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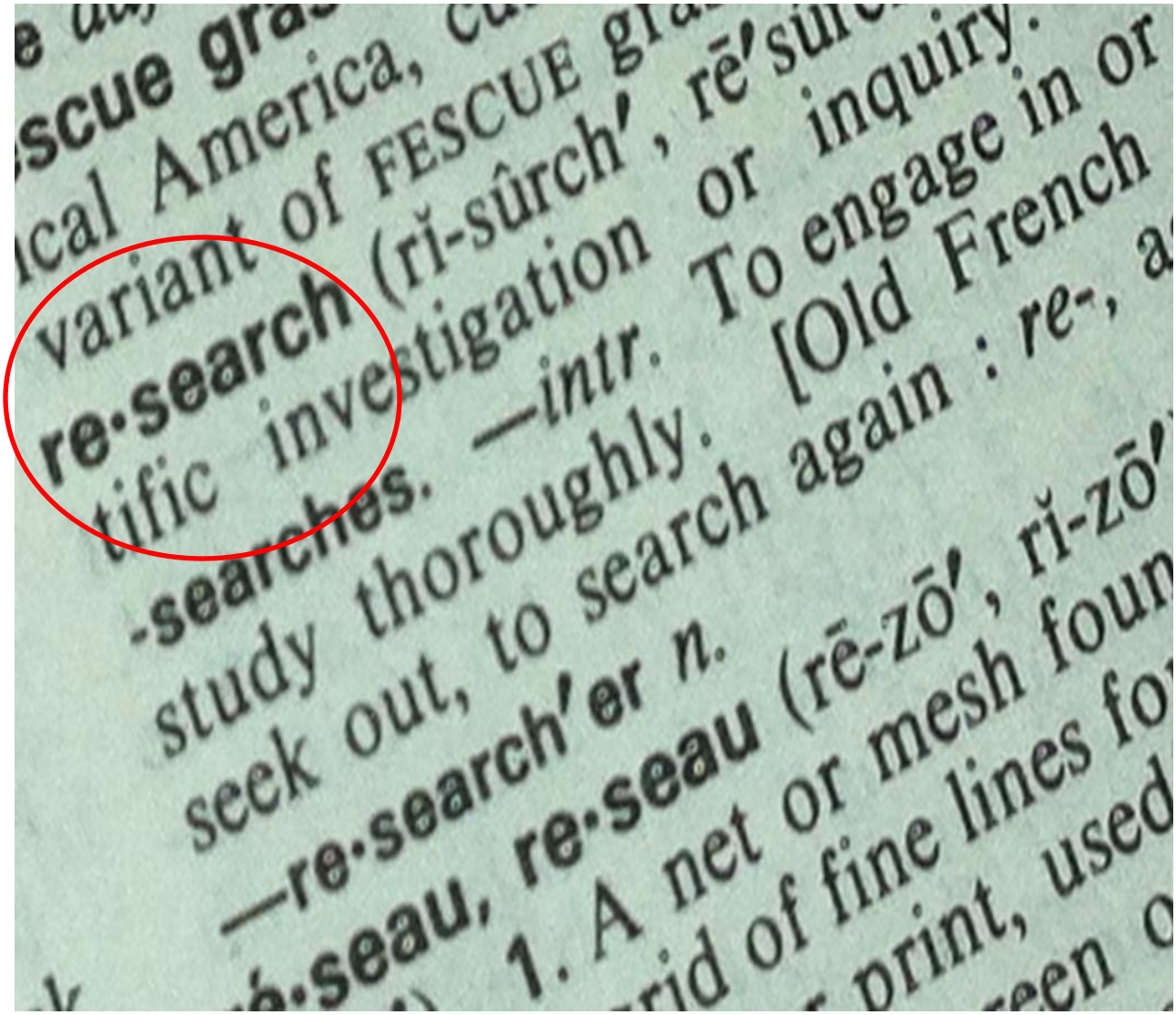

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A Study of Filter Design for Noise Reduction in Speech Signals

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Abstract

Speech signal de-noising plays an important role in numerous applications, including speech synthesis, recognition and also a necessary step for speech feature extraction, e.g., pitch track estimation. To acquire an accurate pitch track, the speech signal should have minimum noise interruption. Noise is usually generated by environments or electronic equipments during the recording of the speech signals. In this paper, we investigate several filter design algorithms and apply them on the recorded noisy speech signals. The experiment results show that the presented methods really reduce noise presented in those speech signals.

Introduction

Digital Filters are the most important methodology for de-noising ("Low Pass Filter" Wikimedia Foundation 2012). Audio and speech signal can easily be distorted with background noise due to electronic equipment or people talking. Digital filters have the functions of separating and restoring distorted signals by reducing and/or masking the noise ("Low Pass Filter" Wikimedia Foundation 2012). This can be accomplished by sending a signal through a filter to reduce the noise without affecting the quality of the desired signal (The Global Social Learning Network 2012). In this article, the digital filter designs consist of a low-pass filter, followed by a band-pass filter, Spectral Subtraction filter, and finally a Wiener filter. All filters are Finite Impulse Response (FIR) Filters. The decision to use FIR filters is based on the fact that the input audio is of finite length and settled to zero in infinite time (Ekstein and Kral 2012).The remainder of this paper is ordered as follows: the filter design and results is entailed in section 2 and the summary and discussion is presented in section 3.

Results

Lowpass Filters to the speech signals

Human voice frequency ranges from about 60 to 7000 Hz ("Human Voice" Wikimedia Foundation 2012). With the help of MATLAB, a noisy speech signal on a spectrum scope is shown in Fig. 1. From this plot, we notice that the magnitude of the speech signal declines dramatically from 6000 to 8000 Hz, which implies that the frequency of a high proportion of noise is above

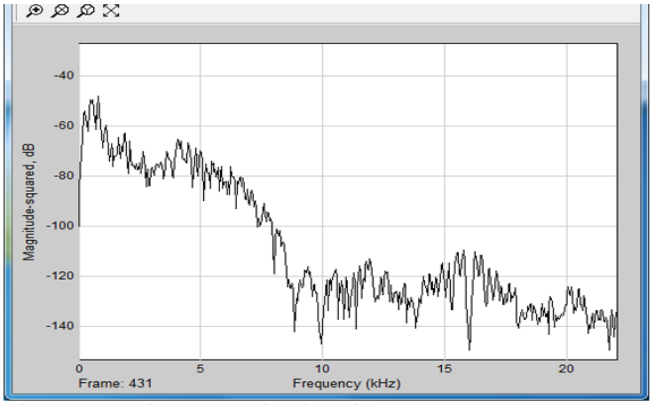

Figure 1. Noisy Speech Signal

A low-pass filter has a sampled frequency (Fs) of 44100 Hz. The noisy speech signal is recorded in 44100 Hz. The frequency pass band (Fpass) is 6000 Hz and frequency stop band (Fstop) is 8000 Hz. The magnitude specifications: Apass is 1dB and Astop is 100 dB. **Fig. 2** shows the speech signal after passing through the specific low-pass filter. The spectrum scope shows that the noises with frequency above 8000 Hz are filtered out of the noisy speech signal. However, some noises with low frequency are still present in the speech signal. This required further research with extended filtering.

