceh following the tsunami disaster. He has been associate editor of JEHR for the past seven years and is a member of the Environmental Health Officers Association of Ireland.

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Editorial

Dr Marie Vaganay
Editor

The Environmental Health team at the University of Ulster is pleased to launch this edition of the Journal of Environmental Health Research. This edition contains six peer-reviewed papers, covering a wide range of issues of national and international public health importance, which we hope will interest most, if not all, readers.

Over the years the Journal has been very successful. It has provided momentum in highlighting the importance of research-based work and an evidence-based approach to practice for Environmental Health professionals. This has resulted in an increased number of submissions, both at national and international level, and the number of peer-reviewers willing to contribute.

We would like to take this opportunity to acknowledge the work and contributions of staff to the journal over the last nine years and we would especially like to thank Professor Harvey for his efforts and dedication as editor-in-chief. We would in particular wish to place on record our recognition of Professor Harvey's vision, stewardship and guidance in the production of this Journal which he saw as pivotal in promoting research in the field of environmental health.

The Environmental Health team at Ulster is committed to produce and maintain a high quality Journal and will strive to continue with its improvement and development.

In order to attract high quality papers from the academic community at both national and international level there is a need to consider and prepare bids for listing on key publication databases. This is essential to gain further international recognition in the wider academic community. This would also increase the impact factor of the journal and undoubtedly attract papers of the highest quality continuing Professor Harvey's work.

It is anticipated to review the submission process and the web interface for contributors in order to facilitate submissions and encourage first time authors. In the next few months, therefore, there will be a review of the web-page and the development of an online submission system.

It is our hope that the Journal will continue to make a significant impact in showcasing both the work of environmental health practitioners and their contribution to public health.

The dissemination, via publication, of good practice and evidence from research will strengthen the role of environmental health practitioners, as we move towards a new public health agenda, and it will also assist the profession in adapting to changing circumstances, whatever they may be.

We look forward to your contributions and hope that you enjoy this, and future, editions.

Dr Marie Vaganay and the Environmental Health Team at The University of Ulster

Guest editorial

David Kidney
Head of Policy,
Chartered Institute of
Environmental Health

Why we should all sign up to sustainable development

Scientific consensus tells us that climate change is real, that human activity is the main contributor and the consequences will be devastating if we do not take mitigating action to address it now.

Even if you doubt the science, you can agree that finite natural resources and a rising global population mean that we cannot go on living the way we do today.

So there is an urgent need for all the world's citizens to take action. Action to protect our communities and nature's biodiversity against the effects of climate change already happening (adaptation), action to reduce emissions of greenhouse gases in the future (mitigation) and action to take greater care of our planet's natural resources (by living our lives more sustainably).

The challenges are so great that it will require major changes to our economic, environmental and social systems to address them. Social justice suggests that the developed nations and their citizens should make the greatest contribution to addressing climate change, as we in the developed nations have consumed more of the Earth's natural resources, emitted more of the greenhouse gases and have accumulated more of the wealth from which action will be funded.

If we don't act collectively on the scale necessary, the situation will become critical both for our planet's biodiversity and for humankind's survival.

The challenges are global – so they must be met with solutions at a similar scale.

Internationally, many nations and citizens are convinced of the need to change. There have been pressures for action, from the Rio Earth Summit via Kyoto and Copenhagen to the recent United Nations-led search for a comprehensive treaty, in Mexico in December 2010.

Politicians discuss allocating responsibility for carbon reductions according to each nation's stage of development: the biggest reductions from developed nations, stabilisation by developing nations and positively lending a hand to least-developed nations, including substantial funding supplied by the developed nations. They say they will put in place mechanisms for sharing technological know-how as well as monitoring, verification and policing by international institutions.

So far, 120 nations have signed a less comprehensive Accord, promising some action but not as far-reaching as the deal sought at Copenhagen last year.

The focus may be on cutting carbon emissions, addressing global warming and helping the poorest countries defend themselves and their citizens from the climate change that is already happening, but we need a strategy that embraces all aspects of sustainable development.

Nationally, the UK is making the transition from a high to low carbon economy. The Climate Change Act sets legally binding targets for cutting carbon emissions and puts in place the structures to keep us on track: carbon budgeting, the independent Committee on Climate Change, the development of sustainability indicators and regular reports to Parliament.

Guest editorial

The UK Low Carbon Transition Plan complements the law by setting the policies needed to help us meet the legal targets. Reducing carbon emissions is a major objective, but action is proposed also to reduce overall energy consumption, promote public transport and reduce waste, especially waste going to landfill.

So much of what needs to happen depends on winning the hearts and minds of individuals and communities. That is why we need public information campaigns like "Act on CO_2", by traditional media methods, social media and intermediaries.

Some of the actions we must take will cost more money, they may result in higher or new taxes or higher energy bills – but others can save us money, for example energy efficiency measures that cut energy consumption. In addition to these actions economies can reap the benefits of new green jobs.

To maintain public support, as we make the transition to a low carbon, more sustainable, economy, we must ensure that it will be a just transition: sharing the costs and the benefits fairly.

That's why, when I was a government Minister in the Department for Energy and Climate Change in 2009, we established a forum for a just transition, offered help tackling fuel poverty, made recycling easier and promoted green skills, so that we all benefit from the opportunities in a low carbon and resource-efficient economy.

There is good practice already in the UK and in other countries. Our challenge is to make the good practice mainstream so that we all cut carbon emissions and live our lives more sustainably.

Environmental health practitioners already take on these challenges. In communities everywhere, they work on every aspect of sustainable development. Air quality, food that is safe, nutritional and from sustainable sources, occupational health and safety, public health and warm homes – you name it, they do it.

Many organisations, groups and individuals will help make the difference as nations and communities strive to become more sustainable – you can be sure that environmental health practitioners will be among them.

The efficacy of industry self regulation of the use of UV skin tanning equipment

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Abstract

Although there is considerable guidance available, the skin tanning industry in the UK is unregulated, although legislation currently in force in Scotland will regulate tanning salons with effect from October 2009. Commercial tanning salon operators through The Sunbed Association (TSA) has, and continues to resist, calls for regulation of the industry claiming that they are regulating it themselves and that customers are appropriately advised of all of the potential issues surrounding the use of the equipment. For this study 120 premises offering sunbeds were identified and 69 of them visited by a researcher posing as a potential customer to determine what advice and controls were in place to prevent misuse of the facilities. None of the premises surveyed made all of the appropriate inquires or gave all of the advice identified in the existing guidance, including those premises that were members of the TSA, and no meaningful controls were exerted over the users or the use of the facilities. It is therefore suggested that self regulation of the UV skin tanning industry is not effective and that given the potential short and long term skin damage that can be caused by UV skin tanning equipment, the industry should be subject to legislative control and enforcement.

Key words: *sun beds, The Sunbed Association, skin cancer*

Introduction

The sun bed industry in England, Wales and Northern Ireland is unregulated insofar as use of the skin tanning facilities is concerned. Regulation came into force in Scotland in October 2009. The industry relies on self regulation. There is a considerable volume of advice on use of sunbeds, most of which is directed at the industry, including advice from the British Medical Association (Policy on the use of sunbeds, 2003), the Health and Safety Executive (HSE Guidance Note INDG209) and European Guidance (European Standard EN60335-2-27:2003). The advice, however, is not consistent, varying across the organisations, and the industry is free to disregard it should it wish to do so.

The Sunbed Association (TSA) is the professional body representing sunbed operators. It has issued a Code of Practice, "The Sunbed Code" with which its members are required to comply. It is based on European Standard EN60335-2-27:2003 and on HSE Guidance Note INDG209 and is implemented through a safety manual and training programme. Membership of TSA is voluntary and currently TSA represents approximately 22% of sunbed operators in the UK.

Research into the efficacy of self regulation in this industry in Australia suggests that it is not an effective control mechanism (Paul *et al.,* 2005, Dobbinson *et al.,* 2006, Gordon *et al.,* 2008). In 2004 the Australian Government Radiation Health Committee issued a position statement that encouraged compliance with the Australian/New Zealand Standard on solaria for cosmetic purposes (AS/NZ 2635:2002) a voluntary code of practice designed to provide solarium operators with procedures to minimise health risks associated with indoor tanning. All of the studies found that compliance with industry-led voluntary Codes of Practice was poor and all concluded that other forms of harm minimisation were necessary, citing variously taxation strategies and mandatory staff training programmes but with the introduction of regulation being the preferred option.

Current guidance

Age of user

Both the British Medical Association (BMA) and Cancer Research UK advise that no-one should use sunbeds for cosmetic purposes, and both organisations advise that no person under the age of 18 should use a sunbed. HSE Guidance Note INDG209 and TSA recommend that individuals under the age of 16 years should not use sunbeds.

Skin type

Skin type is divided into six categories, depending on how the skin reacts to sun.

- **Skin type I:** Always burns, never tans, sensitive to sun exposure. Redhead/freckles.
- **Skin type II:** Burns easily, tans minimally, fair skinned, blue/green/grey eyes.
- **Skin type III:** Burns moderately, tans gradually to light brown.
- **Skin type IV:** Burns minimally, always tans well to moderately brown, olive skin.
- **Skin type V:** Rarely burns, tans profusely to dark, brown skin.

- **Skin type VI:** Rarely burns, least sensitive, deep pigmented skin

The TSA Code of Practice states that individuals with Type 1 skin should never use sunbeds. HSE Guidance Note INDG209 advises that sunbeds should not be used by individuals who have fair, sensitive skin that burns easily or tans slowly or poorly; a history of childhood sunburn; a large number of freckles and/or red hair or a large number of moles.

Intensity of use

There is considerable confusion surrounding intensity of use. The BMA in its policy statement on use of sunbeds says that sunbeds should not be used for cosmetic purposes at all. However, The British Photodermatology Group (BPG) recommends that, should people choose to use sunbeds, they should not exceed two courses a year of no more than 10 sessions per course. The HSE recommends no more than 20 sessions a year and suggests that the user consult with the operator of the sunbed to determine how long a session should be, depending on their skin type. TSA advises that two to three sessions a week is acceptable but that skin should be rested for 24 hours between each session where user is of skin type 3 and 4 and 48 hours where the user is of skin type 2.

While some operators claim that they will not allow users to exceed recommended limits either in respect of frequency of use or cumulative use over a period of time, it is not at all clear how such a policy can be enforced. This is particularly the case as some operators offer reduced rates for block booking, such as discounted offers – '24 sessions for the price of 20' being on example identified during the study. Meaningful control over frequency of use of the sunbeds in a salon would require some form of customer identification and registration such that use over a prescribed number of times could be prohibited.

Use while taking medication or with pre-existing medical conditions

Some skin conditions and medication increase the photosensitivity of skin, making it more likely to burn. The BMA recommends that sunbed users should be screened to ensure that they are not taking any medication that would induce photosensitivity reactions or suffer from abnormal sensitivity to ultraviolet and/or visible radiation. This advice is also given in HSE Guidance Note INDG209. The training given to TSA members includes the advice given by the HSE in INDG209 and therefore should include advice regarding medication and pre-existing skin conditions and the use of sunbeds.

Use of eye protection

The adverse health effects of UV radiation on eyes is well documented (NRPB 2002). All of the guidance regarding use of sunbeds is clear that eye protection should be used. HSE Guidance note INDG209 states that where eye protection is not provided, the facility should not be used. TSA requires that its members provide eye protection.

Availability of advice

There are a number of disparate sources of advice. General advice is available from sources such as the internet, television and health and women's magazines, with very specific advice being available from specialist websites such as the SunSmart pages of the Cancer Research UK web site. For those who have made the decision to use a sunbed, however, the advice should be obtainable at the salon, either from posters or leaflets or from the staff. For users to be fully aware of the potential risk they may be taking when using a sun bed it is essential that information is readily available and staff are able to give appropriate advice.

The sunbed industry claims that it is capable of managing the risk that exists from the use of UV skin tanning equipment by self regulation. It claims that advice for customers is readily available and that customers are protected from over exposure by its ability to prevent them accessing sunbeds for too long or too frequently. This research project sought to establish the validity of those claims and to determine whether the public are adequately protected by the practice of self regulation.

Methodology

The research was carried out in South East Wales and took the form of secret shopping at premises offering sunbeds. Secret shopping was the preferred method of information gathering as it was considered that advice given to a secret shopper would be more 'real' than would be given if the researcher revealed the purpose of the visit, which may be more likely to provoke the giving of model answers. At each site the researcher sought advice regarding safe use of the equipment either from staff or by checking whether advice in the form of a poster or leaflet was available. Where staff could be questioned, a pre-planned interview script was used to ensure

consistency of questioning; follow-up questions were used where additional clarification was required. All visits were cold calls, the researcher posing as an individual wishing to get a sun tan before going on holiday and seeking advice about how best this could be achieved.

Study sample

The study was carried out across eight local authority areas in South East Wales: Blaenau Gwent, Caerphilly, Cardiff, Monmouthshire, Newport, Rhondda Cynon Taf, Torfaen and the Vale of Glamorgan. From a desk top study and telephone survey, 120 premises were identified as having sunbeds within the study area. These included manned and unmanned premises and premises operated by local authorities, private members clubs and those available for public use.

Of the 120 premises, six were found to be entry/use by private members only and were therefore excluded from the physical survey.

Fifteen unmanned facilities were identified. Of these, three were stand-alone independent businesses, the remaining 12 being operated by two multi-outlet groups. Each of the stand-alone unmanned premises was visited; one each of the premises operated by the two multi-outlet groups was visited as being representative of the remainder.

The 104 manned general access facilities identified fell into definite types, being sunbed salons, beauty salons, fitness centres/gyms and hairdressers. Sixty-nine premises were visited (66%), being a representative sample each group.

Information sought

A series of questions was designed that the researcher asked at each premises. Where the premises was unmanned, the researcher used the available literature to source the answers. If that information was available it was recorded as a positive response. It is, however, recognised that users of the facility may chose to ignore such literature in the absence of staff to draw it to their attention, so this may artificially inflate the results.

The information sought was:

1. Must users register with the premises?
2. Were they asked if they had used a sun bed before?
3. Were they asked when they last used a sun bed?
4. Was a skin type assessment carried out?
5. Was a suggested tanning period recommended?
6. Were they were suffering any illness/skin condition?
7. Were they asked if taking medication?
8. Was advice on display?
9. Was safety advice given?
10. Were under 16s allowed to use the sunbeds?
11. Was eye protection provided?
12. What time period was recommended between sessions – 24h, 48h or freeflow?
13. Is salon a member of TSA?

The first question asked (was it necessary to register at the premises) was asked to determine whether the salon had any method of measuring how many sessions an individual user had had within a finite time period.

The final question asked was whether the salon was a member of TSA. In the light of the training and guidance given by TSA to its members, it would have been reasonable to expect higher levels of knowledge and awareness to be demonstrated by TSA members. This question was asked as the final question since it was considered that an ordinary member of the public was unlikely to have been aware of the existence of TSA and, that being the case, to ask the question earlier in the exchange might have caused suspicion and the offering of model answers.

At the end of the interview the researcher indicated that it was not convenient for them to have a sunbed session at that time and that they might return later. No sunbed sessions were purchased.

Results

Manned facilities

Q1.Must users register with the premises?
Of the salons visited, some did offer a registration card. This was not, however, linked to any form of detection of overuse; it was generally linked to an offer of discounted or block booked sessions and was used to record the number

of sessions remaining unexpired. In the view of the researcher, where block sessions were purchased there would have been little to prevent the user taking all of the purchased sessions in a very short period of time.

Q2 Was the user asked about previous sunbed use?
At 25 (36%) premises the researcher was asked about previous sunbed use. Where this issue was addressed it was in relation to determining the user's need for advice and ability to operate the equipment rather than as an attempt to elicit information about previous health reactions to exposure to UV.

Q3 Was the user asked when they last used a sun bed?
None of the 69 premises surveyed asked the researcher when they had last had a sunbed session. This meant that overuse of sunbeds was not restricted or prevented.

Q4 Was a skin type assessment carried out?
At 16 (23%) of the premises the researcher was given a formal skin type assessment. In the remainder, it is possible that the operator formed an informal judgement based on the colouring of the hair, eyes and skin of the researcher, but in no case was there formal acknowledge that this had been done.

Q5 Was a suggested tanning period recommended?
As noted, 25 (36%) of the premises asked the researcher if they had used a sunbed before. The researcher indicated that there had been previous use but that it was years before. This led to varying periods being suggested as a first session, usually based on the strength of the individual tanning equipment. Seventeen (25%) of the 69 premises surveyed did not offer a suggested tanning period, leaving it for the researcher to decide how long to purchase.

Q6 Was the researcher asked about pre-existing illness?
Of the 69 premises where interviews were conducted only three (4%) asked whether the researcher was suffering any form of illness. Of those premises displaying notices regarding illness, none verbally confirmed whether the researcher was suffering any form of ill health.

Q7 Was the researcher asked whether taking medication?
On only two (3%) occasions, the researcher was asked whether they were taking any form of medication. As with pre-existing illness, where notices were displayed giving advice about use of prescription medication there was no attempt to confirm verbally whether the advice had been read and was applicable.

Q8 Was advice on display?
Advice that can be displayed in sunbed salons is available from a number of sources. It can be downloaded from the internet from a number of skin cancer web sites and is also a free-standing attachment to HSE Guidance Note INDG209. This notwithstanding, only 19 (28%) of the premises displayed clearly legible warning notices in prominent places, both in reception and in the individual cubicles.