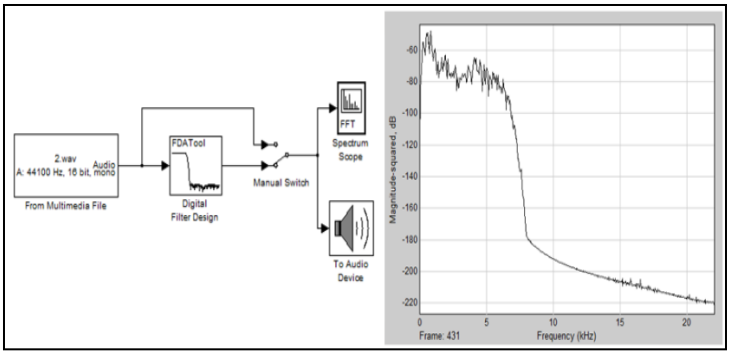

Figure 2. Low pass filtered Speech Signal

Applying a specific designed Band-Pass Filter to the noisy speech signals

The green plot (Fig. 3) is the wave form of a noisy speech signal and the power spectrum of the noisy speech signal is presented in the lower part of this picture. The bright orange is the desired speech signal that is between 2 to 7 seconds. The rest of the bright orange is the noises of the signal that needs to be filtered out.

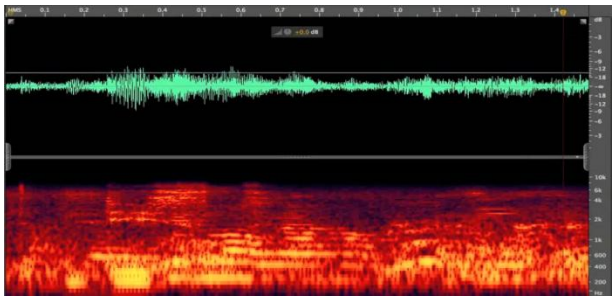

Figure 3. Noisy speech signal power spectrum

The frequency of the noise signal ranges from 0 Hz to 500 Hz and 8000 Hz +. The F_S is 44100 Hz, Fstop1 is 500 Hz, $F_{pass}1$ is 1000 Hz, F_{pass} 2 is 6000 Hz, and F_{stop} 2 is 8000 Hz. The magnitude specifications are: Astop1 is 30 dB, Apass is 1 dB, and Astop2 is 150 dB. The output signal of the band-pass filter is shown (Fig. 4).

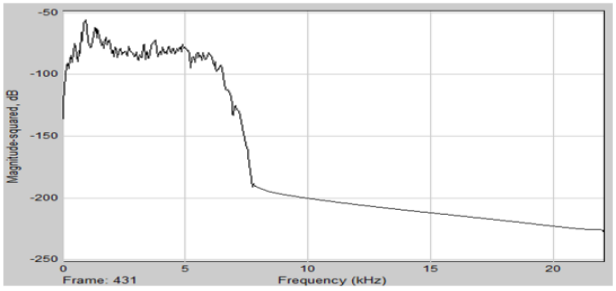

Figure 4. Band pass filtered speech signal

Applying spectral subtraction and Wiener Filters to the noisy speech signals

Zheng et al. in the paper "Background Noise Cancellation for Speech Communication" proposed an approach of using the Spectral Subtraction and Wiener filters to de-noise speech signals, and approach even more effective than those proposed above.

Discussion

We have investigated three filter design methods for the reduction of the noise presented in the speech signals. Results showed that they are effective in some spectrum ranges. However, it's difficult to find a perfect scheme to remove all noises and keep the original signals unchanged. This is because noise generally covers different audio spectrum. The filter algorithms can remove the noise and also some components of the speech signals in the same time. We have to strike a balance between removing noise and leaving speech.

Acknowledgements

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Remote Sensing of Soybean Stress Indicator of Chemical Concentration of Biosolid Applied Soils

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Abstract

The accumulation of heavy metals in the biosolid amended soils and the risk of their uptake into different plant parts is a topic of great concern. This study examines the accumulation of several heavy metals and nutrients in soybeans grown on biosolid applied soils and the use of remote sensing to monitor the metal uptake and plant stress. Field and greenhouse studies were conducted with soybeans grown on soils applied with biosolids at varying rates. The plant growth was monitored using Landsat TM imagery and handheld spectroradiometer in field and greenhouse studies, respectively. Soil and plant samples were collected and then analyzed for several elemental concentrations. The chemical concentrations in soils and roots increased significantly with increase in applied biosolid concentrations. Copper (Cu) and Molybdenum (Mo) accumulated significantly in the shoots of the metal-treated plants. Our spectral and Landsat TM image analysis revealed that the Normalized Difference Vegetative Index (NDVI) can be used to distinguish the metal stressed plants. The NDVI showed significant negative correlation with increase in soil Cu concentrations followed by other elements. This study suggests the use of remote sensing to monitor soybean stress patterns and thus indirectly assess soil chemical characteristics.

Introduction

There are several concerns about the potential buildup of heavy metal concentrations in soils and there by affecting the crop growth from repeated biosolid applications. Without extensive and time consuming field work, it is often difficult to obtain an overview of the spatial distribution of soil chemical concentrations and its effect on crop growth. As the agricultural fields are covered with vegetation for most of the year, remote sensing of crop-covered biosolid applied fields helps in making indirect assessment of soil concentrations through mapping the spatial and temporal heterogeneity in the crop growth. Hence the objectives of this research are; 1) to determine the accumulation of metals and nutrients in soybeans grown on biosolid amended soils and 2) to study the relationship between soybean vegetation reflectance and different levels of soil contamination

Materials and Methods

Greenhouse study

A greenhouse study was conducted in parallel with a field study, to assess the phytotoxic effects of increase in soil chemical concentrations on soybean growth in a controlled environment and to study the changes in spectral reflectance at individual plant-level. A total of 30 potted plants consisting of five sludge treatments and a control or untreated group (P0) were arranged in a completely randomized design, with 5 replicates in each treatment. Sludge treatments were made by adding the biosolids at the rate of 2, 5, 10, 25 and 50 t/acre on dry weight basis to the potting-soil and these groups were subsequently referred as P2, P5, P10, P25 and P50 respectively. The soils were air dried, homogenized and about 2.0 kg of the soil was weighed and transferred into plastic pots. The seedlings were thinned to two plants per pot at the 2-3-leaf stage (Fig.1). Two plants per pot were allowed to grow up to 57 days after sowing (Sridhar et al. 2011). **Fig 1:** Green house setup for soybean plant growth.

Fig. 1: Green house setup for soybean plant growth.