Q9 Was safety advice given?
In 18 (26%) of the premises, the researcher was given a safety sheet or card to read. Some of the sheets were leaflets containing questions that if answered positively by the potential user, should cause them to reconsider having a sunbed session, e.g. Are you pregnant? Are you taking prescription medication? The researcher was given the sheet to read but was not questioned in any premises as to whether the answer to any of the questions was positive.

Q10 Were under 16s allowed to use the sunbeds?
In order to gain a response to this question, the researcher indicated that they had a 15 year old daughter who would be accompanying them on holiday, and wanted to know whether she could have a sunbed before travelling. Of the 69 premises surveyed, 30 (43%) refused to allow under 16s to use the sunbeds. Thirty-nine (57%) were willing to allow under 16s to use the sunbeds, subject to them being accompanied by an adult or having parental consent. Three of the 39 premises were members of TSA.

Q11 Was eye protection provided?
Sixty-eight out of the 69 manned premises provided eye protection in the form of goggles. Some additionally provided adhesive patches which were offered as an alternative and were seen as preferable by some customers as they prevented 'panda eyes'. In the one premises that did not have eye protection available, the researcher was advised that the business was in the course of being taken over and the provision of eye protection had been overlooked. It was indicated that sale of sessions would be refused until goggles could be provided or unless the customer provided their own eye protection.

Q12 What time period was recommended between sessions?
Of the 69 manned premises interviewed, 61 (88%) would allow a customer to have a session every day, subject to there being 24 hours between uses. Eight (12%) premises required a 48 hour period between sessions. In

all of the cases it was strongly suggested to the researcher, on further detail being requested, that the sunbed should not be used more than once on any day.

Q13 Was the salon a member of TSA?
Of the 69 manned premises where interviews were conducted, 21 operators (30%) were members of the TSA. This is slightly above the national average of 22%.

Unmanned facilities

Unmanned facilities do not offer any form of one-to-one advice to users. As no staff are present at the facility, all advice and information is given in the form of posters or leaflets. Time units on the sunbeds are accessed through purchase of coins representing a number of units of time; it is a matter for the customer to decide how many coins are purchased and used at any one time. Fifteen unmanned salons were identified in the survey area; of those, five were visited by the researcher. The researcher attempted to find readily available information at the unmanned premises to satisfy the questions asked of the manned salons.

The unmanned salons all had signage indicating that the facility should not be used by persons under the age of 16 years. There was, however, nothing to prevent such use and at the time of the visit to one of the salons, the researcher observed a number of customers who appeared to be under the age of 16 years placing coins into a machine in order to access a sunbed session.

There was also no obvious mechanism for controlling the number of sessions purchased by an individual customer. Although CCTV was in evidence at all of the salons, in the view of the researcher it was felt to be unlikely that a recognition of the same user on having repeated uses on the same day would elicit any emergency physical response from the premises operator to deny further use. This would in any event be too late to prevent the use that had already taken place. It was considered more likely that the CCTV equipment was in place to deter damage, vandalism and theft.

All of the unmanned facilities had health advice in the form of posters, but there was no requirement on the part of the potential user to read the advice.

Importantly, in unmanned facilities the provision of goggles was not free. Goggles could be purchased from vending machines, where the cost of purchasing them equated to several minutes of sunbed use. It was also noted that there was nothing to preclude users bringing their own eye protection to use while using the sunbed, but it was a serious concern to the researcher that some users, and particularly those under 16 years, may chose not to use goggles but additional minutes of sunbed time instead and that there was nothing to prevent this happening.

A number of operators, notably some local authorities, justify their continued sunbed offer by claiming that they operate to a higher level of control and restrict users to a fixed number of sunbed sessions within a prescribed period. The researcher therefore set out to establish how many sunbed sessions could be accessed by one user in the course of one day. Manned sunbed salons typically operated between 9.00am and 5.30 pm, although those operated by gyms and health clubs operated for longer hours. Unmanned salons typically operated by the opening of a remote-controlled time lock operated between 7.00am and 9.00pm or 10.00pm.

Clearly, as the user had more sunbed sessions their skin would begin to colour and they would be unlikely to wish to continue to access further sessions, or would be refused further sessions by the operator of manned salons, making all day use unlikely. However, allowing for travel time and time spent undressing, using the sunbed and dressing, it was noted that in Cardiff it was possible to visit up to 20 sunbed salons in the same day, including a number of unmanned facilities where the time spent on the sunbed would not be controlled by an operator. In a smaller town in the South East Wales valleys it would be possible to access six sunbed salons on foot in the same day.

Discussion

The research shows that within the skin tanning industry knowledge of risk attributable to sun beds where it exists is patchy and inconsistent. None of the salons asked all of the questions that were regarded by the research team as important. Where inquiries were made, there was no suggestion that the potential customer would be prevented from accessing a sunbed; rather the choice whether to do so or not would be a matter for the customer.

Knowledge of the importance of skin type and time period since most recent use, which is critical if the operator is going to give meaningful advice regarding overuse and excessive exposure, was poor. Less than 25% of manned premises made adequate inquiry regarding these issues and even where inquiry was made, there was

no guidance given to the potential user about the adverse health effects of overuse. The researchers saw no evidence of any system on operation that would have prevented an individual customer exceeding a maximum recommended of sessions. Indeed, given the prevalence of sunbed salons, both manned and unmanned operating as commercial competitors, it is hard to see how any such system could operate effectively.

While most operators recommended that users should not have more than one session in 24 hours, many of the premises operated on a 'cash over the counter' basis and the researcher felt it would have been possible to access more than one session in a day had the customer been determined to do so. It was also noted that in those premises with longer opening hours such as gyms and health clubs, the receptionists changed during the day, making it possible to access two sessions – one at the beginning and one at the end of the day.

There is considerable evidence to show that salon operators and those operating unmanned facilities are aware that exposure for under 16s is undesirable. It is a cause for concern, however, that over 5% of the manned salons would allow a 15-year-old to have a sunbed session subject to parental approval or the presence of an adult at the time. This suggests that the operators are more concerned with respect to the issue of their own liability in the event of an episode of burning or skin damage than they are cognisant of the potential long term skin damage that could result from the use. There was no control at all over the age of users of unmanned facilities. It is unrealistic to expect those under the age of 16 years to take account of an unenforced notice warning that they may not use any unmanned facility, and indeed the researcher was able to see some individuals who appeared to be under the age of 16 disregarding the notices and accessing the facilities.

It is well established that the use of particular medication and the pre-existence of certain skin conditions can cause skin to be more photosensitive. There was little understanding of this concept or of the need for those taking medication or suffering from the conditions to avoid exposure to UV light. In both cases the customer would have to disclose information that was uniquely known to them, and it is questionable whether an operator in a sunbed salon would have the necessary knowledge to advise as to whether a particular drug or drugs in combination would give rise to adverse health effects. The only meaningful advice that could be given would be for the potential customer to seek advice from their GP. Given the accessibility of sun tanning facilities, it is possible that a customer determined to use a sunbed may leave one facility on being given advice and go to another where the relevant information would not be disclosed.

The need for eye protection was understood and in all but one case in the manned premises was provided. In the unmanned facilities, eye protection could be purchased, but its use could not be required. It is, however, acknowledged that even in manned premises, the operator could not be sure that the customer was using eye protection once they went into the sun-tanning booth.

Twenty-one (30%) of the 69 manned premises surveyed and interviewed were members of TSA. It was the view of the researcher that those premises were better run and the staff appeared, on questioning, to be more knowledgeable. It is, however, a matter of considerable concern that three of the 39 premises that would allow under 16s to have sunbeds were members of TSA even though TSA states clearly in its Code of Practice that under 16s should not use sunbeds. It is also the case that TSA speaks for the sunbed industry while representing only 22% of sunbed operators in the UK. Its requirements of its members are good, if enforced, but on the evidence of this research its own members do not comply with its requirements, calling into question the effectiveness of its role as a regulating body.

This study suggests that the skin tanning industry is not effectively controlled. The guidance available conflicts and is not helpful. Advice, where it is given, is given on the basis of assisting the potential user to access the sunbed rather than on deterring or preventing use. Given that the operation of sunbeds is a commercial one, this is hardly surprising, but it is not appropriate that an industry capable of causing acute short term as well as long term skin damage should be allowed to self regulate.

The results of this research are consistent with results from studies carried out in Australia between 2005-2008 (Paul *et al.,* 2005, Dobbinson *et al.,* 2006, Gordon *et al.,* 2008). In both countries, regulation of the sun-tanning industry was by way of compliance with a voluntary code of practice and in both compliance when tested by way of secret shopping was poor and did not protect the health of the users of the facilities. The failure of the sun tanning industry to regulate itself in Australia and New Zealand has led to calls for the industry to be subject to legislative control. Based on the findings of this research the same case for legislative control can be made for the UK industry.

Conclusions

- Self regulation of the manned skin tanning industry is not effective, and is wholly ineffective in respect of unmanned skin tanning facilities.
- Legislation is required to control the operation of UV skin tanning facilities. Regulators should have the power to close premises that operate in breach of legislation.

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Surface properties and slip resistance of glazed ceramic tiles over-treated, or treated multiple times, with hydrofluoric acid

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Abstract

There are many anti-slip treatments that claim to reduce the risk of slips and falls. In a previous investigation, it was shown that the normal treatment of smooth and glazed ceramic tiles with an aqueous solution of hydrofluoric acid increased only slightly the dry and wet friction and the average roughness but made the tiles more fragile and more difficult to clean. In this article, we report the impact of multiple cycles of hydrofluoric acid treatment and abrasive wear and the impact of over-treating the tiles with 10 times the amount of hydrofluoric acid normally used. The overtreatment removes more glaze than the normal treatment and it leads to a significantly rougher and more slip resistant surface. On the other hand, the surface properties and slip resistance of the tiles exposed to multiple cycles of hydrofluoric acid treatment and abrasive wear do not evolve much.

Key words: Wet friction, dry friction, wear, abrasion, etch, gloss, roughness, flooring.

Introduction

There are many anti-slip treatments based either on ammonium bifluoride or hydrofluoric acid that claim to increase the slip resistance of glazed tiles and hence reduce the risk of slips and falls. In a previous article (Quirion *et al.*, 2009), it was shown that normal hydrofluoric acid treatments had a positive but limited impact on the average roughness and slip resistance of glazed ceramic tiles. At the same time, the tiles lost their gloss and became less resistant to abrasion and more difficult to clean. The consequences of such treatments would be to slightly improve the slip resistance in the short term but with a faster deterioration of the surface due to the decreased resistance to abrasive wear. Thus, the owner might be tempted to reapply the treatment. This investigation addresses the question of the impact of multiple cycles of hydrofluoric acid treatment and abrasive wear on the surface properties of glazed ceramic tiles.

As observed in our previous investigation (Quirion *et al.*, 2009), the slight increase in slip resistance obtained after the normal treatment of the glazed ceramic tiles is somewhat contradictory with other studies that report a significant increase of the slip resistance following the chemical treatment of glazed ceramic tiles (Grönqvist *et al.*, (1992), Di Pila (2000), Bowman *et al.*, (2002), Carpenter *et al.*, (2006)). However, it is difficult to compare the results of these studies with our results because the authors do not report the nature of the chemicals used and/or the application procedure. Recently, Houlihan (2009) applied a commercial product containing hydrofluoric acid at 0.5% for 20 minutes on glazed ceramic tiles with only a limited improvement of the slip resistance and almost no changes in the roughness of the tiles. These results agree with ours obtained with hydrofluoric acid at 8.6% for 20 minutes. Thus, maybe it takes a more aggressive treatment to significantly improve the slip resistance of glazed tiles. In this study, we report the surface properties and the slip resistance of glazed ceramic tiles treated with 10 times the amount of hydrofluoric acid used previously.

Methodology

Chemical and abrasive wear of the glazed ceramic tiles

All the results presented in this investigation were obtained with Cecrisa glazed ceramic tiles (White Basic Matte, PEI = 4, 200mm x 200mm). The tiles were used as received or cut into 100mm x 100mm samples. The chemical wear refers to the amount of glaze removed by one treatment with a given amount of hydrofluoric acid solution. The abrasive wear refers to the amount of glaze removed by abrading a sample tile under given conditions.

In this article, a treatment always refers to the application of a solution of hydrofluoric acid on the glazed tiles. The procedure described in our earlier study (Quirion *et al.*, 2009) is referred to as normal and it was used for the investigation of multiple treatments. A more aggressive procedure, described below, was used for the investigation of overtreatment. Note that hydrofluoric acid (CAS: 7664-39-3) and its vapour are considered as corrosive and extremely hazardous. Treatments using hydrofluoric acid should be performed only by trained and qualified personnel.

Multiple treatments

Two small sample tiles (100mm x 100mm) were exposed to 20 cycles of normal hydrofluoric acid treatment and abrasive wear. Cycle 0 corresponds to the cleaning of the tiles with water followed by abrasive wear. The following treatments with hydrofluoric acid and abrasive wear correspond to Cycles 1, 2, etc.

The normal treatment corresponds to the application of the hydrofluoric acid solution (8.6% w/w) on the glazed

ceramic tiles (~400ml/m^2) for 20 minutes. During that time, the tiles were scrubbed gently with a piece of red floor pad to keep the surface wet. After the treatment, the tiles were rinsed thoroughly under running water, without any neutralisation and dried at room temperature for 18 hours. The chemical wear corresponds to the mass loss due to the chemical reaction of the hydrofluoric acid with the glaze and it is expressed in g/m^2.

To simulate the wear from light traffic, the sample tiles were sanded twice for 20 seconds each time with an orbital sander (Grit 220, Al_2O_3, with only the pressure due to the weight of the sander, 1.2Kg). This light abrasion allows us to identify fragile surfaces. The abrasive wear corresponds to the mass loss from abrasion and it is expressed in g/m^2.

It is very difficult to compare the aggressiveness of these cycles with real life. While the chemical wear should be essentially the same, our choice of a light abrasive wear certainly underestimates abrasion caused by real life traffic and maintenance. It is thus reasonable to assume that, in real life, a cycle of hydrofluoric acid treatment and abrasion will result in higher wear (chemical and abrasive) than in the laboratory.

Overtreatment

For the overtreatment, the hydrofluoric acid solution was applied undiluted (17.2 % w/w) at ~400ml/m^2 with gentle scrubbing for 20 minutes. The solution was then replaced with a fresh solution and the procedure was repeated until five consecutive treatments were performed. Overall, the surface of the tile was exposed to 10 times the amount of hydrofluoric acid used for the normal treatment. The resulting over-treated tile was dried and analysed. It was not exposed to abrasive wear.

Gloss

The gloss is a measure of the intensity of the light reflected from a surface. In this study, a red light beam (630nm) is projected at an angle of 45° on the surface of the tiles and the intensity of the light reflected at 45° is measured using a photo resistive cell. The gloss of a given tile is expressed as the percentage of the reflectivity of a new and untreated tile (100 %).

Average roughness

The average roughness, Ra, was determined with a DekTak 3030 equipped with a diamond stylus having a tip radius of 12.5µm. The measurement proceeded at low speed over a length of 5mm with a 0.05mN (5mg) force applied on the stylus. Each value of Ra corresponds to the average of at least five measurements in five different locations of the sample tile.

Friction coefficient

The dry friction coefficient of the small tiles used for the investigation of multiple treatments was determined using the horizontal pull method. The larger tiles used to compare the dry, wet and oily friction of the over-treated tile with the untreated tile and the tile treated only once with HF were characterised using the Brungraber Mark II apparatus.

Horizontal pull

The dry friction coefficient of the small sample tiles (100mm x 100mm) was determined by pulling a small slider (126g) mounted on three stainless steel cap nuts on the dry tiles at a velocity around 22mm/sec. The ratio of the pulling force (horizontal force, F_H) to the weight of the slider (vertical force, F_V) gives the dynamic friction coefficient ($\mu_{dry,SS} = F_H/F_V$) of the stainless steel cap nuts as it slides on the surface. The friction coefficient was determined at least four times for each tile, after the treatment with hydrofluoric acid and after the abrasion of the surface.

Brungraber Mark II

The friction of Neolite on the large tiles (200mm x 200mm) was determined using the Brungraber Mark II. The friction was first measured on the dry tiles ($\mu_{dry, Neo}$). It was then measured as a function of the surface concentration (g/m^2) of a detergent solution (Sodium Lauryl Sulphate, SLS = 0.15 % in water) to get the wet friction ($\mu_{wet, Neo}$). Finally, the tiles were rinsed and dried and the friction was measured as a function of the surface concentration of vegetable oil (canola) to get the oily friction ($\mu_{oil, Neo}$). In this investigation, the notation $\mu_{wet, 25, Neo}$ and $\mu_{oil, 10, Neo}$ refers to the friction at 25g/m^2 of detergent solution and 10g/m^2 of vegetable oil, respectively.

The Mark II consists of a 10lbs articulated foot that is dropped on the floor at an angle corresponding to a friction coefficient. If the foot slides forward, then the friction of the floor is lower than the friction indicated on the Mark II. The foot is raised, the angle is adjusted to a lower friction value and the foot is dropped again. If the

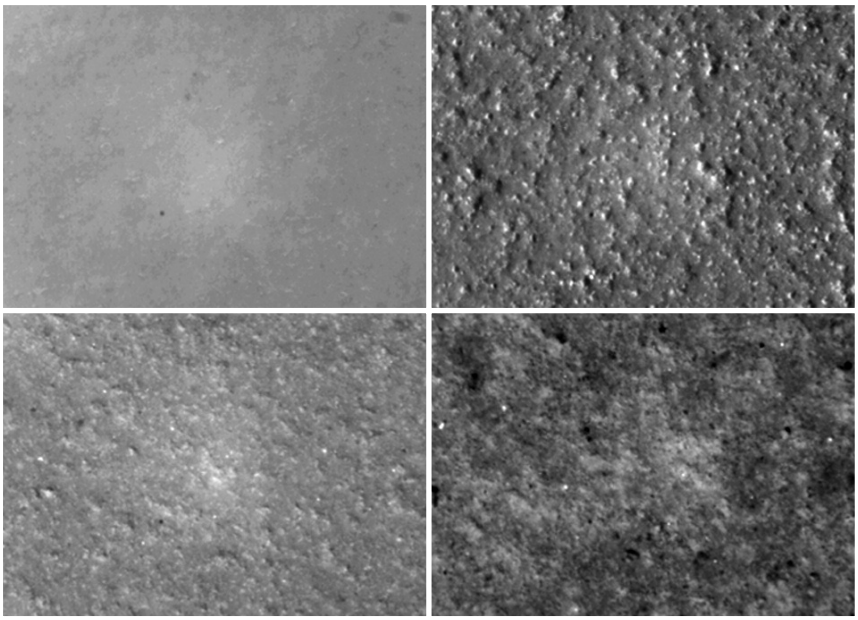

Figure 1.0
Images (3.36mm x 2.53mm) of the surface of the cleaned glazed ceramic tile (cycle 0, top left) and after the hydrofluoric acid treatment corresponding to cycle 10 (top right), cycle 15 (bottom left) and cycle 20 (bottom right).

foot does not slide, then the friction of the floor is higher than the friction indicated on the Mark II. The foot is raised, the angle is adjusted to a higher value and the foot is dropped again. The floor friction corresponds to the highest Mark II friction that does not lead to a forward slip of the foot.

In our procedure, the size of the Neolite sole is 64mm x 41mm and before each drop, the foot is tilted so that its rear end is in contact with the tile before the drop. For the wet and oily friction, the liquid is spread homogeneously over the surface of the tile and after each drop, the Mark II is repositioned on an area of the tile where the liquid was not disturbed by the previous drops.

Results and discussion

The results will be presented in three parts. The first part presents the evolution of the surface properties of glazed ceramic tiles exposed to multiple cycles of hydrofluoric acid treatment and abrasive wear. The second part compares the surface properties of the over-treated glazed ceramic tile with those of the untreated or normally treated tiles. The third part compares the surface properties of the over-treated tile with the tiles exposed to 10 cycles of hydrofluoric acid and abrasive wear.

Multiple treatments

As mentioned in an earlier article (Quirion *et al*., 2009), the treatment of glazed ceramic tiles reduces their resistance to wear so that, shortly after the application of the treatment, the owners may have to either replace the flooring tiles or treat them again and so on. This raises the question of the impact of multiple hydrofluoric acid treatments on the surface properties of glazed tiles.

To explore that question, two sample tiles were exposed to successive cycles of hydrofluoric acid treatment and abrasive wear. Cycle 0 corresponds to the cleaning of the tiles with water followed by abrasive wear. As seen in Figure 1.0, the new and cleaned surface (cycle 0) becomes quite rough after 10 cycles of hydrofluoric acid treatment and abrasive wear. At this stage, the surface of the glaze shows peaks that contribute to its macroscopic roughness. The following cycles seem to eliminate these peaks so that after 15 cycles, the surface looks smoother than after 10 cycles. Finally, after 20 cycles, the glazed layer has been almost completely removed, exposing the darker and porous bisque of the tile.

A detailed analysis of the evolution of the surface properties of the tiles is presented in the next sections. After each step (hydrofluoric acid treatment and abrasive wear) of the 20 cycles, the mass loss, the gloss,

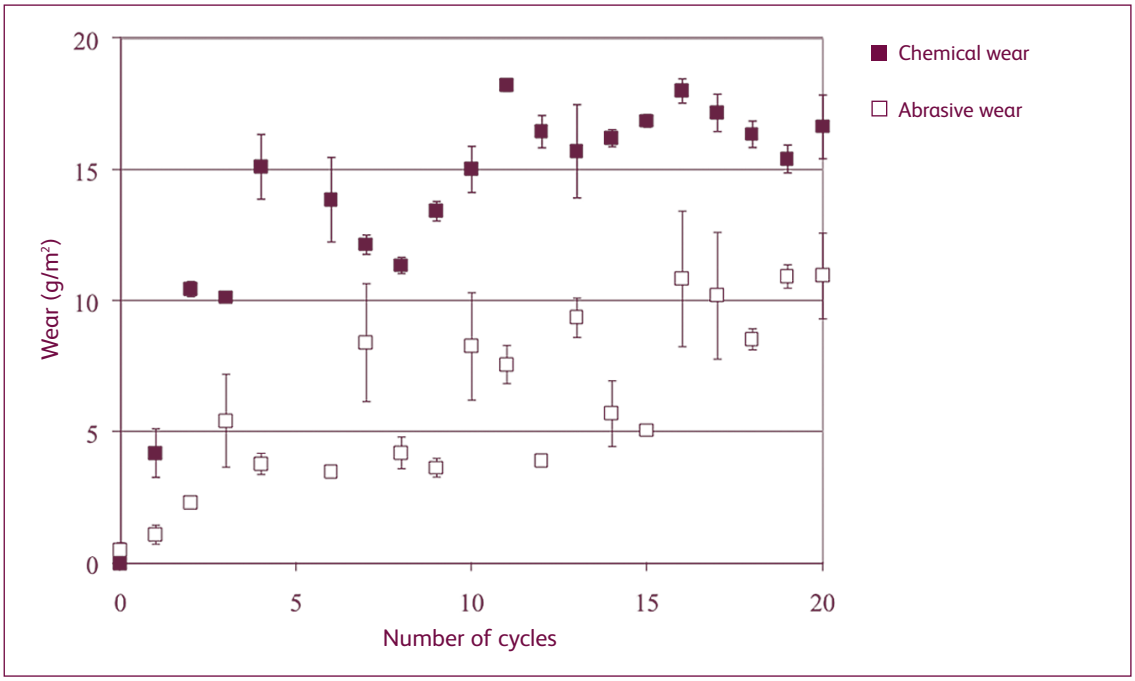

Figure 2.0
Chemical and abrasive wear corresponding to each step of the hydrofluoric acid treatment and wear cycles.

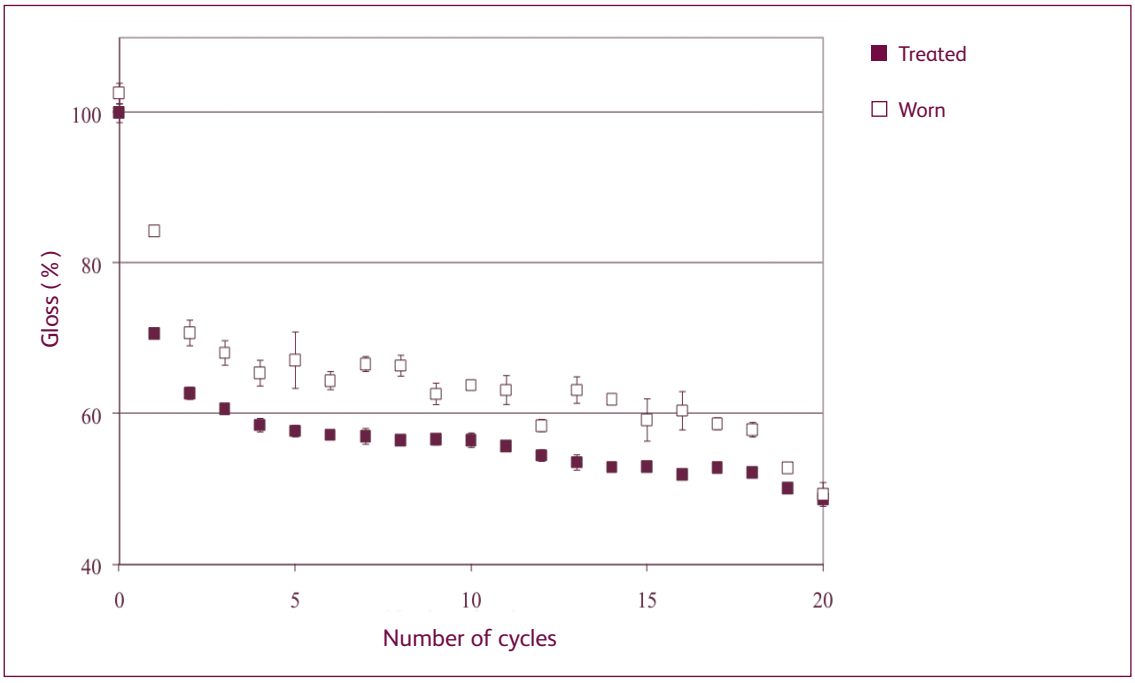

Figure 3.0
Gloss of the tiles after the hydro-fluoric treatment (■) and the abrasive wear (☐) as a function of the number of cycles of hydrofluoric acid treatment and wear.

the average roughness and the dry friction were measured. These surface properties were averaged over the two tiles and their evolution is shown in Figures 2.0 to 5.0 as a function of the number of cycles. The difference between the surface properties of the two sample tiles was larger than the standard deviation of the surface properties of each tile. Hence, the figures show the standard deviation of the average between the two tiles.

Wear

Figure 2.0 shows that the chemical wear (mass loss caused by the dissolution of the glaze in the presence of hydrofluoric acid) increases from 4 to 16g/m^2 for the first 12 cycles. Experimentally, all the treatments correspond to the application of the same amount of hydrofluoric acid molecules for the same period of time. Thus, the only way to increase the amount of glaze dissolved is to increase the reaction rate of the hydrofluoric acid solution with the glaze. As mentioned in the previous section, this can be done by increasing the area of contact of the hydrofluoric acid solution with the glaze, in agreement with the apparition of macroscopic roughness from Cycle 0 to Cycle 10 (see Figure 1.0).

The plateau after 12 cycles suggests that either the area of contact does not increase anymore or that it is large enough for the completion of the reaction within the 20 minutes of the treatment.

The average chemical wear at the plateau is 16.4 ± 0.8g of glaze per m^2 and it corresponds to the removal of 0.48 ± 0.02g of glaze per g of hydrofluoric acid. This mass loss may come from the transformation of silicone dioxide (SiO_2), the main component of glazes, into hexafluorosilicic acid (H_2SiF_6) through the reaction:

$$SiO_2 + 6HF \rightarrow H_2SiF_6 + 2H_2O$$

The hexafluorosilicic acid formed is very soluble in water and it would be washed away during the rinsing operation resulting in a mass loss of 0.5g of glaze (SiO_2) per g of hydrofluoric acid (HF), in excellent agreement with the plateau value obtained experimentally.

Figure 2.0 also shows that the abrasive wear (mass loss caused by light abrasion) increases steadily from 0.5g/m^2 for the clean and untreated glaze up to 10g/m^2 for the abrasive wear performed after the twentieth treatment with hydrofluoric acid. This indicates that the treated surface becomes more and more fragile after each consecutive treatment and that the resistance to wear decreases as the amount of glaze removed increases.

After 20 cycles, the cumulative wear caused by the hydrofluoric acid treatments and the abrasion leads to a glaze loss of 408g/m^2. Assuming that the density of the glaze corresponds to that of fused silica (2,190kg/m^3, Handbook of Chemistry and Physics, 1977), then the apparent thickness of glaze removed would correspond to 186µm. That value is in very good agreement with the measured thickness of the glaze 185 ± 20µm, suggesting that after 20 cycles, the glazed layer is completely removed, exposing the porous bisque of the ceramic tile (see Figure 1.0).

Gloss

As seen in Figure 3.0, the consecutive cycles of hydrofluoric acid and abrasive wear decrease the gloss of the glaze. The gloss decreases rapidly for cycles 1 to 4 and slowly up to 18. For cycles 19 and 20, the glaze is almost completely removed and the tiles become darker (see Figure 1.0), resulting in a faster decrease of the reflectivity.

From cycles 1 to 15, the gloss of the treated tiles increases on average by 8% after the abrasive wear, suggesting a polishing effect on the treated glaze. From cycles 16 to 20, the abrasive wear has less and less impact on the gloss, in agreement with the disappearance of the glaze from the surface.

Average roughness

Figure 4.0 shows that, overall, the consecutive cycles of hydrofluoric acid treatment and abrasive wear have little impact on the average roughness which remains between 1.1 and 1.8 for the treated tiles and 0.8 and 1.4 for the worn tiles.

For the first three cycles, the average roughness of the tiles treated with hydrofluoric acid decreases from 1.5 to 1.1 µm. The abrasive wear of these cycles further decreases the average roughness to Ra = 0.8µm. In other words, during the first three cycles, the treated tiles have an average roughness equal to or lower than the original tiles and when they become worn. From cycle 4 to 11, the average roughness of the treated tiles slowly increases from 1.1 to 1.8µm, a value higher than the roughness of the untreated and unworn tiles (Ra = 1.5µm). This increase of the average roughness agrees with the observed macroscopic roughness at the surface of the tiles after the treatment of the tenth cycle (see Figure 1.0).

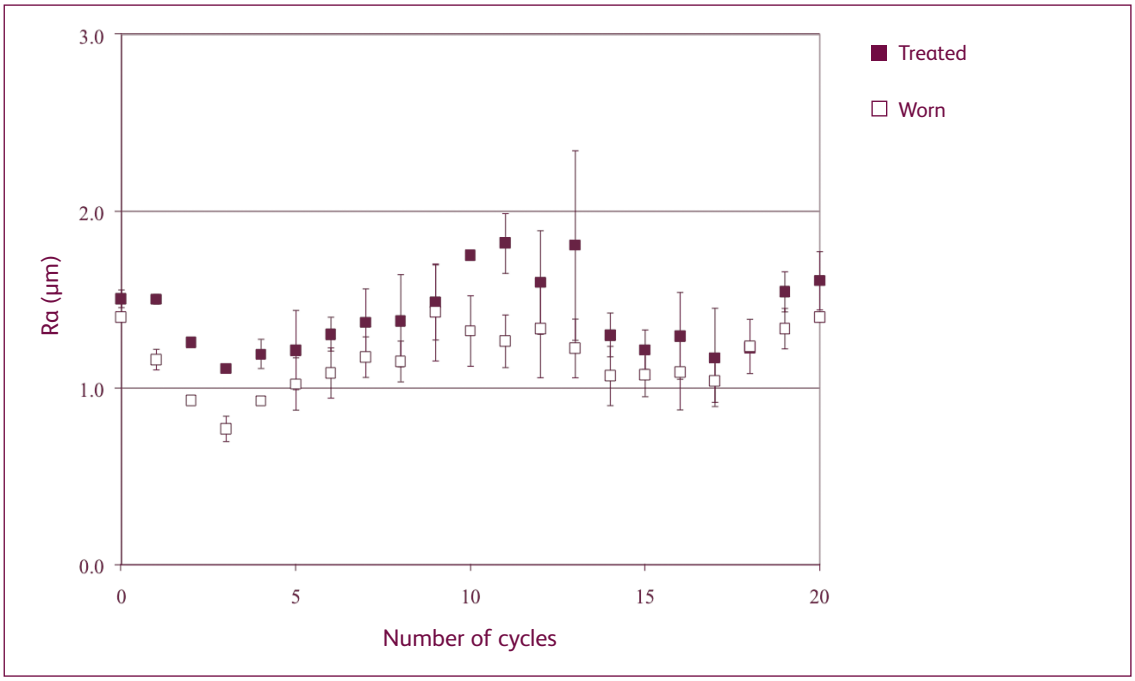

Figure 4.0
Average roughness of the tiles after the hydrofluoric treatment (■) and the abrasive wear (☐) as a function of the number of cycles of hydrofluoric acid treatment and wear.

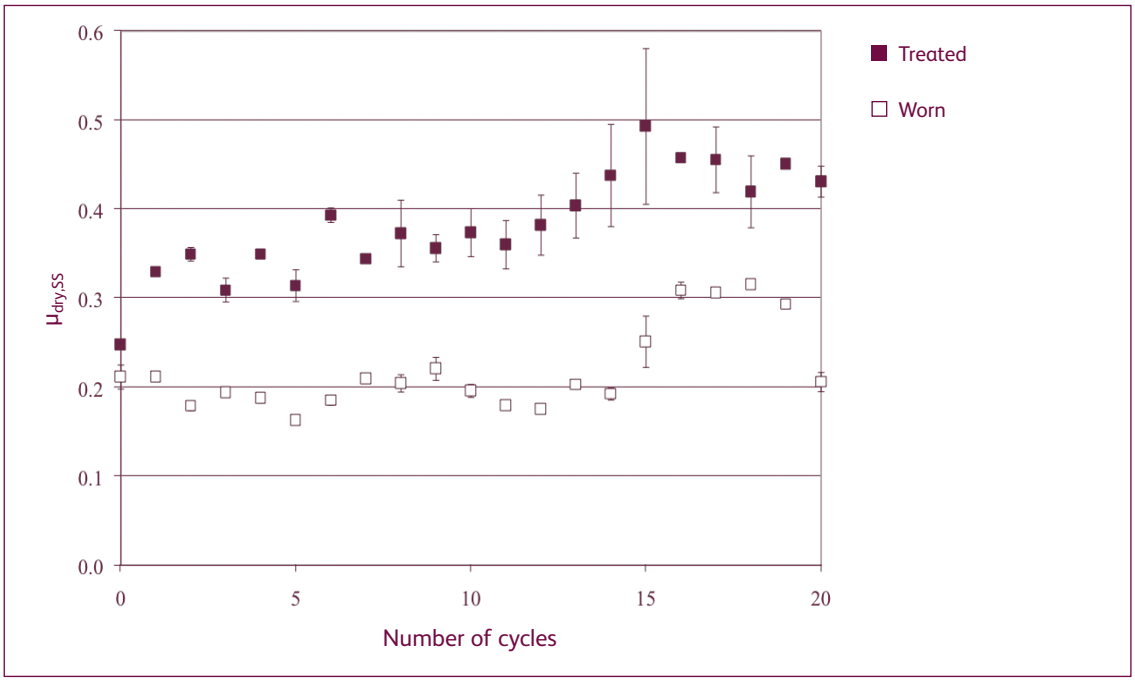

Figure 5.0
Dry friction ($\mu_{dry, SS}$) of the tiles after the hydrofluoric treatment (■) and the abrasive wear (☐) as a function of the number of cycles of hydrofluoric acid treatment and wear.