Fig. 2: Spectral reflectance of soybean plants.

Spectral reflectance

A Fieldspec Pro spectroradiometer (ASD Inc., Boulder, CO, USA) with a spectral range of 350–2500 nm was used to obtain the reflectance spectra of each individual potted plant in the green house, with a quartz-tungsten-halogen (QTH) lamp as a light source. The spectral reflectance procedure was reported in detail elsewhere by Sridhar et al. (2009, 2011). The spectral reflectance of the plants was collected at periodic intervals during the entire experiment period for the greenhouse study (Fig 2).

Results

The soybean plants in the P0, P2, P5, P10, P25 and P50-treated groups grew steadily and chlorosis was not visually observed during the pot study. The metal and nutrient accumulation in soils increased significantly (p<0.05) with increase in applied sludge concentration in all the sludge-treated groups. Accumulation of Ba, Cd, Cu, Mo, S and Zn in roots increased with increase in their soil chemical concentrations. Accumulation of Cu and Mo in shoots increased with increase in the applied sludge concentrations. Metal concentrations remained high in soils, followed by roots and shoots while the nutrient (P and S) concentrations remained high in shoots and roots followed by soils.

The averaged (n = 5) reflectance spectra of canopies of all the treated groups from the last day (57^{th} day) of green house study are given in (Fig. 3). The reflectance spectra of the P25 and P50-treated groups showed a decrease in reflectance in the 800–1300nm, 1470–1850 nm, and 2000–2500 nm regions. The reflectance spectra from the P5- and P10-treated groups showed less decrease in the 800–1300nm and 1470–1850 nm regions compared to untreated (P0) plants.

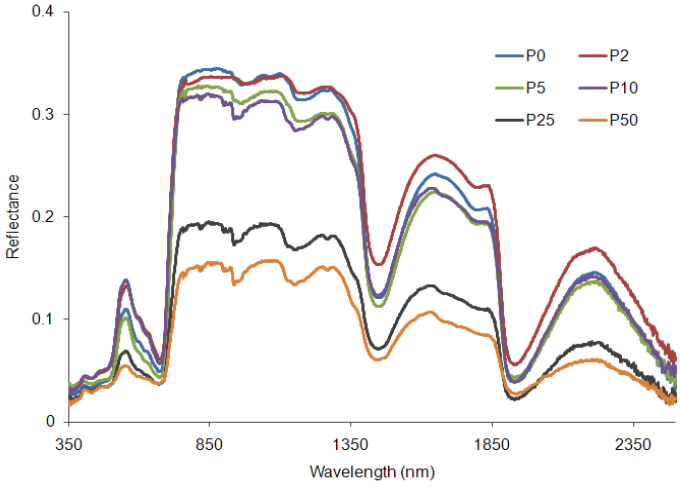

Fig 3: Changes in spectral reflectance of plants treated with different concentrations of biosolids.

Discussion

The results of our study show the potential of remote sensing to monitor the effect of elevated chemical concentration in soybeans grown on biosolid amended soils. The EPA part 503 regulations require monitoring of heavy metal additions to biosolid amended soils. However, they do not require any testing and monitoring of the crops grown on these soils. The effects of biosolid application on soil and crop quality have rarely been monitored in non-research field conditions, such as the regular production agriculture farm selected in this study.

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Silymarin and Related Favonoids Counteract Stress of Simulated Microgravity in the Nematode *Caenorhabditis elegans*

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Abstract

Flavonoids present in many herbal edibles possess a remarkable spectrum of biochemical and pharmacological actions and they are assumed to exert beneficial effects to human health. Many of the protective properties of flavonoids are attributed to their antioxidant effects. The flavonoids Silymarin, silibin, quercetin and narengenin were tested as antioxidants to counteract the effect of simulated microgravity using *C. elegans*. The flavonoids were added as food supplement to the food source *E. coli* OP50. Treated *C. elegans* populations were exposed to simulating microgravity utilizing the wall rotating vessels, high aspect ratio vessels (HARV). The tested flavonoids increased survival, increased reproduction and reduced mortality for *C. elegans* populations that were exposed to simulating microgravity. Also progeny and the lifespan increased in hermaphrodites isolated from populations exposed to simulated microgravity and treated with different food supplements.

Introduction

Silymarin, the active extract from *Silybum marianum*, commonly known as milk thistle is one of the oldest and thoroughly researched plants of ancient times for the treatment of liver disorders including hepatitis, cirrhosis, jaundice, and protection from other toxin poisonings. Silymarin, is a complex of flavonoids and polyphenols. Much of milk thistle's protective effect is due to flavonoid complex which act as a powerful antioxidant, combining with and thus neutralizing harmful free radicals that result from normal metabolic processes and from breakdown of toxic substances, (Noroozi et al.1998; Middleton et al. 2000; Kamkotter et al. 2007). The most active compound in silymarin is silibinin (SB), a powerful antioxidant aid to protect liver cells (and other cells in the body and brain) from toxins. Quercetin, an isomer derived from Silymarin is a main representative of flavonoids found at high concentrations in herbal edibles (Kamkotter et al. 2007; Senoo et al. 2001).

Naringenin (flavonoid) isolated from grapefruit offers prevention against the hepatitis C virus, (Nahmias et al. 2008). Several epidemiological studies have found an inverse association between the intake of diets rich in polyphenols such as fruits, vegetables, and grains, and the risk of age-related diseases in humans (Halliwell 2011; Gutteridge, and Halliwell 2010; Williams et al. 2004). *C. elegans* with many biological functions and systems such as: reproductive, muscular, nervous and digestive systems allow researchers to find protection for human in space. NASA adopted *Caenorhabditis elegans* as a model organism to study the effects of space and space like conditions (Johnson and Nelson 1991). Most importantly, *C. elegans* can reveal the biological effects of microgravity and provide clues to protect future human astronauts headed to the space (Spitz et al. 2004; Szewczyk et al. 2003). In this research the Silymarin and its related flavonoids, Silibinin, Quercetin and Naringenin have been tested to investigate their impact on C. elegans survival, reproduction, brood size, and lifespan when exposed to stress caused by exposure to simulated microgravity.

Materials and Methods

Strains and Reagents

Caenorhabditis elegans wild type (N2) was obtained from the *Caenorhabditis* Genetics Center (CGC, Minneapolis). Worms were maintained at 20°C in petri dishes containing Nematode Growth Medium (NGM), seeded with *Escherichia coli* strain OP50 (Brenner, 1974). Silymarin, Silybin (Silybinin), Quercetin, and Naringenin were purchased from Sigma- Aldrich, St. Louis, MO, USA). Each chemical was dissolved in dimethyl sulfoxide (DMSO) at a final concentration 1mg/mL (1000 ppm) as the stock solution.