Surface properties and slip resistance of glazed ceramic tiles over-treated, or treated multiple times, with hydrofluoric acid

Surface Property	Untreated	Treated once	Over-treated
Chemical wear (g/m²)	0	4.2 ± 0.9 (3.5 ± 1.3)	134
Gloss (%)	100	71 ± 1 (65 ± 1)	57 ± 1
Ra (µm)	1.5 ± 0.3 (1.4 ± 0.1)	1.5 ± 0.3 (1.6 ± 0.2)	4.0 ± 0.3
$\mu_{dry, Neo}$	0.68 (0.68)	0.65 (0.78 ± 0.07)	>1.09
$\mu_{wet, 25, Neo}$	0.05	0.06	0.35
$\mu_{oil, 10, Neo}$	0.03	0.02	0.41

Table 1.0
Comparison of the chemical wear, surface properties and slip resistance of the glazed ceramic tiles untreated, treated normally and over-treated with ten times the amount of hydrofluoric acid. The values in parenthesis correspond to the values previously published (Quirion *et al*., 2009).

From cycle 12 to 16, the average roughness of the treated tiles decreases again, also in agreement with the observation that the surface of the glaze after the cycle 15 is smoother than that after cycle 10 (see Figure 1.0). Finally, the roughness increases again up to 1.6µm as the glaze disappears from the surface, exposing more and more of the porous bisque.

Dry friction

In this investigation, we are only interested in the evolution of the dry friction and not in its absolute value. As seen in Figure 5.0, the hydrofluoric acid treatment of cycles 1 to 12 increases the dry friction of the tiles by an average of 42% relative to the untreated tiles (cycle 0). However, the abrasive wear drops it about 10% below the friction of the worn and untreated tile (cycle 0). In other words, treating worn tiles with hydrofluoric acid increases their dry friction but the effect disappears rapidly after the treatment has worn out leaving tiles with a dry friction smaller than the untreated and worn tiles.

From cycle 15 to 20, the dry friction of the treated tiles increases by almost 80% when compared to that of the untreated tiles. This time, the abraded surfaces still have a dry friction about 45% higher than the dry friction of the worn and untreated tiles. This could be the result of the disappearance of the glaze and the exposure of more and more of the porous biscuit.

Overtreatment

The objective of this section is to evaluate the impact of over-treating glazed ceramic tiles with 10 times the normal amount of hydrofluoric acid. The chemical wear, the surface properties and the slip resistance of the over-treated tile are compared with the properties of untreated tiles and tiles treated normally in Table 1.0.

The results for the untreated tiles and the tiles treated once are in fair agreement with our previous results (Quirion *et al*., 2009). Interestingly, the overtreatment, which exposes the tile to 10 times more hydrofluoric acid than the normal treatment, removed 32 times the amount of glaze removed by the normal treatment. This suggests that the reaction rate of the hydrofluoric acid with the glaze increases as the etching progresses. Otherwise, treating the glaze with 10 times the amount of hydrofluoric acid used normally would result in the removal of 10 times the amount of glaze (and not 32 times).

A possible explanation would be that the area of contact of the glaze with the hydrofluoric acid solution increases as the etching progresses. The increase in the area of contact would increase the reaction rate of the reaction. This hypothesis is in accordance with the appearance of macroscopic roughness at the surface of the over-treated tile (see Figure 6.0 and Table 1.0) and the significantly higher average roughness for the over-treated tile (Ra = 4.0µm) compared to the tile treated normally (Ra = 1.5µm).

As observed for the roughness, the dry friction of the tile does not change much after one treatment (from $\mu_{dry, Neo}$ = 0.68 to 0.65) and it increases significantly to $\mu_{dry, Neo}$ > 1.09 after the application of the over-treatment (see Table 1.0).

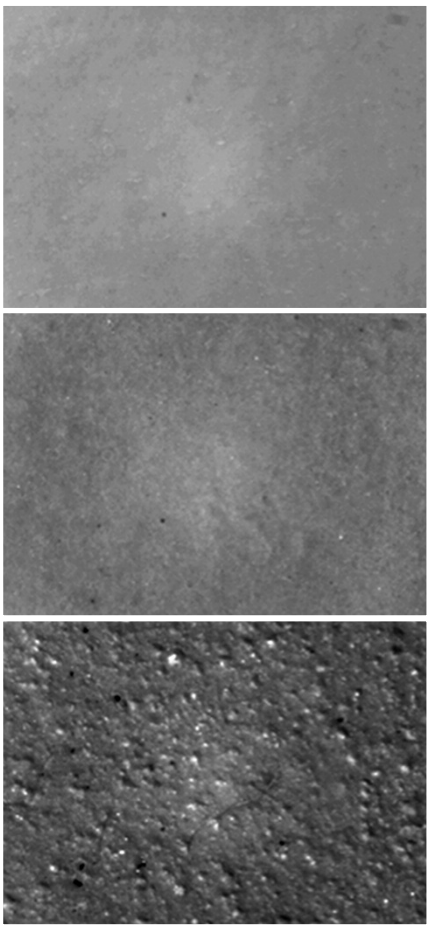

Figure 6.0
Images (3.36mm x 2.53mm) of the surface of the glazed ceramic tiles. Untreated (top), treated normally (center) and over-treated with 10 times the amount of hydrofluoric acid used for the normal treatment (bottom).

The gloss of a surface is often associated with its roughness. However, Table 1.0 show that the gloss of the tiles treated normally with hydrofluoric acid has dropped drastically to 71 % of the gloss of the untreated tile while its roughness did not change. A possible explanation would be that the gloss, which is a measure of the reflection of light, is more sensitive to micro roughness while the DekTak meter (tip radius of 12µm) is more sensitive to macroscopic roughness. Hence, the over-treated tile, with a significantly higher average roughness (Ra = 4.0µm) and a lower gloss (Gloss = 57 %), would be rougher than the untreated or normally treated tiles at both the macroscopic and microscopic levels.

These results suggests that, as the amount of hydrofluoric acid in contact with the glaze increases, the morphology of the surface evolves from a rather smooth surface with a microscopic roughness to a macroscopically rough surface.

Figure 7.0 compares how these roughness changes affect the wet friction of Neolite with the tiles. As the surface concentration of the detergent solution increases to 5g/m^2, the wet friction of the untreated and normally treated tiles both decrease rapidly to a plateau value around $\mu_{wet,\ Neo}$ = 0.05. In the same condition, the wet friction of the over-treated tile is always significantly higher, in agreement with its higher average roughness. For instance, at 25g/m^2 of the detergent solution, the wet friction of the over-treated tile is seven times higher ($\mu_{wet,\ 25,\ Neo}$ = 0.35) than the friction of the untreated tiles ($\mu_{wet,\ 25,\ Neo}$ = 0.05). This suggests that the micro roughness generated with the normal treatment has little impact on the dry and wet friction of Neolite but that the macroscopic roughness generated by the overtreatment improves significantly the slip resistance the glazed ceramic tiles.

Figure 8.0 compares the evolution of the friction as a function of the amount of vegetable oil at the surface of the tiles. As for the wet friction, the normal treatment with hydrofluoric acid has little impact on the friction of oily glazed ceramic tiles. At 3g/m^2 of vegetable oil, a surface concentration typical for food services (Underwood, 1992), the Neolite friction increases only slightly from 0.06 to 0.08 after the normal treatment with hydrofluoric acid. On the other hand, the overtreatment generates tiles with a significantly higher friction at low oil concentrations. It was not possible to measure the friction at 3g/m^2 because the oil could not be spread homogeneously on the rough surface. But even at three times the typical amount of oil on a food service floor, (Oil = 9g/m^2), the friction is still quite high at $\mu_{oil,\ Neo}$ = 0.52. Notice however that at very high oil concentrations (> 20g/m^2), the three states of the glazed ceramic tile all have a very low friction, i.e. $\mu_{oil,\ Neo}$ = 0.02. This suggests that even the over-treated tiles would be quite slippery on an oil spill.

In summary, over-treating smooth and glazed ceramic tiles with a large amount of hydrofluoric acid leads to a significant improvement of the slip resistance in moderately wet and oily conditions. However, that improvement comes with the dissolution of a large amount of the glaze and the appearance of macroscopic roughness, which will make the treated glazed surface more fragile and more difficult to clean (Quirion *et al*., 2009; Hupa *et al*., 2005). In other words, it is reasonable to anticipate that the improvement of the slip resistance owing to the overtreatment will wear off rapidly.

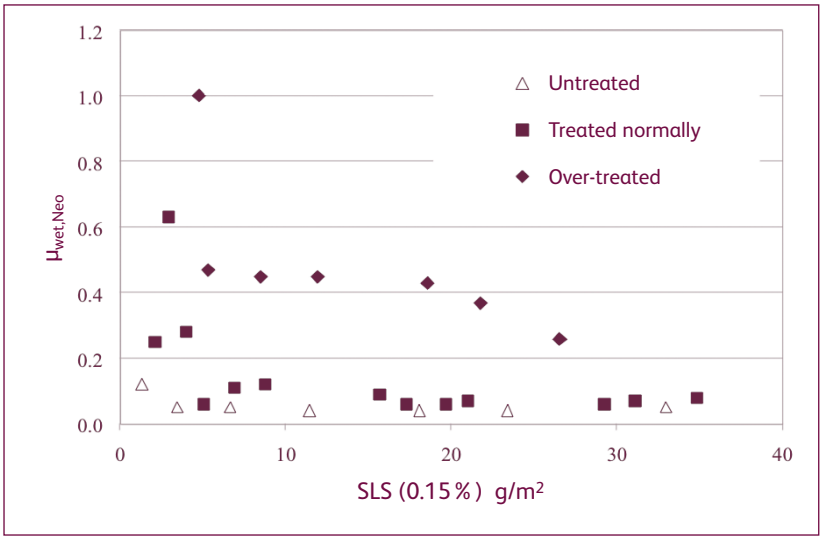

Figure 7.0
Brungraber Mark II friction of a Neolite slider on wet glazed ceramic tiles. Untreated and clean tiles (Δ), tiles treated normally with the hydrofluoric acid solution (■) and tiles over-treated with the hydrofluoric acid solution (♦).

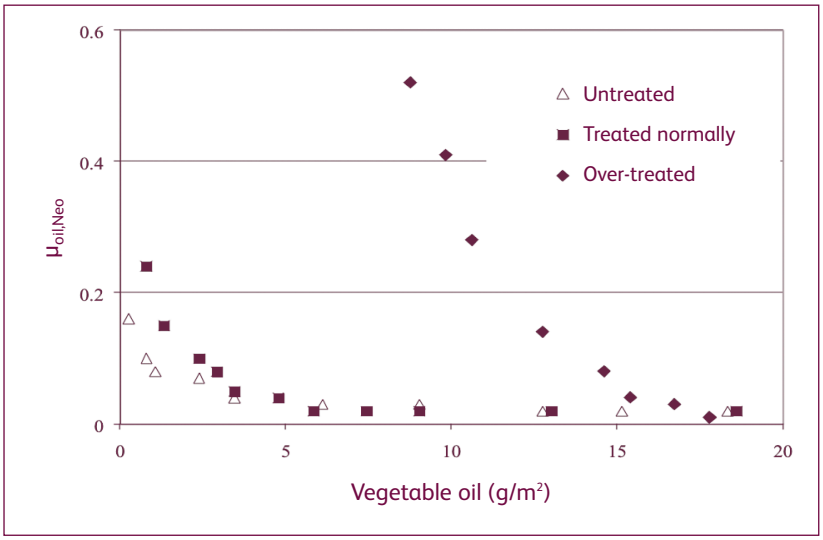

Figure 8.0
Brungraber Mark II friction of a Neolite slider on oily glazed ceramic tiles. Untreated and clean tiles (Δ), tiles treated normally with the hydrofluoric acid solution (■) and tiles over-treated with the hydrofluoric acid solution (♦).

Comparing the over-treated tile with the tiles after 10 cycles

The over-treated tile and the tiles after the tenth hydrofluoric acid treatment have been exposed to the same amount of hydrofluoric acid. The main difference between them is that the over-treated tile has never been exposed to abrasive wear while the other tiles were sanded at each cycle. Both tiles look similar (see Figures 1.0 and 6.0) with rather large peaks protruding from the surface. Table 2.0 compares their surface properties.

The first ten treatments removed almost the same amount of glaze than the overtreatment, in accordance with a dissolution mechanism based on the amount of hydrofluoric acid molecules in contact with the glaze. Both treated surfaces have the same gloss, i.e. approximately 56 % of the gloss of the original tile. However, the over-treated tile has a significantly higher average roughness resulting in a much higher dry friction than the tiles exposed to multiple treatments.

Table 2.0
Comparison of the surface properties of the over-treated tile with the properties of the tiles after 10 cycles of hydro-fluoric acid treatment and wear.

Surface Property	Over-treated (10 x the single dose)	Ten cycles of wear and treatment
Chemical wear (g/m²)	134	110 ± 2
Gloss (%)	57 ± 1	56 ± 1
Ra (µm)	4.0 ± 0.3	1.7 ± 0.3
$\mu_{dry, SS}$	0.67 ± 0.01	0.37 ± 0.03

A possible explanation would be that the macroscopic peaks are higher and sharper for the over-treated tile than for the tiles exposed to multiple cycles of treatments and abrasive wear. Indeed, the height of a peak should be smaller and its top should be wider after it has been abraded. This could also explain the lower chemical wear after the 10 cycles of hydrofluoric acid treatment because, after each abrasion, the surface peaks are cut off, leaving a flattened top having a smaller area of contact with the hydrofluoric acid solution.

Conclusions

This investigation reports the impact of multiple cycles of hydrofluoric acid treatment and abrasive wear (traffic) on the surface properties and slip resistance of glazed ceramic tiles and the impact of over-treating smooth and glazed ceramic tiles with a large amount of hydrofluoric acid.

The conclusions presented in this section apply to the tiles and procedures described in the methodology. It is possible that other glazed ceramic tiles and other experimental procedures lead to different results and conclusions.

Multiple treatments

The consecutive treatments consisted of 20 cycles of normal hydrofluoric acid treatment followed by light abrasive wear. It is observed that:

- The chemical wear caused by a normal treatment increases with the number of cycles, suggesting that the area of contact of the hydrofluoric acid solution with the glaze increases with the number of cycles of treatment and abrasive wear.
- The chemical wear reaches a plateau corresponding to the dissolution of silicone dioxide (SiO_2) by hydrofluoric acid (HF) into the form of hexafluorosilicic acid (H_2SiF_6) which is completely soluble in water and removed from the surface during the rinsing step.
- The abrasive wear increases with the number of cycles suggesting that the resistance to wear diminishes with the number of cycles of hydrofluoric acid treatment and abrasive wear.
- After the completion of three cycles of normal hydrofluoric acid and light abrasive wear, the average roughness, the dry friction and the gloss have decreased to values below those obtained for the worn and untreated tiles.

Based on these results, it appears that the repetition of a treatment with hydrofluoric acid removes more glaze than the preceding one and generates a surface that is more fragile than the one generated by the previous treatment. Eventually, the glaze will be completely removed exposing the porous bisque of the tile, which may raise sanitary and food hygiene considerations. The number of treatments required to reach that state will depend on the traffic, the initial thickness of the glaze and the amount of hydrofluoric acid used for each treatment.

Overtreatment

The overtreatment consisted in treating a tile with 10 times the amount of hydrofluoric acid used for the normal treatments. The tile was then rinsed, dried and tested. It is observed that:

- Increasing the amount of hydrofluoric acid also increases the amount of glaze removed, suggesting that the impact of the treatment depends on the amount of hydrofluoric acid molecules in contact with the glaze.
- The over-treated surface presents many macroscopic peaks, an indication that the glaze is not removed homogeneously throughout the surface of the tile.

- The average roughness, the dry friction and the wet friction are significantly higher when compared to the tiles treated normally.

- In moderately oily conditions, the over-treated tiles have frictions significantly higher than the friction of the tiles treated normally. However, at concentrations greater than $15g/m_2$, the tiles are as slippery as the untreated tiles with a very low oily friction.

Based on these results, it appears that over-treating a glazed ceramic floor with a large amount of hydrofluoric acid removes large amount of glaze, resulting in a much rougher and less slippery floor than treating the same ceramic floor with a normal amount of hydrofluoric acid. If the initial thickness of the glaze is too small, the over-treatment will expose the porous bisque which may raise sanitary and food hygiene considerations. The over-treated floor will initially perform well in moderately wet and oily conditions. However, the macroscopic roughness will make the floor much more difficult to clean. Assuming that the resistance to wear decreases with the amount of glaze removed, the effect of the over-treatment should wear off quite rapidly. Finally, the use of large amounts of hydrofluoric acid raises safety concerns.