Exposing C. elegans to simulated microgravity

High Aspect Ratio Vessel (HARV) is used to expose *C. elegans* to simulated microgravity. Each vessel

received 10 ml of nematode suspension in S. medium seeded with *E. coli*. Each vessel received one of the tested chemicals as food supplement with a final concentration of 5 µg/ml, DMSO only added to the control vessels. The initial population was determined before starting the experiment. The bioreactor was maintained running continuously on speed of 13 rev. / minute for 5 days. Tissue culture vessels each received similar treatments served as normal gravity control. All treatments including the bioreactor were maintained in an incubator on 21 °C. After exposure for five days the test was terminated and the following tests were performed:

Determination of final populations

Final populations were obtained also the rate of increase (reproduction), and mortality percentage was calculated.

Brood size (Progeny) assay

Twenty larvae (L3 - L4) from each were selected and moved into separate NGM plates seeded with E. coli (4 larvae/plate), and allowed to lay eggs. Once ggs observed were counted also count all hatched eggs, adult hermaphrodite were moved to fresh plates daily to prevent overlapping of the populations until the egg laying was stopped. The number of progeny was determined for each hermaphrodite.

Lifespan assay

As above 20 larvae (L3 - L4) were selected and moved to 5 NGM plates seeded with *E. coli*, moved daily until egg laying was stopped, then moved to fresh plates and watched daily until every hermaphrodite died. They considered dead when the worm is touched with a probe and did not move any part of its body.

Statistical analysis

The comparison between control and different treatments was done using Student's t test. Figures indicate means and standard errors of the mean. $P<0.05$ was regarded as statistically significant.

Results

Continuous exposure of *C. elegans* to the HARV bioreactor environment with the addition of Silymarin, Silybin, Quercetin, and Naringenin with the food increased the final populations and the rate of reproduction in all treatments than the control with DMSO only. The final population of each vessel nematode-suspension treated with silymarin, silybin, quercetin, and naringenin was 313±21, 384±50, 372±40, and 408±26, respectively versus DMSO control 228±72. The final population of the control that was kept on normal gravity with DMSO only was slightly higher than the HARV control 329±6.0 and 228±72 respectively. Mortality percentage was much lower in all of the treated population than the control, mortality in treatments of silymarin, silybin, quercetin, and naringenin was, 4.8, 3.2, 3.3, and 8.6 respectively vs. the control 28% mortality. Reproduction rates were much higher in treated populations the rate of reproduction for silymarin, silybin, quercetin, and naringenin treated populations was 4.8, 5.2, 4.9, and 5.7 respectively vs. the control 2.6

Brood size, progeny of adult hermaphrodites that were borne (hatched) under simulated microgravity with and without the food supplements was much higher in all silymarin and isomers treated populations compared to the control with DMSO. The number of progeny for each hermaphrodite was 270, 181, 251, 235, respectively for silymarin, silibin, quercetin and naringenin vs. 171 for DMSO only (HARV) and 200 for the brood size of a population kept on normal gravity with no food supplement (DMSO) (Fig. 1).

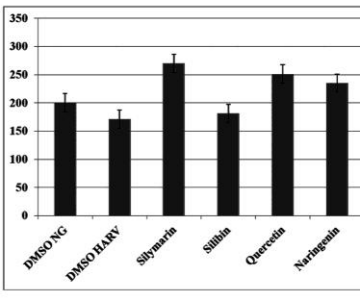

Figure 1. Brood Size (progeny of Hermaphodites obtained from larvae borne under HARV environment with or without food supplemnet, Standard error of the mean are shown.

Worm lifespan was longer when silymarin and other isomers were added as food supplements during the exposure to simulated microgravity than the control (DMSO). The lifespan and the range for silymarin, silibinin, quercetin, and naringenin respectively were 14 (13-15), 15 (12-17), 13(12-14), 15 (11-17), versus the DMSO control 10 (8-11), and DMSO control at normal gravity (NG) 11 (10-13) (Fig. 2).

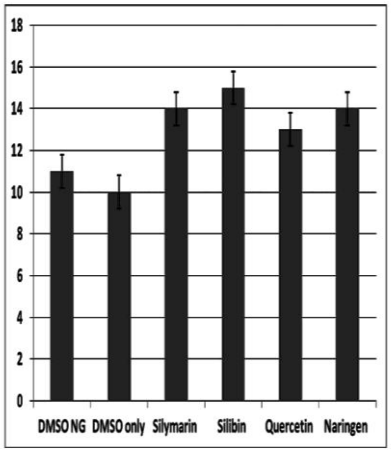

Figure 2. Lifespan of adult hermaphrodites that were born under the HARV environment with or without food supplement. Standard error of the mean is shown.

Discussion

Adding the flavonoids phytochemicals silymarine, silibin, quercetin, and naringenin as food supplements with different populations of *C. elegans* when exposed to simulated microgravity improved survival, increased progeny, and lifespan. Also the mortality percentage was much lower in all of the treated population than the control, and the reproduction rates were much higher. The reproductive system was less affected when exposed to simulated microgravity using different flavonoids as food supplement, than the control. Nematodes from cultures exposed to HARV environment without flavonoids took longer time to develop to the first larval stage and to hatch. Flavonoids caused higher reproduction and decreased mortality when compared to the control in all treatments. The mortality percentage was the highest in the control without flavonoids.

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The Perception of Time as Experienced Through Tango

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Introduction

Tango, in terms of time and structure can be manipulated to become a different dance. In the animated short film En Tus Brazos (Goby et al. 2006), the importance of a tango dancer maintaining his passion and creativity, and how they can psychologically transport the dancer to relive moments in time is explored.

How can tango and time as the Hegelian concept (Murray 1981) relate to each other? If time is used to measure a more complex thought, then according to Einstein's Theory of Relativity (Einstein 1919), time is nonexistent except in relation to distance and speed; therefore, what we think of as time does not exist by itself. It actually depends on another entity to exist. Furthermore, time and timing, both being abstract thoughts used to measure a moment, can become infinite as timing expands from seconds in the present to years in the past. Present and past are connected through memories, and memories are all we have to perceive who we were. In the film, emotions transmitted to the audience are very realistic, but time is handled is secretively, guardedly, and disingenuously.