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Biochemical effects of pesticides on crop sprayers in Western Maharashtra (India) vineyards before and after fifteen days of taking vitamin C supplements

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Abstract

The aim of this study was to investigate the biochemical effects of pesticides on sprayers of vineyards in Western Maharashtra (India), before and after 15 days of taking vitamin C supplements, who were occupationally exposed to various pesticides over a long period of time (about 5 to 15 years).

Blood samples were collected from all study-group subjects for biochemical parameters assays before and after 15 days of vitamin C supplements. The study clearly showed that giving vitamin C supplements protects liver damage in vineyard crop sprayers, which results in decreased aspartate transaminase and alanine transaminase activity. In addition to its antioxidant activity, vitamin C is known to perform other actions that enhance its protective effect in OP (organophosphate)-induced toxicity.

Therefore, it is suggested that farmers, pesticide applicators, workers in the pesticide industry and other pesticide users, who come in regular contact with pesticides, may benefit from pretreatment with vitamin C.

Key words: Acetylcholinesterase, C-reactive protein, Aspartate transaminase, Alanine transaminase, Lipid peroxidation, Superoxide dismutase, Catalase, Ceruloplasmin, Glutathione S-transferase, Zinc, Copper.

Introduction

Grape cultivation is increasing, mainly in Western Maharashtra (India). Nowadays, grape growers are using more pesticides to increase the yield and reduce the post-harvest losses. The environmental pollution and poisoning caused by widespread use of pesticides during grape cultivation may be important factors affecting the socio-economic status of uneducated farm workers in rural areas [Dave,1998].

Pesticides are ubiquitous contaminants of the environment and are found in air, soil, water, human and animal tissue samples from all over the world. The common organophosphorous and carbamate pesticides used in vineyards are Basathrin 25 EC (Cypermethrin 25% EC), Nuvan (Dichlorovas 76% EC), Nuvacron (Monocrotopho 36% EC), Dimethoate 30% EC (CHAMP 30 EC), Phosphamidon 85% SL (Dimecron), Kilex Endosulfan 35% EC, Carbaryl, Cypermethrin 25% EC (JAWAA), Monocrotophos 36% SL, and Methomyl.

The principal classes of compounds used as insecticides are organochlorines, organophosphorous, carbamates and pyrethroid compounds, and various inorganic compounds. Pesticides uptake occurs mainly through the skin and eyes, by inhalation, or by ingestion. The fat-soluble pesticides, and to some extent, the water-soluble pesticides are absorbed through intact skin. Sores and abrasions may facilitate uptake through the skin. The fumes from pesticides or aerosol droplets smaller than 5µm in diameters are absorbed effectively through the lungs. Larger inhaled particles or droplets may be swallowed after being cleared from the airways [WHO, 1990].

Occupational exposure occurs in the mixing, and loading of equipment and in the spraying and application of the insecticides. There are several factors affecting the levels of exposures that occur while mixing and handling during the agricultural application of pesticides [Wolfe *et al*., 1967].

Other factors are wind, equipment used, duration of exposure, and individual protection [MacCollom *et al*., 1986]. Absorption resulting from dermal exposure is the most important route of uptake for exposed workers. Acute toxic effects are easily recognised, whereas the effects resulting from long-term exposure to low doses are often difficult to distinguish. In particular, the effects of a regular intake of pesticide residues in food are hard to detect and quantify [WHO, 1990].

Signs and symptoms associated with mild exposure to organophosphate and carbamate insecticides include: headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps, diarrhoea, blurred vision associated with excessive watering of the eyes, contracted pupils, excessive sweating and salivation, slowed heartbeat (often fewer than 50 per minute), and rippling of surface muscles just under the skin [WHO, 1990; Al-Saleh, 1994].

The adverse effect from exposure to pesticides depends on the dose, the route of exposure, how easily the pesticide is absorbed and persistence in the body. The toxic effect also depends on the health status of the individual. Malnutrition and dehydration are likely to increase sensitivity to pesticides. Pesticides have been known to affect a number of enzymes and physiological systems, which results in a wide variety of changes in humans.

Pesticides have been shown to affect mammalian reproduction, nervous, immune, and blood coagulation systems and they have carcinogenic and mutagenic potential. Exposure affects several organs in humans, but

the liver is most susceptible [WHO, 1992; WHO, 1993]. Disorders of the cardiovascular system, nervous system, sensory organs, respiratory system, and reduced lung function have been reported after exposure to pesticides. Skin disorders including dermatitis, headache and nausea have also been reported [IARC, 1991].

Genotoxic effects are considered to be the most serious possible side effects of agricultural chemicals. If the chemical reacts with nuclear DNA, it is usually mutagenic and carcinogenic to the exposed organisms. The effects include inheritable genetic diseases, carcinogenesis, reproductive dysfunction and birth defects [Wagida, 1997].

Accumulation of acetylcholine in the CNS (central nervous system) is believed to be responsible for the tension, anxiety, restlessness, insomnia, headache, emotional instability, neurosis, excessive dreaming, nightmares, apathy and confusion described after organophosphorus (OP) pesticide poisoning. Slurred speech, tremors, generalised weakness, ataxia, convulsions and coma are the other CNS effects [Ecobichon, 1996].

Metabolic disturbances, fluid and electrolyte imbalance are also reported in several OP and carbamate-exposed populations. The increased formation of reactive oxygen and nitrogen species results in an increase in lipid peroxidation in the brain, musculo-skeletal system, RBC, etc. and depletes antioxidant status reported in several studies of various pesticide-exposed populations [Dave, 1998; FAO Rome, 1986; WHO, 1990; Wolfe *et al*., 1967; MacCollom *et al*., 1986; Al-Saleh, 1994].

The pesticides may irritate lung macrophages, encouraging them to generate superoxide radicals and deplete antioxidants status. The biochemical effect produced by certain pesticides can be enzyme induction or inhibition. Several pesticides inhibit cholinesterase, altered liver and kidney functions, decreased haemoglobin, impaired oxidative stress, antioxidants imbalance and altered drug metabolism of liver enzymes has been reported among pesticide-exposed workers [WHO, 1992 and 1993; Patil *et al*., 2003].

In an earlier study, we found altered haematological parameters, liver and kidney functions, along with impaired mixed-function oxidase systems, oxidative stress and antioxidants imbalance [Patil *et al*., 2003 and 2008]. Ascorbic acid (Vitamin C) is an important antioxidant that significantly decreases the adverse effect of reactive oxygen and oxides of nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins. Vitamin C plays a crucial role in wound healing and reducing inflammation, and may partially prevent certain types of hepatic cellular damage.

Ascorbic acid also regenerates other small molecule antioxidants such as α-tocopherol, glutathione, urate and β- carotene from their respective radical species [Frei *et al*., 1990; Halliwell, 1996]. It also enhances protein biosynthesis. The chief source of vitamin C is citrus fruits, which are predominantly available in the field. Therefore, this study was undertaken to investigate the biochemical effects of pesticides on vineyard crop sprayers before and after 15 days of giving vitamin C supplements.

Materials and methods

This study comprises 30 subjects with occupational pesticides exposure, i.e. sprayers of vineyards. All the study-group subjects had ages in the range of 20 to 45 years and came from Tasgaon taluka (an administrative unit of a district), Sangli district, (Western Maharashtra) India. For all study-group subjects, a 500mg vitamin C tablet/day for 15 days was given to each participant. Both growers and sprayers were informed of the study objectives and health hazards of pesticides exposure prior to data and biological specimen collection. Written consent was obtained from all sprayers.

Demographic, occupational and clinical data were collected by using questionnaire and interview. Most of the crop sprayers had major problems of watering eyes, nausea, salivation, sniffing, headache, breathlessness, itching and vomiting. All the subjects of the study groups belong to agricultural families with similar socio-economic status. None of the subjects had a past history of major illness. Dietary intake and food habits of all subjects were normal, which was confirmed periodically by checking what they ate at lunchtime. It was also verified that they had their routine breakfast and dinner.

Any subjects who were on drugs for minor illnesses were excluded from this study. Non-smokers, non-alcoholic healthy males, occupationally exposed to various pesticides - i.e. vineyard sprayers for between 5-15 years duration of exposure - were selected for this study. The entire experimental protocol was approved by the institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration of 1964 [Helsiniki, 1964]. Blood samples were taken from crop-sprayers' veins into tubes containing heparin solution as anticoagulant for

biochemical parameters assay before and after 15 days of vitamin C supplementation

From all subjects within the study group, serum acetyl cholinesterase (AChE), C reactive proteins (CRP), aspartate transaminase (AST), alanine transaminase (ALT), total proteins (TP), albumin (ALB), globulin (GLB), A/G ratio, lipid peroxide, and antioxidants status parameters, i.e. RBC-superoxide dismutase (SOD), RBC-Catalase(CAT), plasma ceruloplasmin (CP), glutathione S-transferase (GST), serum zinc (Zn) and serum copper (Cu) were measured before and after giving vitamin C supplement to crop sprayers, using standard methods.

Serum Acetyl Cholinesterase was measured by the Knedel *et al*. [1989] Accucare kit method. The Butyrylthiocoline is hydrolysed by serum cholinesterase to produce thiocoline in the presence of potassium hexacyanoferrate (III). The absorbance decrease is proportional to the cholinesterase activity of the sample.

Serum C-Reactive Proteins was measured by the Anderson and McCarthy [1950], Lothar Thomas [1998] method. TURBILYTE- CRP™ is a turbidimetric immunoassay for the determination of C- reactive protein in human serum and based on the principal of agglutination reaction. The serum sample is mixed with activation buffer (R1), TURBILYTE- CRP™ latex reagent (R2) and allowed to react. Presence of CRP in the serum sample results in the formation of an insoluble complex producing a turbidity, which is measured at 546 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the serum specimen.

The liver function tests were measured by using a fully automated biochemistry analyser (Eurolyser) on the same day of sample collection. The SGOT (AST) and SGPT (ALT) were measured by the UV-kinetic method [Committee on Enzymes of the Scandinavian Society, 1974] using reagents from M/S Accurex Biomedical Ltd. The conversion of NADH to NAD in both transaminase (SGOT, SGPT) reactions was measured at 340nm, as the rate of decrease in absorbance.

Serum total proteins were measured by the Biuret method [Henry *et al*., 1974] using an M/S Accurex Biomedical Kit. Serum proteins react with cupric ion in alkaline pH to produce a coloured complex; the intensity of the colour complex was measured at 546nm and directly proportional to the protein concentration in the specimen. Serum albumin was measured by the BCG method [Doumas *et al*., 1971] using reagents from M/S Beacon Ltd. Serum albumin binds with 3,3′,5,5′-tetra bromocresol sulfonapthalein (BCG) in acidic medium at pH 4.2, and the blue-green coloured complex formed is measured at 600nm. Serum globulins and the A/G ratio were calculated by using serum total proteins and albumin values.

Lipid peroxidation was measured spectrophotometrically by the Satoh [1978] method. Serum proteins were precipitated by trichloroacetic acid (TCA) and the mixture was heated for 30 min with thioburbituric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of malondialdehyde (MDA) nmol mL^{-1} using 1, 1, 3, 3, tetraethoxy propane as the standard.

The activity of erythrocyte superoxide dismutase (SOD) was measured by the Marklund and Marklund [1988] method. Superoxide anion is involved in the auto-oxidation of pyrogallol at alkaline pH 8.5 and is inhibited by SOD, which can be determined as an increase in absorbance per two minutes at 420mm. The SOD activity was measured as units mL^{-1} hemolysate. One unit of SOD is defined as the amount of enzyme required to cause 50 % inhibition of pyrogllol auto-oxidation.

Erythrocyte catalase was measured by the Aebi [1983] method. Heparinised blood was centrifuged and plasma was removed, and the erythrocytes were washed 2-3 times with saline (0.9 % NaCl) and then haemolysed in 10 volumes of cold deionized water. The whole mixture was centrifuged for 10 min at 3,000rpm. The cell debris was removed and the clear haemolysate was diluted 500 times with phosphate buffer (60mM) pH 7.4. Catalase decomposes H_2O_2 to form water and molecular oxygen. In the UV range, H_2O_2 show a continual increase in the absorption with decreasing wavelength. At 240nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240nm for every 15 seconds interval up to 1 min and the difference in absorbance (ΔA at 240 nm) per unit time is a measure of the catalase activity. The unit of catalase activity was expressed as mM of H_2O_2 decomposed/mg Hb min^{-1}.

Plasma ceruloplasmin was measured by the Herbert and Ravin [1961] method. Ceruloplasmin oxidizes P-phenyl-enediamine in the presence of oxygen to form a purple-coloured oxidised product. The ceruloplasmin concentration was determined from the rate of oxidation of P-

Table 1.0
Depicts mean values and correlation coefficient [r] of serum acetyl cholinesterase (AChE), C reactive proteins (CRP), liver functions tests of sprayers of vineyards before and after vitamins C (500 mg/Tab/day for 15 days) supplementation.

Parameters	Vitamin C administered		Correlation Coefficient [r]
	Before (N=30)	After (N=30)	
AChE [U/L]	4667± 1313 (876 – 6844)	4768 ± 1281** (950 – 6926)	0.801
CRP [mg/dl]	0.116 ± 0.082 (0.045 – 0.34)	0.101±0.067** (0.032 – 0.28)	0.895
AST [U/L]	29.40 ± 14.53 (14 – 78)	26.20 ± 9.04* (17 – 54)	0.950
ALT [U/L]	35 ± 17.16 (14 – 88)	27.45 ± 10.1** (18 – 60)	0.653
TP [gm/dl]	7.29 ± 0.35 (6.45 – 7.9)	7.38 ± 0.27• (6.9 – 7.8)	0.702
ALB [gm/dl]	4.17 ± 0.18 (3.9 – 4.5)	4.29 ± 0.21** (3.9 – 4.7)	0.553
GLB [gm/dl]	3.17 ± 0.18 (2.8 – 3.6)	3.08 ± 0.17* (2.8 – 3.5)	0.416
A/G Ratio	1.32 ± 0.08 (1.11 – 1.46)	1.39 ± 0.10** (1.2 – 1.6)	0.354

Figures indicate Mean ± SD values and those in parenthesis are range of values.
** P < 0.01, * P < 0.05, • Non significant with respect to before vitamins supplementation of the sprayers of vineyards.
Acetyl cholinesterase (AChE), C Reactive proteins (CRP), Aspartate transaminase (AST), Alanine transaminase (ALT), Total proteins (TP), Albumin (ALB), Globulin (GLB).

Table 2.0
Mean values and correlation coefficient of lipid peroxide, antioxidant enzymes and trace elements of sprayers of vineyards before and after vitamin C (500 mg/Tab/day for 15 days) supplementation.

Parameters	Vitamin C administered		Correlation Coefficient [r]
	Before (N=30)	After (N=30)	
LP [nmol/ml]	3.28 ±0.59 (2.27 – 5.34)	2.64 ± 0.41*** (1.85 – 3.49)	0.579
SOD [a]	9.64 ± 1.14 (7.89 – 12.09)	12.03 ± 1.5*** (9.50 – 16.27)	0.598
CAT [b]	8.39 ± 4.12 (4.23 – 16.9)	10.12 ± 3.8*** (4.22 – 18.46)	0.710
CP [mg/dl]	73.81 ± 12.31 (38.8 – 93.5)	79.25± 13.3*** (40.45 – 99.60)	0.890
GST C	0.099 ± 0.055 (0.022 – 0.211)	0.077± 0.047• (0.011 – 0.22)	0.230
Serum Zn [µg/dl]	83.65 ±12.8 (61.5 – 115)	88.27 ± 12.5** (68 – 118)	0.853
Serum Cu [µg/dl]	79.90 ± 15.3 (51 – 110)	84.67± 15.6*** (55 – 119)	0.798

[a] Unit/ml of hemolysate, [b] mM H_2O_2 decom/mg Hb/min, µmol of conjugate form/min/mg of protein.
Figures indicate Mean ± SD values and those in parenthesis are range of values. *** P<0.001, ** P<0.01, * P<0.05,
• Non significant with respect to the before vitamins supplementation of the sprayers of vineyards.
Lipid peroxide (LP), RBC- Superoxide dismutase (SOD), RBC – Catalase (CAT), Plasma Ceruloplasmin (CP), Glutathione S-transferase (GST)

phenylenediamine at 37°C at pH 6.0, which has an absorption peak at 530nm.

Serum Glutathione S-Transferase (GST) was measured by using the Habig *et al*., (1974) method. Glutathione S-transferase (GST) activity was determined by measuring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation was accompanied by an increase in absorbance at 340nm. The rate of increase is directly proportional to the GST activity in the sample.

Serum zinc and copper were measured using a Perkins Elmer model 303 graphite furnace atomic absorption spectrophotometer, which was connected to a Hitachi 165 recorder; values were shown in μg dL^{-1} [Mert and Henkin, 1971; Parson and Slavin, 1993].

Statistical comparisons between before and after vitamin C dosing of vineyard crop sprayers were made by paired t-test. Pearson's correlation equation was also carried out to evaluate correlation between biochemical parameters of before and after vitamin C dosing.

Results

The mean values, percentage change, statistical significance and correlation coefficient (r) value of serum acetyl cholinesterase (AChE), C reactive proteins (CRP), liver functions tests, lipid peroxide, antioxidant enzymes and trace elements of vineyard crop sprayers before and after vitamin C supplements are given in Tables 1.0 and 2.0 and Figures 1.0 and 2.0.