As in the film, when Jorge returns to reality following his delirious, but tangible experience, he asks Elba not to stop dancing, and that he wants to remain in her arms. Jorge must relive his experience. Beyond the dancing, the emotions running through Jorge and Elba while they are still, wrapping themselves in time, have an enormous impact on their next dance step sequence. When Elba thinks Jorge is hurting himself by reminiscing through the photo album she comes to realize that is through memories that people live again. In realizing this, she is able to connect the present-day Jorge to the Jorge of the past and undertakes the task of reviving his spirit. Elba becomes like the West African goddess Oshun, "Mother of the Mirror, Owner of the Dance, who Transforms" (Fatunmbi 14). Oshun's love brought her husband back from madness and transformed him. Similarly, Elba, instead of letting Jorge go mad by reminiscing, picks him up and makes him dance one more time. In the physical world, we perceive a still moment in dance as time, but in the spiritual world everything is timeless. Since dance, our thoughts, and memories, all come from the spiritual world, tango, like any other form of dance, in the spiritual sense, is timeless. What is perceived, through time, in this physical world is nothing but an imperfect shadow; the true reality remains in the spiritual world. We can think of these two reflective worlds as reverse realities. Eric Maple examined Lewis Carroll's Alice Through the Looking Glass, "that contradictory world where left is right and time and space run counter to reality. Alice must walk backwards to meet the Red Queen, while the Queen screams with pain even before her finger has been pricked" (Maple 1859). This is why mirrors, which allow us to see reality, are extremely important for the couple while they are dancing: "if all existence was but a reflection of a hidden truth, the looking through the 'doorway' of the mirror would reverse the process and reveal reality instead" (Walker 146). This doorway Walker talks about is represented by the mirrors' reflections of their souls while the couple is dancing.

Conclusion

Tango, in terms of time and structure, can be manipulated to our benefit, and its effects are not just psychological, but, as we have seen, physical as well. Whatever the dancer's choice of timing and whatever reason or tactic he/she uses, the dancers will experience a connection that will bind them in a timeless space. A space where their bodies become aware of each other's energy, providing their audience with an amazing display of interconnectedness through the majestic tango dance.

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Probabilistic Gerber Bending Fatigue Design

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Abstract

This paper presents a probabilistic design approach for the Gerber bending fatigue failure rule using sensitivity-based analysis. The design model parameters are considered as random variables that are characterized by mean values and coefficients of variation (covs). The coefficient of variation of a design parameter is obtained by using first order Taylor series expansion for strength and stress. A reliability factor is determined based on the coefficients of variation and a failure probability which is then used for design sizing and analysis. Probabilistic design allows a quantification of risk that can help to avoid over- or under-design problems thus ensuring that safety and quality levels are economically achieved. This study shows a 51% reduction in component size and hence significant savings in product cost can be obtained through probabilistic design. Probabilistic design seems to be the most practical approach in product design due to the inherent variability associated with service loads, material properties, geometrical attributes, and mathematical design models.

Introduction

In today's market environment, quality is taken for granted. A quality product is associated with rare unexpected and unpleasant events which result from uncertainties in design. Customers are usually satisfied if a product performs as expected or better (http://www.kxcad...). In deterministic design, safety or design factors are usually subjectively assigned in product design so as to assure reliability. However, the safety factor method does not give insight about individual variation or the actual margin of safety in a design (Koch 2002), hence some authors prefer the term design factor to safety factor (Mott 2008).

Probabilistic design deals with the consideration of the effects of random variation on design model parameters. Very few real engineering problems are void of uncertainty because variation is inherent in material properties, loading conditions, geometric properties, simulation models, manufacturing precision, actual product usage, etc. (Koch 2002). The use of probabilistic design methods requires some appropriate probability distribution (Johnson 1980). It is known that products of variates from any distribution tend asymptotically to lognormal (Mischke 1996). Also, products, quotients, and exponents of lognormal variates are also lognormals. Since design formulas generally contain products and quotients of design parameters or sum of terms of these parameters, the lognormal distribution is a good candidate for probabilistic design applications.

The most problematic design situation is dynamic where fatigue failures may result. Fatigue failure normally takes the form of brittle fracture at stresses well below the static strength of the materials (Hidgon et al. 1967). About 80% to 90% of the failures of machine and structural members result from fatigue (Kravchenko 1964; Sachs 1999). Because of the predominance of fatigue failure, the objective of this study is to develop probabilistic design model for the traditional Gerber bending fatigue model by considering design parameters as random variables. The Gerber model is a good candidate for probabilistic application because it captures experimental data on an average performance (Norton 2000; Shigley and Mischke 1996) basis and thus can be associated with a 50% probability. This study uses the sensitivity analysis approach which is considered appropriate and cost effective in component design for structural and mechanical applications.

Lognormal Reliability Model

In the physical domain, is the random variable for strength and is the random variable for stress. Assuming that and have lognormal distributions, respectively, then lognormal random variables and can be defined. By definition, the reliability factor in a design, x and y; are:

$$n_z = \frac{\mu_S}{\mu_\sigma} \quad (1a) \qquad x = In(S) \quad (1b) \qquad y = In(\sigma) \quad (1c)$$

The failure zone is defined by the probability density function q, the difference between random variables *x* and *y*. That is: q = x-y (2)

Based on the properties of the lognormal probability density function (http://itl.nist.gov...):

$$z = \frac{\mu_q}{s_q} = \frac{In(n_z) - 0.5In\left(\frac{1+v_S^2}{1+v_\sigma^2}\right)}{\sqrt{In\{(1+v_S^2)(1+v_\sigma^2)\}}} \quad (3a) \qquad R_z = 1 - \Phi(-z) = \Phi(z) \quad (3b)$$

Eqn. 3b gives the reliability for the unit normal variate z, with the value of or read from an appropriate table. The reliability factor for a desired reliability level defined by *z* is:

$$n_z = \exp\left[z\sqrt{In\{(1+v_S^2)(1+v_\sigma^2)\}} + 0.5In\left(\frac{1+v_S^2}{1+v_\sigma^2}\right)\right] \quad (4)$$

Thus, from equation (4), if a desired reliability or failure probability is specified, then *z* is known and the necessary reliability factor for achieving this reliability can be obtained. According to Wang, Kim and Kim (Wang et al. 2006), it is common to use the unit normal variate (Eqn. 3a) for failure probability assessment because probability values (Eqn. 3b) can change by several orders of magnitude over small changes in the unit normal variate. The task in using the reliability model of equations 3 and 4 is to develop expressions for and for specific design models. The model application is not limited to stress-based design; it can be used for any serviceability criterion of interest such as lateral stability, transverse deflection, torsional rigidity, critical speed, etc.

Gerber Bending Fatigue Model

The design space of the Gerber bending fatigue model is divided into two regions called dynamic fatigue failure regime and static fatigue failure regime (Osakue 2012; Osakue et al. 2012). In the dynamic fatigue failure region, component failure results from the predominant influence of the alternating stress and **.** The design load capability in dynamic fatigue failure is determined by the service fatigue strength. From (Osakue 2012; Collins et al. 2010), for tradition Gerber rule is:

$$\sigma_{ef} = \frac{k_\sigma \sigma_a}{1 - \frac{1}{n_m^2}} \quad (5a) \qquad n_m = \frac{S_u}{\sigma_m} \quad (5b)$$

The cov of σ_{ef} is:

$$v_\sigma = \left[v_k^2 + v_F^2 + v_l^2 + 9v_h^2 + \left(\frac{2}{n_m^2 - 1}\right)^2 (v_F^2 + v_l^2 + 9v_h^2 + v_u^2)\right]^{\frac{1}{2}} \quad (6)$$