After 15 days of giving vitamin C supplements to vineyard crop sprayers, we observed significantly decreased serum C-reactive protein (12.78%, $P<0.01$, $r = 0.89$), aspartate transaminase (11.69%, $P<0.05$, $r = 0.95$), alanine transaminase (21.57%, $P<0.01$, $r = 0.65$), globulin (2.83%, $P<0.05$, $r = 0.41$) and increased serum acetyl cholinesterase (2.16%, $P<0.01$, $r = 0.80$), albumin (2.87%, $P<0.01$, $r = 0.55$), albumin/globulin ratio (5.3%, $P<0.01$, $r = 0.35$), whereas no statistical significant change was found in total proteins compared with before vitamin C dosing (Table 1.0 and Figure 1.0).

Vitamin C supplements given to the vineyard crop sprayers significantly decreased serum lipid peroxide (19.51%, $P<0.001$, $r = 0.57$), serum glutathione-S-transferase (21.58%, NS, $r = 0.23$), and increased RBC-superoxide dismutase (24.79%, $P<0.001$, $r = 0.59$), RBC-catalase (20.61%, $P<0.001$, $r = 0.71$), plasma ceruloplasmin (7.37%, $P<0.001$, $r = 0.89$), serum zinc (5.52%, $P<0.01$, $r = 0.85$), serum copper (5.96%, $P<0.001$, $r = 0.79$) compared with before vitamin C dosage (Table 2.0 and Figure 2.0).

In all above parenthesis, the percentage change, P values, and correlation coefficient (r) with respect to before vitamin C dosing of vineyard crop sprayers are given. Correlation coefficient (r) values above 0.5 of various biochemical parameters show significant correlation between before and after vitamin C supplements were given to the vineyard crop sprayers and it indicates consistency of biochemical parameters before and after vitamin C supplementation.

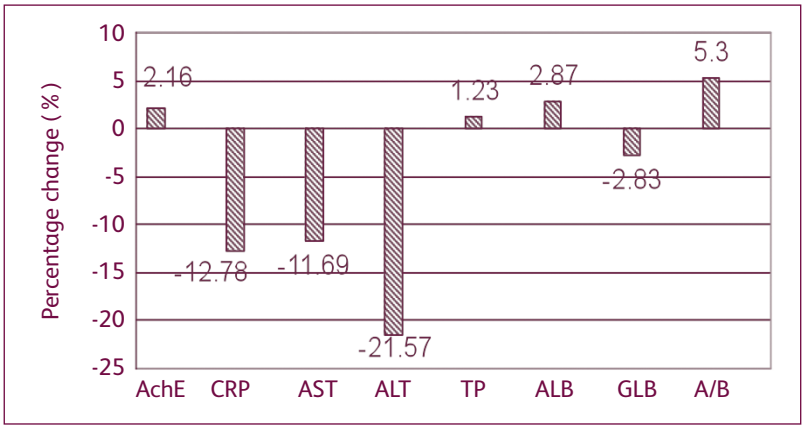
Discussion

Ascorbic acid is an important antioxidant that significantly decreases the adverse effect of reactive oxygen and oxides of nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and protein, which are implicated in chronic diseases, stroke, cancers, neuro-degenerative diseases and cataracto-genesis [Halliwell and Gutteridge, 1993]. Additional dosage of L-ascorbic acid was found to be beneficial in heavy metals (nickel) induced alteration of testicular nucleic acid concentration, hepatic lipid peroxidation and histopathology of the liver [Das and Das, 2004; Das *et al.,* 2007].

Several studies are under way to determine the effects of antioxidant supplementation following heavy metals and pesticide exposure. The data suggests that antioxidants specifically play an important role in abating certain pesticides. It is well documented that various pesticides cause membrane disruption via lipid peroxidation and alter the antioxidant status of the body, ultimately resulting cell death [Zavodnik *et al*., 2002; Koryagin *et al*., 2002; John *et al*., 2001] and we also found the same results in earlier studies [Patil *et al*., 2003, 2009b].

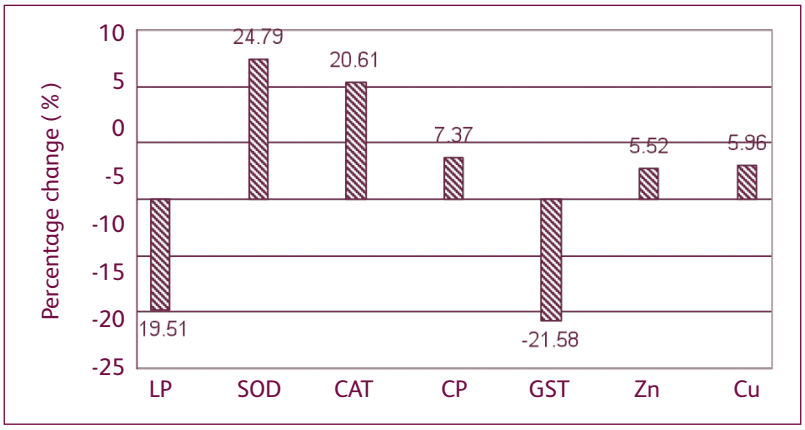
At the same time, supplementation of L-ascorbic acid was found to be effective in the prevention of oxidative damage in erythrocytes induced by various pesticide exposures, which resulted in significantly lowering serum lipid peroxide concentration (19.51%, $P<0.001$, $r = 0.57$) and increased RBC-SOD (24.79%, $P<0.001$, $r = 0.59$), RBC-catalase (20.61%, $P<0.001$, $r = 0.71$), and plasma ceruloplasmin (7.37%, $P<0.001$, $r = 0.89$) in vineyard crop sprayers compared with before vitamin C supplementation.

Figure 1.0
Percentage change of mean values of serum acetyl cholinesterase (AChE), C reactive proteins (CRP), liver functions tests of sprayers of vineyards after vitamin C (500mg/Tab/day for 15 days) supplementation with respect to before vitamins C supplement.



Acetyl Cholinesterase (AChE), C Reactive proteins (CRP), Aspartate transaminase (AST), Alanine transaminase (ALT), Total proteins (TP), Albumin (ALB), Globulin(GLB).

Figure 2.0
Percentage change of lipid peroxide, antioxidants enzymes and trace elements of sprayers of vineyards after vitamin C (500mg/Tab/day for 15 days) supplementation with respect to before vitamin C supplementation.



Lipid peroxide (LP), RBC- Superoxide dismutase (SOD), RBC – Catalase (CAT), Plasma Ceruloplasmin (CP), Glutathione S-transferase (GST), Zinc (Zn), Copper (Cu).

These results indicate that the L- ascorbic acid protects the erythrocyte antioxidant defence system and may eliminate toxic effects of various pesticides on the activity of these enzymes. L- ascorbic acid can be oxidised by most reactive oxygen and oxides of nitrogen thought to play roles in tissue injury associated with various diseases. These compounds include superoxide, hydroxyl, peroxyl and nitroxide radicals, as well as non-radical reactive compounds such as singlet oxygen, peroxynitrite and hypochlorate. By virtue of its scavenging activity, ascorbate inhibits lipid peroxidation, oxidative DNA damage and oxidative protein damage [Das *et al*., 2007].

Serum glutathione-S-transferase level was markedly increased (80.55 %) before vitamin C dosage of vineyard crop sprayers as compared with the control group reported in our earlier study [Patil *et al*., 2009a]. However, GST activity decreased (21.58 %) after vitamin C dosage of vineyard crop sprayers as compared with before vitamin C dosage.

Glutathione-S-transferase (GST) consists of a large family of GSH-utilising enzymes that play an important role in detoxification of xenobiotics in mammalian systems. Increased GST activity before vitamin C dosage in this study might be caused by the pesticides, which generally

combine with GSH and form less toxic, water-soluble products that are excreted in urine. Gene expression of GSH-related enzymes in human cells is increased under conditions of oxidative stress [Wang *et al*., 1997]. Increased regulation of GSH-dependent enzymes is necessary for an adaptive/protective response [Kaplowitz et. al., 1985].

In this study, we found increased oxidative stress because of various pesticides used in vineyards. Therefore, gene expression of GST enzyme might have increased through oxidative stress, resulting in increased GST activity in this study. Vitamin C reduces oxidative stress owing to its antioxidant property. Hence, GST activity might have been decreased after vitamin C dosage because of decreased oxidative stress, which may lead to decreased gene expression of GST and reduce the GST activity.

Serum C-reactive protein level is increased significantly by 117.6% in vineyard crop sprayers as compared with the control group reported in our earlier study [Patil, 2009b]. However, CRP activity is significantly decreased (21.58%, NS, r = 0.23) after vitamin C dosage of vineyard crop sprayers compared with before vitamin C dosage.

C-reactive proteins (CRP) are serum proteins, synthesised in the liver. CRP level increases within hours of an acute injury or the onset of inflammation and may reach as high as 20 times the normal levels. Increased CRP in vineyard crop sprayers before vitamin C dosing clearly indicates the hepatic cell damage caused by various pesticides. Vitamin C may be partially preventing certain types of hepatic cellular damage [McDowell, 1989; Parola *et al*.,1992; Sies *et al*., 1992; Burtis and Ashwood, 1994; Netke *et al*., 1997]. In addition, vitamin C also plays a crucial role in wound healing and reducing inflammation. Therefore, CRP activity might have been decreased after vitamin C supplementation in this study.

Liver function marker enzymes, i.e. serum aspartate transaminase (AST) and alanine transaminase (ALT), were increased by 57 and 37.36% in vineyard crop sprayers respectively as compared with control subjects reported in our earlier study [Patil *et al*., 2009b]. However, aspartate transaminase (11.69%, $P<0.05$, r = 0.95) and alanine transaminase (21.57%, $P<0.01$, r = 0.65) were decreased after vitamin C dosage of vineyard crop sprayers compared with before vitamin C dosage. High levels of these enzymes are usually indicative of hepatic damage in this study by various pesticides.

Several procedures have been used to protect the liver from damage by administering antioxidants such as β-carotene [Olmez and Karakilcik, 1994], vitamin C [Netke *et al*., 1997; Mitra *et al*., 1991], vitamin E [Parola *et al*., 1992; Harvey *et al*., 1994; Durak *et al*., 1996; Naziroglu, 1999] and selenium–vitamin E combination [Sies *et al*., 1992; Naziroglu, 1999; Brucato *et al*., 1986]. Therefore, from past reports and present results it clearly indicates that the vitamin C supplementation protects liver damage in vineyard crop sprayers, which results in decreased AST and ALT activities in this study.

In addition to its antioxidant activity, vitamin C is known to have other effects that enhance its protective effect in OP-induced toxicity. For example, vitamin C has shown to increase the activity of paraoxonase [Jarvik *et al*., 2002], an enzyme known to aid in the detoxification of OP and carbamate compounds.

Conclusion

The slight increase in serum AChE, albumin, and A/G ratio after vitamin C supplementation in this study might be owing to its role in protein biosynthesis, or its protective role in hepatic cell damage. Significantly decreased serum LP, GST, and increased RBC-SOD, catalase, and plasma ceruloplasmin in vineyard crop sprayers as compared with before vitamin C dosage may be owing to the antioxidant property of vitamin C. The decreased serum CRP activity in this study may be caused by vitamin C preventing hepatic cell damage, which is also supported by decreased serum AST, ALT. Therefore, the study concludes that daily intake of citrus fruits (vitamin C) is beneficial to reduce the toxicity of various pesticides on vineyard crop sprayers because of its antioxidant property, role in wound healing, reducing inflammation, preventing hepatic cellular damage and its role in protein biosynthesis.

Proper precautions like wearing protective clothes, taking a bath after pesticides spraying, wearing eye protectors and not wiping eyes with contaminated gloves or hands during spraying the pesticides will reduce the exposure to pesticides, and daily intake of antioxidant vitamins like ascorbic acid, α-tocopherol, and β- carotenes may reduce the toxicity of pesticides on vineyard crop sprayers. Minimum daily intake of two glasses of lemon juice will be very useful in decreasing adverse effects of pesticides.

In the future, a detailed study will be conducted to see the effect of these antioxidants on pesticides-exposed populations.

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Oxidative stress and antioxidant status in acute organophosphorous pesticides poisoning cases of North Karnataka (India)

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Abstract

This study was conducted to assess oxidative stress and antioxidant status of organophosphorus (OP) poisoning cases before and after therapy with atropine and pralidoxime of north Karnataka (India). For this study 30 male OP poisoning cases (study group) and 30 male normal healthy subjects (control group) having age range 17-40 years were taken. All organophosphorus poisoning cases were diagnosed by physicians.

In OP poisoning cases plasma cholinesterase levels were significantly decreased (83%, P<0.001) as compared to controls and increased (264%, P<0.001) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Serum malondialdehyde (MDA) level was significantly increased (142%, P<0.001) as compared to controls and significantly decreased (20.47%, P<0.05) as compared to before treatment of these drugs. Plasma total antioxidant capacity was significantly decreased (18%, P<0.001) in study group as compared to controls and increased (17%, P<0.05) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Increased erythrocyte superoxide dismutase (54.70%, P<0.05), catalase (40.30%, P<0.05), and glutathione peroxidase (15.70%, P<0.001) were observed in OP poisoning cases as compared to controls. Erythrocyte superoxide dismutase and catalase were decreased after treatment with atropine and pralidoxime as compared to before treatment of these drugs which was not statistically significant but only erythrocyte glutathione peroxidase (4.9%, P<0.001) was significantly decreased.

Organophosphorus pesticides inhibit the cholinesterase, induce oxidative stress and alter antioxidant status in OP poisoning cases. The biochemical effects of organophosphorus pesticides were reduced after treatment of atropine and pralidoxime. Therefore, along with these drugs, antioxidant supplementation may be useful to reduce toxic effects in OP poisoning cases.

Key words: Atropine, Catalase (CAT), Cholinesterase (ChE), Glutathione Peroxidase (GPx), Lipid peroxidation, Organophosphorus (OP) Poisoning, Pralidoxime (PAM), Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD).

Introduction

Organophosphorus compounds (OP) are highly toxic to human beings. Poisoning caused by OP compounds is steadily increasing in India because of their easy availability and potent toxicity. Among the OP compounds the most commonly used are: Dimethoate (Roger), Monocrotophos, Chlorpyrifos, Paraoxan, Mevinphos, Triazophos.

The toxic effects of OP compounds in acute poisoning cases result from inhibition of blood ChE (cholinesterase) activity including plasma and erythrocyte. Also there is evidence that AChE inhibition correlates with OP-induced symptoms of toxicity (Ranjbar *et al*., 2005). Toxicities of OP pesticides cause adverse effects on many organs and systems such as liver, pancreas, muscles, immune system, urinary system, reproductive system and hematological system (Teimouri *et al*., 2006).

In acute poisoning the main mechanism of toxicity of OP compounds is irreversible binding of these compounds to AChE (acetylcholinesterase) and inhibiting its activity which results in accumulation and prolonged effect of ACh (acetylcholine) and consequently follows with acute muscarinic and nicotinic effects (Ranjbar *et al*., 2004). Mild poisoning includes muscarinic and nicotinic signs only, while severe cases always show central nervous system involvement. The symptoms can vary in time of onset, sequence and duration depending on the chemical, dose and route of exposure (WHO, 1986).

The imbalance between production of free radicals and antioxidant defences in the body is called oxidative stress which has important health implications. Oxidative stress is a major mechanism in the pathophysiology of several toxins and diseases. In addition oxidative stress is also a process related to xenobiotic exposure and different levels of environmental contamination (Banerjee *et al*., 1999). In such cases peroxidation of membrane lipids seems to be an unavoidable process in tissue injury and may impair antioxidant defences leading to oxidative damage by changing the balance between oxidants and antioxidants (Halliwell B. *et al*., 1999; Banerjee *et al*., 1988).

Measurement of lipid peroxidation product e.g. malondialdehyde (MDA) and total antioxidant capacity (TAC) of blood is an effective marker to study oxygen free radicals effects in the body; hence this study was conducted to evaluate the existence of oxidative stress, antioxidants and total antioxidant capacity levels in acute organophosphorus poisoning cases before and after atropine and pralidoxime drugs therapy.

Materials and methods

This study comprises 30 OP poisoning cases and 30 normal healthy control subjects. All the study and control group subjects were in the age 17-40 years range. OP poisoning cases admitted to BLDEU'S Shri. B. M. Patil Medical College Hospital Bijapur, North Karnataka (India) were taken for the study. OP poisoning cases were diagnosed by physicians by observing clinical signs and symptoms and taking detail history from family members and the patient. All the patients were given 1gm of PAM by slow intravenous injection. After that a bolus dose of atropine was administered till signs of atropinisation. Also, improvement in signs and symptoms were monitored after treatment with atropine and pralidoxime. Before the biological specimen collection, the demographic, occupational and clinical data were collected from the study group and control subjects by questionnaire and interview. The entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration (1964).

10ml venous blood samples were collected from the OP poisoned cases under aseptic conditions.

> **Group I:** Immediately after admission to the hospital, before starting the appropriate (atropine and PAM) treatment.
>
> **Group II:** After complete recovery of the OP patient and before the patient is discharged from the hospital (i. e. on the last day of hospitalisation).

Serum and plasma were separated by centrifugation at 3,000rpm for 10 minutes, at room temperature. Then all samples were immediately placed at 4°C until they were processed to get accurate and reproducible results.

Plasma cholinesterase (ChE) was estimated by butyrylthiocholine kinetic method using standard kit of Agappe Diagnostics. Cholinesterase act on butyrylthiocholine to form thiocholine, which acts on dithio-bis-nitro benzoic acid giving pink-coloured 2-nitro, 5-mercaptobenzoate (Kendel and Bottger, 1967; Tietz, 1986).