In the static fatigue failure regime, component failure results from the predominant influence of the mean stress. The design load capability in static fatigue failure is determined by the service tensile strength. From (Osakue 2012), for traditional Gerber bending fatigue rule is:

$$\sigma_{ef} = \frac{\sigma_m}{\sqrt{1 - \frac{1}{n_a}}} \quad (7a) \qquad n_a = \frac{S_f}{k_\sigma \sigma_a} \quad (7b)$$

The cov of σ_{ef} is:

$$v_\sigma = \left[v_F^2 + v_l^2 + 9v_h^2 + \frac{1}{4}\left(\frac{1}{n_a - 1}\right)^2 \left(v_k^2 + v_F^2 + v_l^2 + 9v_h^2 + v_f^2\right)\right]^{\frac{1}{2}} \quad (8)$$

Applications of Models

Two solutions are developed and three solutions are analyzed in this section. The design problem is a case of possible dynamic fatigue failure taken from Norton (2000 pp. 391 – 397). The first example is a design analysis of the solution provided in the reference from a probabilistic perspective based on the design model equations developed in this paper. The second and third examples are attempts to redesign the components of example 1 using probabilistic fatigue approach as presented in this paper. This example is used because it is described as a typical design problem (Norton 2000).

Figure 3 shows one of two brackets attached to a machine frame. The brackets carry a combined fluctuating load varying from a minimum of 890 N to a maximum of 9,786 N (Norton 2000) (data converted to SI Units by author). The load is shared equally by the brackets; the maximum allowed lateral deflection is 0.51 mm for each bracket, and each should be designed for 10^9 load cycles. The load-time function is sinusoidal, maximum cantilever length is 152 mm, and the operating temperature is 50°C. Trial dimensions are *b* = 51 mm, *h* = 25.4 mm, *H* = 28.6 mm, *r* = 12.7 mm

and l = 127 mm. The brackets will be machined to size from stocks. From [13], the value of k_σ = 1.16 and Z_x = 5463.45 mm^3. The brackets are to be made from SAE 1040 steel with S_u = 550 MPa and S_f = 150 MPa at 99.9% reliability.

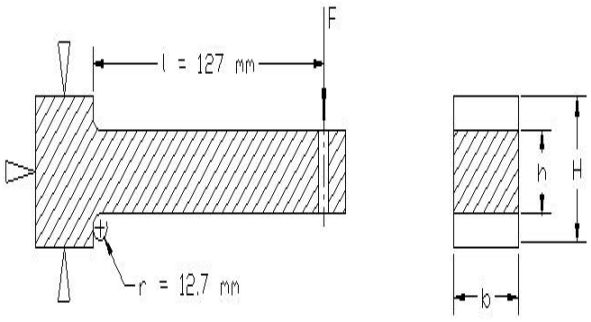

Figure 1. adapted from Fig. 3: (After Norton, 2000)

Solutions

The model formulas developed in this study were used to analyze and size the components in the problem. A literature research provided the coefficients of variation of the design parameters used. Design formulas for deterministic design are applicable once the reliability factor is determined.

Table 1 is a summary of the three solutions to the example problem. It shows the deterministic solution gave very conservative results in all design parameters considered. The next conservative results are from the 2.5 minimum reliability factor constrain. The optimum results are based on the desired reliability goal of 99.9%. The reliability factor of 2.2 for this solution is based on the failure probability of 0.1% and cannot be independently specified. While the design reliability requirement is "three nines", the deterministic solution yields almost "six nines"! Clearly, if these dimensions for the deterministic solution were adopted, it would appear to have been a case of over-design.

Design Parameter	SOLUTIONS		
	Deterministic	99.9% Reliability	2.5 Reliability Factor
Effective stress (MPa)	67	91	80
Reliability factor	2.99	2.20	2.51
Unit normal variate	4.42	3.16	3.71
Reliability (%)	99.9997	99.92	99.989

Table 1. Solutions Summary

The deflections at the point of load application and at the end of the bracket were computed for a cantilever beam. The computed deflection values are compared in Table 2 for the three solutions with the allowable. These values are much lower than the maximum allowable value of 0.51 mm, indicating that the three designs satisfy the deflection requirement.

Table 3 summarizes the cross-sectional dimensions and area of the bar. The cross-sectional area of the optimum solution is a 51% reduction in area compared to the deterministic solution. Practically, this translates to a 51% reduction in weight or material cost per component at 44% of maximum allowable deflection. Certainly a 51% savings in material cost could translate into thousands if not millions of dollars in savings in a large volume production considering that two components per equipment are required!

Design Parameter	SOLUTIONS		
	Deterministic	99.9% Reliability	2.5 Reliability Factor
Deflection at load point (mm) (mm)	0.252	0.174	0.142
Deflection at bar end (mm)	0.300	0.225	0.184
Bar end deflection (%)	59	44	36

Table 2. Deflection comparison

Design Parameter	SOLUTIONS		
	Deterministic	99.9% Reliability	2.5 Reliability Factor
Depth (mm)	25.4	42	45
Width (mm)	51	15	15
Area (mm^2)	1295	630	675
Area comparison (%)	100	49	52

Table 3. Size comparison

Discussion

A probabilistic fatigue design approach based on the Gerber failure rule has been presented. The model design parameters use mean values to estimate expected design results while the reliability of the design is evaluated using the coefficients of variation of the design parameters and desired reliability goal. The coefficients of variation of strength and stress were developed using sensitivity analysis based on first order Taylor series expansion of design relationships. A typical design problem was analyzed and redesigned using the approach.

Acknowledgements

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Determination of Lindane and its Metabolites in Biological Samples

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Abstract

Lindane (hexachlorocyclohexane) is a persistent environmental toxin and a known neurotoxin. High levels of lindane have been detected in postmortem human brain from Parkinson's disease patients. Metabolism of lindane produces several metabolites which are distributed in the body and excreted through various routes like urine, sweats, feces, hairs etc. Detection of lindane and metabolites are still imperfectly identified. Therefore, an easy, fast and sensitive method to detect lindane and metabolites is highly desirable. We have determined lindane and its metabolites in urine, serum, and feces samples using HPLC-UV-Vis and mass characterized with MALDI-TOF. Serum, urine and feces samples were collected following treatment of rats with lindane (17.6 mg/Kg; 1/5 of LD_{50}) or vehicle orally for 4 weeks. Lindane and metabolites were extracted following treatment with hexane. The extracts were subjected to HPLC-UV-Vis analysis and confirmed with MALDI-TOF. HPLC spectrum of standard lindane peaks were determined and compared with that from urine, serum, and feces samples.

Introduction

Lindane (hexachlorocyclohexane) is a well known insecticide and a therapeutic agent used against scabies, pediculosite, and ectoparasitic infections in humans and animals (Budavari et al., 1989). As an insecticide, it is used on fruit, vegetable, tobacco, and for seed treatment; dog dips, house sprays, and in commercial food or feed storage areas and containers (ASTDR, 1998; U.S. EPA,1985). Because of health concern from exposure to lindane, it has been banned in many countries. Lindane has not been produced in the United States since 1977, but it is imported in multiple forms for pharmacologic and industrial use. The use of lindane is restricted; it can only be applied by certified applicators with restricted applications to livestock, structures, and pets (U.S. EPA, 1985b). With these types of use, lindane can easily find its way into the body and adversely affect the biological systems.