Serum lipid peroxide i.e. MDA concentration was measured by the Satoh (1978) method. Serum proteins were precipitated by trichloro-acetic acid (TCA) and the mixture was heated for 30 minutes with thiobarbituric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of nmol/ml of malondialdehyde (MDA) using 1,1,3,3, tetra-ethoxy propane as the standard (Satho, 1978).

The plasma total antioxidant capacity (TAC) was estimated by FRAP (Ferric reducing ability of plasma) assay. The antioxidant power of plasma converts ferric ions to ferrous ions at low pH forming a pink coloured ferrous tripyridyl triazine (Fe^{III} – TPTZ) complex. Ferrous reducing antioxidant power values were obtained by comparing the change in the absorbance at 593nm in mixture with those of ferrous ion of known concentration. The TAC in plasma was expressed as nmol/ml (Iris *et al*., 1999).

Activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund. Superoxide anion is involved in the auto-oxidation of pyrogallol at alkaline pH 8.5. The superoxide dismutase inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance per two minutes at 420nm. The SOD activity was measured as Units/gms of Hb. One unit of superoxide dismutase is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation (Marklund and Marklund, 1974).

Erythrocyte catalase was measured by the method of Aebi (1983). Heparinised blood was centrifuged and the plasma was removed. The erythrocytes were washed three times with 5ml 0.9% sodium chloride and lysed in 10 volumes of cold deionised water. The whole mixture was centrifuged further for 10min at 3,000rpm. The cell debris was removed and the clear haemolysate was diluted 500 times with phosphate buffer (60mM) pH 7.4.

Catalase decomposes hydrogen peroxide (H_2O_2) to form water and molecular oxygen. In the UV range, H_2O_2 shows a continual increase in absorbance with decreasing wavelength. At 240nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240nm at 15 second intervals up to 1 minute and the difference in absorbance (ΔA at 240nm) per unit time was taken to be a measure of the catalase activity. The unit of catalase activity was expressed as mM of H_2O_2 decomposed/mg Hb/min (Aebi, 1983).

Erythrocyte Glutathione peroxidase (GPx) was assayed by Paglia and Valentine (1967) method. GPx catalyses

Paramaters	Controls (N=30)	OP poisoning cases	
		Before treatment (N=30)	After treatment (N=30)
I. Toxicity marker			
Plasma ChE (U/L)	6286 ± 912.53	1045.5 ± 555.6***	3802.6 ± 867***
II. Oxidative stress marker			
Serum MDA (nmol/ml)	1.05 ± 0.37	2.54 ± 0.89***	2.02 ± 0.71**
III. Antioxidant status			
Plasma TAC (nmol/ml)	1264 ± 130	1043 ± 197.8***	1215 ± 229**
Erythrocyte SOD (Units/gms of Hb)	10.14 ± 3.6	15.69 ± 6.4**	14.25 ± 3.7*
Erythrocyte CAT (mM H_2O_2 decomposed/mg Hb/min.)	15.54 ± 5.67	21.81 ± 8.1**	17.49 ± 6.5*
Erythrocyte GPx (U/L)	5351 ± 934.8	6196 ± 588.5***	5890 ± 476.8***

Table 1.0
Mean ± SD values of plasma cholinesterase, serum malondialdehyde, plasma total antioxidant capacity, erythrocyte superoxide dismutase, catalase and glutathione peroxidase in controls and OP poisoning cases before and after treatment.

** indicates P<0.05-significant, *** indicates P<0.001-highly significant and
* indicates P>0.05-Non significant as compared to controls.
ChE – Cholinesterase, MDA – Malondialdehyde, TAC – Total Antioxidant Capacity, SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase.

the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH (reduced NADP+) the oxidised glutathione is immediately converted to the reduced from with a concomitant oxidation of NADPH to NADP+ (nicotinamide adenine dinucleotide phosphate). The decrease in absorbance at 340 nm is measured (Paglia and Valentine, 1967).

Statistical analysis was performed using students 't' test. The values were expressed as mean ± SD. P value less than 0.05 (P<0.05) was considered as significant.

Results and discussion

Poisoning by OP compounds is the most common cause of suicidal deaths in India. Organophosphorus compounds are irreversible inhibitors of both muscarinic and nicotinic acetyl cholinesterase and affect the central nervous system. In this study, 60% of participants were from low socio economic status. The incidence of poisoning is very common in individuals with low economic status (Agrawal, 1993).

In organophosphate poisoning cases plasma cholinesterase levels were significantly decreased (83%, P<0.001) as compared to controls and increased (264%, P<0.001) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. The plasma ChE level of normal healthy control group ranged from 4,500-8,000 U/L and in OP poisoning cases 400-4,800 U/L. With an increase in the severity of poisoning there was a corresponding decrease in plasma ChE activities. This result is consistent with earlier studies (Vidyasagar *et al.*, 2004; Dandapani *et al.*, 2003). Inhibition of plasma ChE at the time of admission but steady recovery after treatment was found, which indicates regeneration of the enzyme and serves as an indicator of clinical improvement in the patient. If the levels do not increase, excessive ACh accumulates at synapses within sympathetic ganglia and skeletal myoneural junctions. Hence plasma ChE could be used as a parameter to monitor the prognosis of OP poisoning. Chances of recovery are greater when the patient is hospitalised at the earliest indication of poisoning.

Serum lipid peroxide (MDA) level was significantly

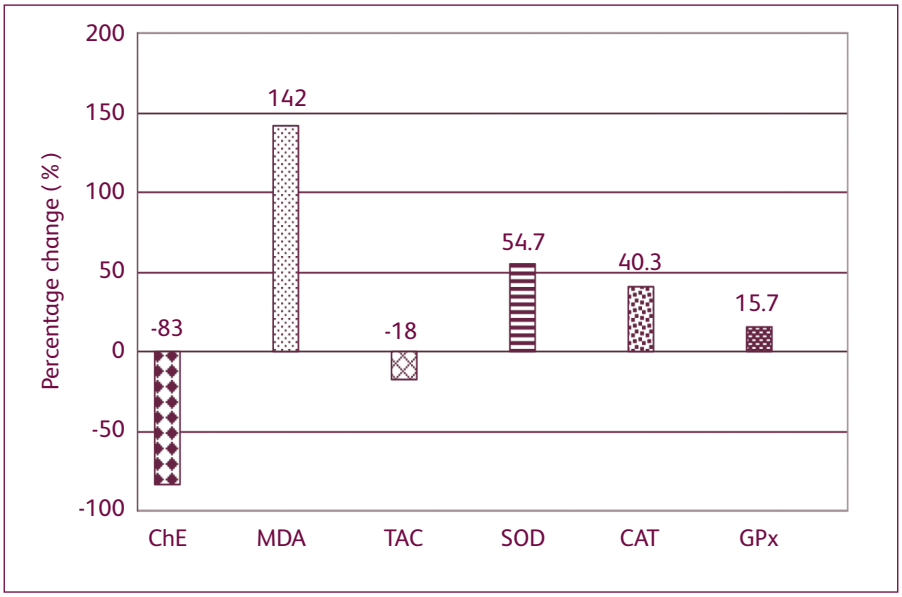

Figure 1.0
Percentage change of plasma cholinesterase (ChE), serum malondialdehyde (MDA), plasma total antioxidant capacity (TAC), Erythrocyte superoxide dismutase (SOD), catalase (CAT) and GPx of organophosphorus (OP) poisoning cases with respect to control group.

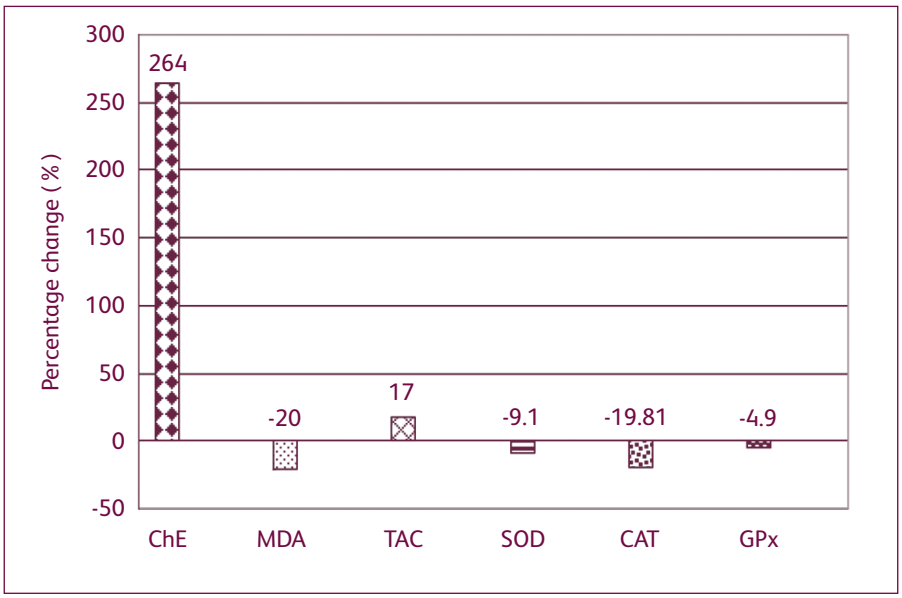

Figure 2.0
Percentage change chart of mean levels of plasma cholinesterase (ChE), serum malondialdehyde (MDA), plasma total antioxidant capacity (TAC), erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of organophosphorus (OP) poisoning cases after treatment with respect to before treatment.

increased (142%, P<0.05) as compared to controls and significantly decreased (20.47%, P<0.05) as compared to before treatment of these drugs. Increased MDA level may be owing to OP poisoning, which is decreased after treatment with atropine and pralidoxime. The inhibition of ChE initiates the accumulation of free radicals leading to lipid peroxidation, which may be the indicator of cell injury. The phospholipids component of biomembranes is believed to be the site of action of OP compounds (Akhgari *et al*., 2003). Toxic manifestations induced by OP compounds may be associated with enhanced production of reactive oxygen species, which induces the oxidative process and lipid peroxidative damage in cell membranes.

Plasma total antioxidant capacity was significantly decreased (18%, P<0.001) in the study group as compared to controls and increased (17%, P<0.05) after treatment with atropine and pralidoxime as compared to before treatment of these drugs. Increased erythrocyte superoxide dismutase (54.70%, P<0.05), catalase (40.34%, P<0.05), and glutathione peroxidase (15.70%, P<0.001) were observed in OP poisoning cases as compared to controls. Erythrocyte glutathione peroxidase (4.9%, P<0.001) was significantly decreased after treatment with atropine and pralidoxime as compared to before treatment with these drugs. Erythrocyte superoxide dismutase and catalase were not altered significantly after treatment with atropine and pralidoxime as compared to before treatment with these drugs. This result indicates that the organophosphorus pesticides altered antioxidants status may be caused by more generation of free radicals.

The human body has several mechanisms to counteract the damage produced by free radicals; the basic and the most prominent defence mechanism of the human body are antioxidant agents. Antioxidants are substances that delay or inhibit the oxidative damage to a target molecule. These molecules are stable enough to neutralise free radicals by donating electrons. Thus, in acute OP poisoning, sudden overproduction of ROS (reactive oxygen species) leads to significant lipid peroxidation and consumption of antioxidant agents for which the body could not compensate in a short period. A previous study involving 22 acute malathion poisoning patients showed significant lipid peroxidation accompanied by decreased levels of TAC, total thiols and ChE activity (Ranjbar *et al*., 2005).

The susceptibility of erythrocytes and lymphocytes to oxidative stress caused by pesticide exposure is a function of overall balance between the degree of oxidative stress and antioxidant defense capability. Thus, the OP compounds may directly or indirectly modify the antioxidant defence capability of exposed subjects and therefore affect their susceptibility to oxidative stress (Banerjee *et al*., 1999). Many intrinsic radical scavenger systems involve enzymatic and nonenzymatic reactions. SOD, CAT and GPx are important components of enzymatic antioxidative systems. Generally, there is an inverse relationship between lipid peroxidation and antioxidant enzymes; however, we found significant increase in erythrocyte SOD, CAT and GPx activities as well as serum MDA concentration. Increase in SOD activities in erythrocytes of OP poisoning cases indicates an increased production of superoxide radical. Increased CAT activities in erythrocytes may be explained by their influence on hydrogen peroxide as substrate, which is formed in the process of dismutation of superoxide radicals (Shaikh *et al*., 1999). Erythrocyte SOD, CAT and GPx efficiently scavenges toxic free radicals and are partly responsible for protection against lipid peroxidation from acute/chronic organophosphorus pesticide exposure. Thus, the increase in these enzymes was probably a response towards increased ROS generation in OP toxicity.

Supporting our results, there is evidence that administration of malathion resulted in increased SOD, CAT as well as MDA concentration in RBCs and livers of rats (Possamai *et al*., 2007). Increase in erythrocyte SOD and CAT activities in dimethoate and malathion treated rats was reported by John *et al*. (2001). The increase in these enzymes was a response towards increased ROS generation in OP toxicity. Banerjee *et al*. (1999) reported that between seven and 14 days after poisoning by malathion or propoxur-altered erythrocyte of AChE, SOD, CAT, GPx levels tend to return towards normal ranges as found in control subjects, probably reflecting the fact that the normal oxidative stress status was achieved easily.

Conclusions

The present findings indicate that cells continually suffer from oxidative stress in spite of over-activity of antioxidant defence mechanism as indicated by increase in erythrocyte SOD, CAT and GPx activity. The higher levels of antioxidant enzymes may be necessary to detoxify increased concentration of lipid peroxidation products that are generated from oxidative stress because of OP toxicity. Consumption of nonenzymatic antioxidants might be so high that the body could not compensate in a short period, hence reduced TAC was observed. Therefore, in emergency treatment of acute OP poisoning, the antioxidants at suitable doses should be given in order to reduce oxidative damage, which could be effective in speedy recovery of acute OP poisoning cases. Plasma ChE estimation determines the severity of poisoning, which can be helpful for predicting the outcome in OP poisoning cases.

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Bacteriological quality of water in garden centre water features

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Abstract

The risk to the public's health from recreational waters has been well documented, with outbreak of disease being associated with contaminated water that has been ingested, such as E coli infection, or inhaled, such as Legionnaires Disease.

Mandatory guidelines, legislation and microbiological standards have mainly focused upon swimming pools, hydrotherapy, spa pools and interactive water features owing to the risk of faecal contamination from humans and animals; however, the guidelines do not extend to covering the potential risk from decorative water features. Where garden centres have ornamental water features on public display, public access to the features is usually unrestricted and the public can come into contact, whether through actively seeking to do so or whether through accidental splashing with the water. The splashing generated by such features when operating creates an aerosol, which discharges at the height of the feature, very often at approximately 0.5-0.75 metres, being at head height for a child restrained in a buggy.

The project sought to identify whether pathogenic bacteria are present in the water contained within these features and whether there is a risk to health that should be managed in order to reduce the risk to both the public and to occupationally exposed employees.

Key words: E coli, Legionella, water borne disease

Introduction

Outbreaks of infectious diseases from waterborne pathogens such as *Legionella*, from Protasoa such as *Gardia* and *Cryptosporidium*, viruses such as *adenovirus* and *norovirus* has led to the development of a regulatory regime requiring the carrying out of risk assessments, monitoring and management based on advisory guidelines and legislation.

Mandatory guidelines, legislation and microbiological standards have been directed towards water features with which users have a direct and intimate association, such as swimming pools, hydrotherapy, spa pools and interactive water features because of the risk of faecal contamination. The guidelines do not extend to water circulating or contained in ornamental water features. Decorative water features are increasing in popularity and such ornaments can be found within buildings such as offices to create a tranquil atmosphere, as a design feature in hotel receptions or displayed in public gardens as well as exhibited for sale in garden centres.

Cases of water borne illness from ornamental water features are well documented. Jones *et al.* (2003) discusses an outbreak of Pontiac Fever, the milder influenza-type symptom of *Legionellasis* which resulted in 25 patrons of a restaurant becoming ill. The cause of the outbreak was found to be a number of decorative fountains and misting machines positioned within the restaurant. An outbreak of Pontiac Fever involving 34 guests was associated with a decorative water fountain positioned in a hotel lobby (Gozt *et al.,* 2001). Nichols (2006) discusses how indoor water features have been implicated in Legionella outbreaks and surmises that this is owing to increased temperatures, lack of sun light and enclosed spaces that enhance aerosol transmission. The water features may also be susceptible to environmental contamination from plants and earth (Havelaar *et al.*, 2001). When the water becomes contaminated and in the absence of a cleaning and disinfection regime bacterial biofilms can establish themselves, subsequent water flow is reduced and invading bacteria are difficult to eradicate with basic cleaning techniques.

Although ornamental water features are not designed to be bathed in or for interaction, and therefore theoretically the risk of direct contamination is less than exists with immersion pools, evidence from the outbreaks discussed above indicates that there may be a risk of contamination from faecal organisms in water or from *legionella ssp* as people and children are drawn to water features. This research seeks to identify whether pathogenic bacteria, specifically E coli and *legionalla ssp,* are present in the water contained within these features and whether if present they appear in sufficiently high levels to pose a risk to health that needs to be assessed and managed in order to prevent the risk.

Methodology

Although there is evidence of water borne disease transmission from ornamental water features in a number of locations, including hotels lobbies and shopping malls, this study focused on water contained and circulating in ornamental water features displayed in garden centres. Where water features are present in hotels and shopping malls, it is suggested that the risk from such features is well known and that the features are usually protected by appropriate maintenance and cleaning schedules. The potential risk posed by ornamental water features displayed for sale in garden centres is not so well

recognised or managed, and there is currently no guidance as to the bacteriological quality of the water. The features are usually readily accessible to the public and there are no barriers preventing individuals from touching the water or preventing them approaching too closely to them. This study therefore focused on water features displayed in seven garden centres to determine what risk from the circulating water exists and how that risk if identified could be minimised.