Lindane present in soil can leach into groundwater, sorb to soil particles, or volatilize to air and can easily find its way into human body. The toxic effects of lindane are primarily neurotoxic, producing rapid response interferes with cations fluxes across nervous membranes leading to convulsions resulting in death due to interference respiratory processes generating severe metabolic acidosis (Cheremisinoff and King, 1994). Other toxic effects of lindane includes chronic fatigue syndrome, infertility characterized by reduced quantity and quality of sperm, spontaneous abortions etc.

Lindane and its metabolites can be detected and measured in blood and body fluids by clinical laboratory tests. But it is difficult ascertain specific exposure levels. Because of the toxic effects of lindane and its persistent properties, there is the need for easy and fast methods for detecting exposure to this organic compound to enable precautions to be taken to preserve health. In this study, we estimated the levels of lindane and its metabolites in urine, serum, and feces from lindane-exposed rats using HPLC, UV-vis spectrophotometry, and MALDI-TOF.

Materials and Methods

Animal treatment

Rats (250-400 g) were treated orally with 17.6 mg of lindane ($1/5^{th}$ of the LD_{50}) in corn oil for 4 weeks. Control animals received only corn oil. At the end of the treatment, animals were placed in metabolic cages for 24 hours, and collected the urine and feces samples; just before sacrifice, blood was collected and serum was prepared after centrifugation. Samples were stored at -80^{o}F until needed for analysis.

Sample Preparation

Urine samples (n=6) and serum samples (n=5) were brought down to room temperature before centrifugation. Lindane and metabolites were extracted with hexane. The organic layer was evaporated with N2 and reconstructed with 1ml of hexane and analyzed. Lindane and metabolites were extracted from 1 gram of feces (n=6) suspended in 3ml of hexane for 6 hours, centrifuged and the supernatant was collected and dried with N2. The samples were reconstructed with 1mL of hexane and analyzed.

HPLC Uv-Vis Analysis

Standard curve was prepared from a stock solution of 1,000 ppm Lindane (0.1gm /100ml) and a series of dilutions were made to get 10, 20, 50, 100, 500ppm. Standard, control and extracted samples were analyzed on Dionex Ultimate 3000 HPLC equipped with Uv-Vis spectroscopy. The analysis was performed on a C-18 column, using an ACN/H2O (50:50) mobile phase and the flow rate was 0.5ml/min for urine, and 1ml/min for serum and feces samples at 254nm wavelength.

MALDI-TOF Analysis

Lindane and Metabolites Mass Confirmation was performed using Mass Spectrum *[Matrix-assisted laser desorption/ionization-Time of Flight]* (MALDI-TOF). Target plate was prepared by adding extracted samples to *sinapinic acid* matrix system, which includes TFA, ACN, MeOH and D.I water.

Results

Urine Analysis

The peak present at 6:00 – 6:16 min retention time (RT) indicates the presence of Lindane, and is confirmed with standard HPLC data. HPLC peaks were quantified and Lindane average concentration was found to be 837 ppm in the urine (Table 1). The peaks at 292-293m/z shows the presence of Lindane and the other peaks at 259, 269, 277, and 318m/z shows the presence of some metabolites on MALDI-TOF (data not shown). The peak 293 ($m+2H^+$) was confirmed with standard Lindane.

Serum Analysis

The peak present at 3.40-4.00 min RT of standard was compared with the peaks at 3.50-4.00 min of samples; control does not have any peak corresponding to Lindane. The HPLC data was quantified and the average Lindane concentration in the serum was 255 ppm (Table 1). The peak at 292-293 m/z confirms presence of Lindane in the samples on MALDI-TOF (data not shown). The minor peaks at 253, 276, 329, 334, 355, 368, and 384 m/z confirm the molecular weights of different metabolites of Lindane.

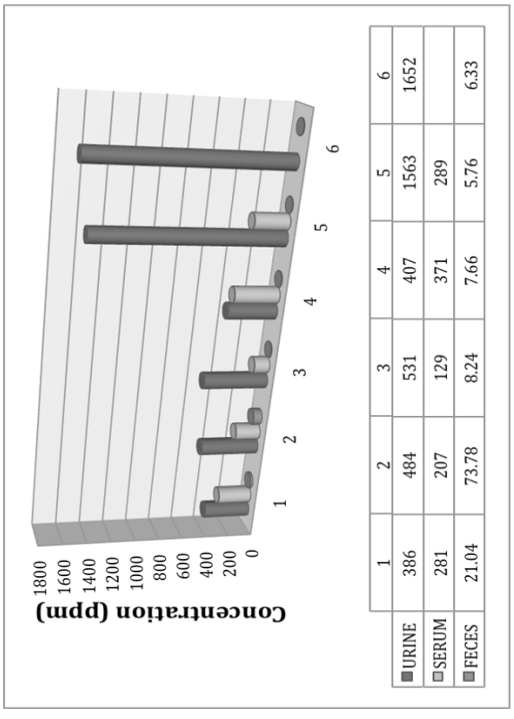
Feces Analysis

Standard Lindane R.T (3.4-3.5 min) was determined and compared with the samples. Controls did not show any peak corresponding to lindane, the concentration was found in the range of 5.76-73.77ppm, with an average concentration of 20.46ppm on HPLC (Table 1). The peak 292-293 m/z

	URINE				SERUM				FECES			
Rx	R.T (min)	Area	Height	Conc. (ppm)	R.T (min)	Area	Height	Conc. (ppm)	R.T (min)	Area	Height	Conc. (ppm)
Std	6.03	0.03	0.16	10	3.68	0.69	0.78	10	3.683	0.69	0.78	10
Cntrl	NO PEAKS				NO PEAKS				NO PEAKS			
Rx 1	6.18	1.10	10.62	386	3.85	1.96	7.65	281	3.44	0.27	2.89	21.04
Rx 2	6.15	1.40	12.46	484	3.67	1.45	1.99	207	3.44	0.92	5.49	73.77
Rx 3	6.14	1.54	12.72	531	3.68	0.9	8.24	129	3.46	0.10	1.37	8.23
Rx 4	6.12	1.14	11.02	407	3.68	2.59	14.43	371	3.447	0.09	1.46	7.65
Rx 5	6.11	4.53	16.07	1563	3.87	2.02	6.15	289	3.447	0.072	1.08	5.76
Rx 6	6.09	4.79	17.54	1652	N/A	N/A	N/A	N/A	3.453	0.07	1.20	6.33

Table: 1 above shows the Retention Times (R.T) in minutes; Area under the curve; Height of the peak; and concentrations of unchanged Lindane in ppm of Urine, Serum and Feces samples.

corresponds to the standard lindane, but the intensity was too low indicative of low concentration as most of the lindane and its metabolites were reabsorbed in the intestines leaving very little in the feces (Fig. 1).