For the purposes of testing, a sample of at least 1,000ml of water was required from each water feature. The study was conducted overtly, with the consent of the garden centre operator being sought when the sample waters were taken. This was for two reasons; it was considered impractical to remove 1,000ml of water from a public water feature covertly, and the researcher was also required to ask the operator a number of questions regarding the operation and cleaning of the feature and therefore was required to declare their presence. No garden centre refused consent for the sampling.

A sample programme worksheet was designed to record sample information. For each feature identified, the following information was recorded.

- Sample code
- Total height of feature in cm
- Height of water discharge in cm
- Photograph of the feature
- Evidence of cleaning procedures
- Evidence of risk assessments or the presence of warning signs

Additional information about the environment

The water features were measured for total height and height of water discharge was taken. Temperature of the water circulated by a feature was taken using a digital probe, which was sanitised between use using antibacterial wipes.

The surrounding environment was observed for warning signs, safety instructions for the public and also for any possible sources of contamination. All information was documented on the worksheet. A minimum of 1,000ml of water was required for laboratory testing which was collected from the water feature at various points depending upon the water's flow. Water samples were collected aseptically into sterile bottles and were identified by an individual code written on the container label along with description of the sample point, date and time of sampling. The samples were transported under refrigeration to an independent laboratory for analysis.

Choice of indicator organisms

In order to demonstrate that the water sampled was contaminated and also potentially posed a risk to health it was necessary to select indicator organisms and to measure the number of the selected organisms present in the sample to determine whether transmission of an infective dose was likely.

Two methods of infection were considered to be possible from operating ornamental water features, being ingestion and inhalation. The potential for ingestion of water came from hand-to-mouth transfer of water where individuals had direct contact with the water by election, and using the same route where accidental splashing of persons or property had occurred. The potential for inhalation arises from the aerosol generated by the splashing of the water feature during operation. This was considered to be of particular relevance in relation to children, either restrained in pushchairs or buggies where the head height of the child was at the same height as that at which the aerosol was being generated and where the potential for inhalation of contaminated droplets was consequently high.

Haavelaar *et al.* (2001) state that E coli is an ideal organism to indicate faecal contamination of water sources, because it is easily detectable, does not grow in natural water and behaves similarly to waterborne pathogens. The water features identified were housed indoors, but were not in any way protected from contamination. In a number of the garden centres there was evidence of the presence of birds overflying, perching on and drinking from the water features. There was little attempt to prevent this; rather it was seen as attractive and in keeping with the ethos of being 'close to nature' in a garden centre. There was also recognition in many of the garden centres of risk of infestation by rodents attracted to the store of seeds and bulbs, and that faecal contamination of water in ornamental water features was likely if rodents were present. E coli is also a pathogenic bacteria; therefore it was considered appropriate to screen samples for the presence of E coli, and to measure the numbers of viable organisms in the

samples collected to determine whether in the dose levels found it posed a realistic risk to health.

The laboratory used the M016 test method for the enumeration of total E coli in water using MLGA. Following incubation relevant colonies were enumerated and results recorded. Limitations to the method are that only 90% of E coli species are detected and the specific species or serotypes were not identified.

The laboratory employed the BS6068-4.12:1998 ISO 117731:1998 analysis method for the detection and enumeration of *Legionella* was based on the membrane filtration technique. Following incubation relevant colonies were enumerated and results recorded.

Results

All garden centres visited agreed to allow sampling to take place and personnel were happy to answer questions regarding cleaning procedures and risk assessments.

The water used in all the decorative features was sourced from the mains supply. The features contained pumps which would circulate the water continuously around the system. An exception to this was where decorative displays were built to demonstrate how they could be incorporated into a garden's landscape. These features differed because water from the various fountains and cascades would stagnate in a shallow pool before re-circulating the system.

Cleaning procedure

The methods and frequency of cleaning was inconsistent across the garden centres. There were no cleaning schedules produced from any of the garden centres. Cleaning was mostly carried out when excess algae growth affected the aesthetics of the decorative feature or blocked nozzles thus restricting its function.

Generally, the methods of cleaning employed involved features being drained and brushes used to scrub away algae growth or they were washed clean without the use of chemicals, refilled and treated with chlorine crystals. The use of chlorine was not encouraged by the manufacturers as the features were designed for the garden and any chemicals used could affect wild life. The water that supplied one of the landscaped features was treated with ultra violet light before being re-circulated.

Risk assessment and warning notices

When asked none of the garden centres personnel was aware of any risk assessments completed for the water features. The Pool Water Treatment Advisory Group (PWTAG, 2001) states that formal risk assessments are required by the Health and Safety Work Act 1974 to assess the microbiological risks from interactive water features and the PWTAG recommends that decorative water features be included in order to protect the health and safety of the employees. This is especially pertinent when there was clear evidence that many of the decorative features created aerosols and caused splashing, creating a slippery surface around the display area that could result in a slip injury as well as the risk contracting an infectious disease.

Two of the seven garden centres visited were aware of the possible dangers and displayed warnings signs around the features with the following statements:

- All children must be accompanied
- Unaccompanied children may result in injuries
- Water fountains may contain bacteria.... wash hands after immersion

Microbiological analysis results

The samples were analysed for the presence of *Legionella spp* – the results concluded that the organism was not detected in any of the water samples.

The samples were also analysed for the presence of E coli. Thirteen of the fifty-two samples were found to contain E coli, all of which exceeded the microbiological standards recommended for water by Barrell *et al. (*2000) in a communicable disease and public health review. Barrell *et al. (*2000) recommend guidance levels of 0cfu/100ml E coli for swimming baths, spa pools and hydrotherapy pools (standard A of Figure 1.0). Three of the samples were >100cfu/100ml which exceeds the recommended value of faecal coliforms in bathing beach water, which is 100cfu/100ml (see standard B of Figure 1.0).

Nine of the 13 water samples analysed and found to be contaminated with E coli were taken from decorative features from decorative features in the same garden centres. The water features themselves were in a poor state of cleanliness, and many of the features were heavily

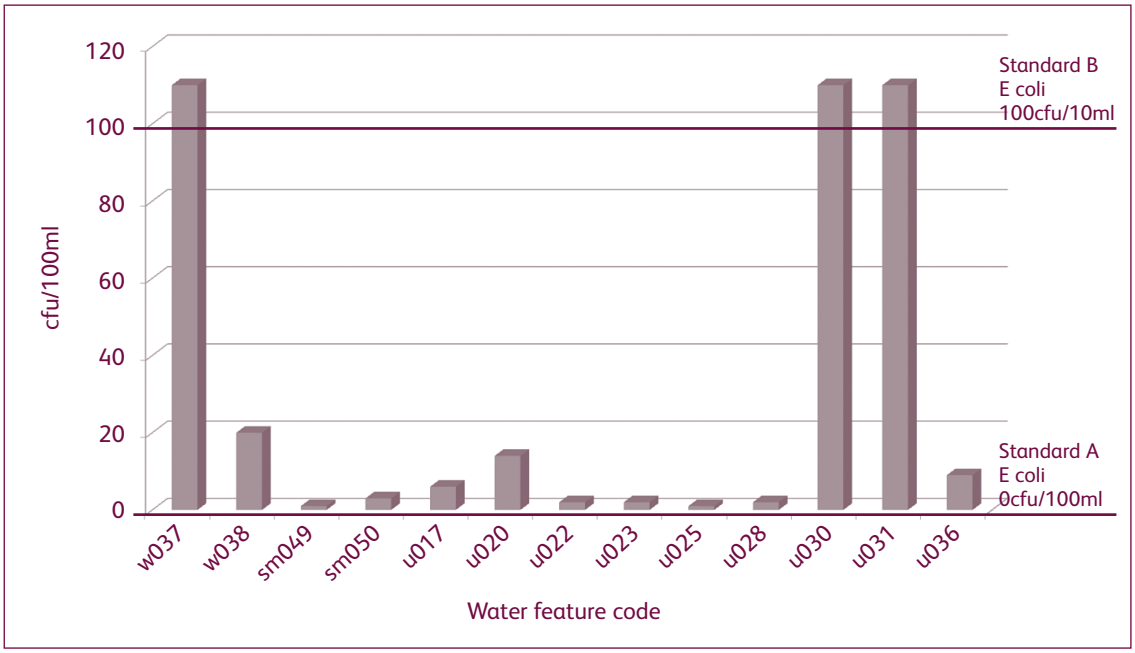

Figure 1.0
Enumeration of E coli isolated from decorative water features.

contaminated with algae. One feature was not working correctly because a number of the pipes were clogged.

Two of the samples that exceeded 100cfu/per 100ml were taken from a display that included a collection of decorative features. Water from all the features was pooled before re-circulating; also the display was landscaped with plants which it is suggested was the source of the high level of contamination. A further two samples that contained *E.coli* were situated outside and were unprotected which increased the risk of contamination.

Discussion

Legionella or E coli were not detected in the samples taken from the decorative features which demonstrated an awareness of the need to carry out a degree of cleaning to improve the features' aesthetics and also the garden centres that had a recognition of the potential for infection and health and safety by displaying public notices.

The water samples with a detectable level of E .coli were taken from features which were landscaped including plants and soil in the display and also those which were situated in the open air. The source of contamination is likely to be environmental from the planting material, birds and rodents faecal shredding since the features were not protected from incidental contamination.

The *E.coli spp.* are a vast group of bacteria, some of which are harmless and others are pathogenic. In order to identify their pathogenicity it would have been necessary to serotype the E coli detected. The study did not extend to serotyping but aimed to be an indicator of water contamination. In addition, the presence of E coli does not always result in illness or disease (CDC, 2008).

The World Health Organisation (2001) states that it is difficult to deduce an infective level of any particular pathogen found in water because the risk largely depends on the infectivity and invasiveness of the pathogen as well as the susceptibility and immunity of the individual exposed. In light of this, the WHO (2001) concludes that any water containing pathogenic organisms cannot be considered safe.

The majority of the decorative water features were prone to splashing and creating water aerosols at heights at which children may make contact and inhale the aerosols. Aerosolisation spreads pathogens to the surrounding environment and the WHO (2001) states that it is unclear precisely about the infective dose of

Legionella but is aware that susceptible humans exposed to a low dose for only a few minutes have become ill.

The absence of *Legonella* in any of the water features sampled while encouraging does not mean that these features may not potentially pose a risk. The general lack of awareness of the organism and the steps necessary to prevent inoculation and reduce the risk of its proliferation demonstrated by garden centre operators and its low infective dose suggest that where a water feature does become infected with *Legionella*, there is a potential risk of infection.

Recommendations

The results identify that there is a potential risk to the public's health from these decorative water features if they are not managed and maintained correctly. Nichols (2006) and Jones *et al.* (2006) agree with the recommendations made by the PWTAG that the foremost effective way of minimising the risk to the public's health from outbreaks of infectious diseases is through risk assessment and risk management.

Correct management of the features would ensure that cleaning schedules and methods employed for cleaning minimise the risk of contamination. Caution must be taken when treating the water with disinfectants because of splashing.

All decorative water features exhibit areas should display warning signs notifying the importance of washing hands if in contact with the water.

Conclusions

The study confirmed that ornamental water features that are not maintained, not managed in terms of risk assessed and not cleaned are subject to contamination, and subsequently have the potential to affect the health of the public. In light of this discovery, it is important that environmental health practitioners (EHPs) take this into account during inspections of these premises providing guidance to garden centre operators.

Where garden centres provide food in the form of a café, EHPs may need to take into consideration the water features as a potential source of infection. EHPs may need to provide advice and guidance on maintenance, risk management and cleaning procedures. Measures such as displaying clear, concise and visible warning signs are required informing the public of potential risks as well as advising the need to wash their hands and providing hand washing facilities for the public before eating food.

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Contributing to success: A contribution approach for better environmental health regulation.

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Abstract

The Contribution Approach is an evidence-based alternative to cost-benefit analysis for assessing the performance of complex environmental health programmes. Instead of the difficult task of trying to establish cause and effect, it explores causality by assessing the contribution a programme is making to expected results. It provides one of the primary bases for the Local Better Regulation Office report and toolkit on Impacts and Outcomes of Local Authority Regulatory Services.

Key words: Environmental health management.

Introduction

When dealing with complex environmental health or other regulatory problems, it is often not feasible to carry out experiments to determine the precise attribution of programme results. For this reason, the Local Better Regulation Office (LBRO) (2009) report recommends the adoption of a 'contribution' approach (Mayne, 2008). This approach establishes a 'causal chain' (or 'theory of change') describing how an intervention is expected to contribute to a desired outcome via a series of logical steps. The implementation of the programme is monitored along each step in the chain by indicators to provide evidence to justify the initial assumptions. A reasonable inference can then be made that the intervention has actually contributed to the solution of the problem.

The logic model

The contribution approach combines the techniques of a 'logic model' with a 'theory of change'. A logic model represents the important conceptual stages in the transformation process from resource inputs to policy outcomes and impacts. It lists the inputs, activities, outputs, outcomes and impacts in discrete categories but does not on its own indicate how a specific element within one category contributes towards the achievement of an element within another category. It must therefore be supplemented by a theory of change to investigate and explain these relationships.

The theory of change

The theory of change explains how a programme or activity is expected to achieve an agreed set of outcomes and impacts. The concept derives from writers such as Carol Weiss (ActKnowledge, undated) who in 1995 considered that the main reason why complex social programmes are difficult to evaluate is because the assumptions and risks underlying them at the planning stage lack sufficient detail and clarity, specifically in relation to the process of change throughout the programme. Planners need to be clear about the theories that explain the connections between activities and expected results throughout the transformation process. Evaluation of progress would then focus on monitoring whether available evidence validates the original theory of change, thereby ensuring the achievement of expected results.

The LBRO research

The LBRO research aimed to demonstrate the theory of change or 'intervention logic' of Local Authority Regulatory Services (LARS) action by developing 'pathways' of 'causal chains'. To develop the pathways, the logic model was supplemented with information from process mapping in research workshops with local authorities to provide links between the elements of the logic model. An impact 'dashboard' was developed for each case study presenting performance indicators for executive decision-making purposes. Finally, a toolkit was devised for LARS to develop their own pathways and dashboards of indicators.

The pathway – An environmental health case study

The intervention logic was demonstrated in five case studies (three trading standards and two environmental health).

One of the environmental health case studies related to fly-tipping and was used as a model for the toolkit. To produce the pathway, it was necessary to define activities, identify outputs (direct products of activities), explore the outcomes (consequences of activities and outputs) and impacts (longer-term and wider outcomes) that they lead to. The activities were classified as 'reactive work' and 'preventative education work'. The outputs of reactive work were the prosecution of fly-tipping cases and the removal of waste deposited illegally.

For preventative education work, the main output in this case study was participation in an ad hoc 'environment action day' involving other contributors such as the Fire and Rescue Service, Police, other council services and community panels. Three causal chains were developed to illustrate outcomes.

First, the removal of waste had direct outcomes of a cleaner environment, less pollution, the removal of obstructions to rights of way and the prevention of damage to agricultural land. There were also indirect benefits of preventing future fly-tipping by the prompt removal of waste.

Second, educational activities contributed to the prevention of fly-tipping by informing the public about illegal activities and hazards.

Finally, prosecution helped to recover removal costs and contributed to deterrence through publicity about cases and penalties.

These three causal chains then merged as they contributed to impacts which were identified as pride in the area, community cohesion, attractiveness for consumers and tourists, effects on house prices and reduced costs to taxpayers.

Indicators for the pathway

The next step was to identify indicators to evaluate the key elements of the pathway in a dashboard. Input resource costs were fairly straightforward to identify in terms of staff, running costs and capital costs. Output indicators for the removal of waste were the number of fly-tips reported and removed and the number and type of prosecutions. There was no quantitative output indicator currently available for education activities, though a suggested measure was the number of people reached through educational activities (such as environment action days) over a period of time.

Outcome indicators were related to the three causal chains. For the first chain on removal outcomes, there were no specific indicators identified for a cleaner environment, less pollution, etc, though it was considered that data on removal of waste could be used as the causal relationship between removal and cleanliness was clear. Time taken for removal would be an indicator for prevention. For the second chain on education outcomes, it was suggested that target groups could be surveyed for changes in attitude. For the third chain on prosecution outcomes, recovered costs would be known but there was no measure of deterrence. A survey of attitudes was recommended. Finally, indicators to assess impacts were explored.

Community impacts of pride, cohesion and attractiveness would have to be established through surveys of residents, businesses and tourists. House price movements would be readily available. Taxpayer savings would be indicated by clearance costs and money recovered through prosecutions. For each of the indicators chosen, a 'traffic light' symbol of red, amber or green was suggested to show to what extent the evidence supports the assumptions made in the pathway.

Conclusions

It is recognised that this approach falls short of a full cost-benefit analysis, which attempts to measure benefits and costs in the same monetary terms in order to calculate net benefits or 'profits'. This level of analysis may never be achieved for environmental health regulatory services. Nevertheless, it is suggested by the LBRO report that the contributions approach is 'an important step' in this direction. It will be an invaluable tool for the environmental health service to demonstrate to decision makers that their resource inputs, activities and outputs make a significant contribution to their local communities and businesses, thereby enhancing their profile in the planning and allocation of financial resources.

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Invitation to peer reviewers
Letters to the editor

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