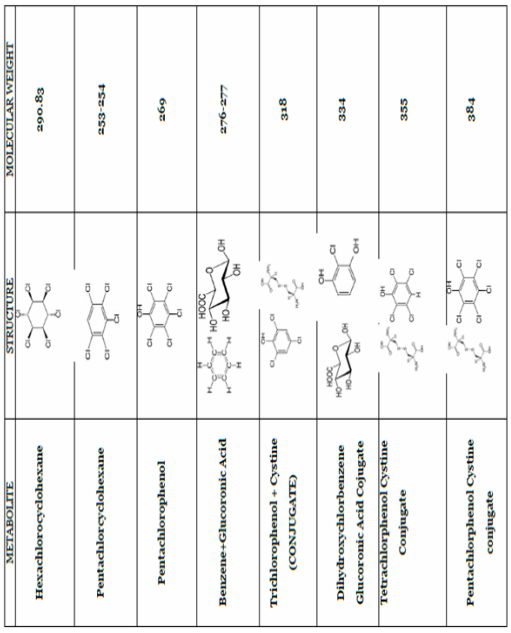


Figure 1: MALDI-TOF, Concentration of Lindane in Biological Samples, and Lindane Structure and MW

Controls did not show any peak corresponding to lindane, the concentration was found in the range of 5.76-73.77ppm, with an average concentration of 20.46ppm on HPLC (Table 1). The peak 292-293 m/z corresponds to the standard lindane, but the intensity was too low indicative of low concentration as most of the lindane and its metabolites were reabsorbed in the intestines leaving very little in the feces (Fig. 1).

Discussion

Urine is the main route of excretion for Lindane and very little is excreted unchanged. It is suggested to be excreted as chlorinated phenols and all isomers of di-, tri-, and tetrachlorophenol metabolites (Balikova et. al,1988). Lindane is slowly metabolized through four possible reactions: 1.) Dehydrogenation to hexachlorocyclohexene, 2.) Dehydrochlorination to pentachlorocyclohexene, 3.) Dechlorination to tetrachlorohexene, and 4.) Hydroxylation to hexachlorocyclohexanol. Also phenylmercapturic acid derivatives are formed by conjugation of hexachlorocyclohexene metabolites with glutathione subsequent to dechlorinations and dehydrochlorinations. Figure 1 shows the concentration of un conjugated Lindane in different samples and different metabolites with molecular masses.

In conclusion, These results show that HPLC Uv-Vis coupled with MALDI-TOF are highly sensitive to the point of detecting and confirming very low levels Lindane (5ppm) and its metabolites in urine, serum and feces. Thus, HPLC-UV-Vis-MALDI-TOF analysis can be a reliable, non-invasive method of detecting Lindane exposure and burden.

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Ecological Impacts of Pollutants in San Francisco Bay Tidal Marshes

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Abstract

In San Francisco Bay (SFB), more than 80% of the original tidal marshes have disappeared over the last 150 years and the existing marshes are significantly fragmented and contaminated by numerous organic pollutants and trace metals, which cause habitat quality degradation. Tidal marshes in SFB provide vital food and habitat for clams, crabs, and juvenile fish and offer shelter and nesting sites for waterfowl and shorebirds. These marshes also play a buffering role by retaining contaminants before they reach the estuarine water body, resulting in significant accumulation of contaminants in marsh sediments. Although concentrations of contaminants in the marsh ecosystem have been declining, they are still high enough to threaten the well-being of aquatic life and wildlife. Considering importance of tidal marsh, it is helpful to have an integrated review on temporal trends and current status of contaminants and their adverse effects in the tidal marsh ecosystem of SFB, which can be used as a reference to better understand tidal marsh habitat quality and implement marsh restoration projects. Potential impacts of contaminants on the health of aquatic life and wildlife in the marsh ecosystems are summarized.

Introduction

Tidal marshes are an important part of the SFB wetland ecosystem, which is the most biologically productive habitat in SFB. Tidal marshes, as a transition zone between upland and aquatic system, in SFB play important roles in sustaining ecological diversity by providing vital food and habitat for clams, crabs, and juvenile fish as well as offering shelter and nesting sites for waterfowl and shorebirds (Goals Project, 1999). Tidal marshes also play critical buffering roles by slowing shoreline erosion and absorbing nutrients and contaminants before they reach the open bay.

This buffering role, however, could lead to heightened accumulation of contaminants in marshes near urban and industrial areas. In fact, all contaminated marshes in SFB are located downstream of creeks and rivers, which deliver various contaminants from urban and industrial areas. More than 80% of historic tidal marshes in SFB have been diked, filled, or transformed into salt ponds, agricultural, residential and industrial lands during the last 150 years (Goals Project, 1999). The remaining marshes are highly fragmented, which makes them more vulnerable to human impacts. When these fragmented marshes are heavily contaminated by toxic chemicals, many marsh dwelling animals, which could not migrate to less contaminated marshes, are likely to be more seriously stressed.

Since the mid 1800s, SFB has received enormous amounts of contaminants such as mercury, polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polycyclic aromatic hydrocarbons (PAHs) from historic gold and mercury mining, municipal and industrial wastewater treatment plants, urban and agricultural runoff, and oil spills. A significant decrease in contaminant loading occurred after the San Francisco Bay Regional Water Quality Control Board issued its first Water Quality Control Plan in 1975 for the fulfillment of the Clean Water Act (SFBRWQCB, 2000). Restrictions on the use of contaminants such as PCBs, OC pesticides, and lead also contributed to the decline of their levels in the SFB system.

Impacts of contaminants in marshes on aquatic life and wildlife

Concentrations of contaminants in many marshes of SFB have declined significantly and concurrent reduction of failed gamete production in clam and enhancement of the stability of benthic community structure have been observed (Thompson et al., 2002), which are the signs of the improvement of ecological conditions. However, concentrations of contaminants in many marshes are still high enough to be a cause of adverse effects found in aquatic life and wildlife (Hwang et al., 2013; Schwarzbach et al. 2006).

Concentrations of sedimentary contaminants in many SFB marshes are much higher than background concentrations but chemical concentration itself is less meaningful unless they are linked to adverse effects. Levels of contaminants in sediments can provide insights into potential impacts on aquatic organisms when they are reasonably converted to proxy for toxicity potential through comparison to numerical sediment quality guidelines. Converted data are then more toxicologically relevant and can be used in assessing

the probable adverse effects of contaminants in sediments more accurately (Long et al., 1995; MacDonald et al., 1996). Concentrations of contaminants in many marsh sediments exceed sediment quality guidelines such as effects range-medians (ER-Ms) and probable effects levels (PELs), meaning that adverse biological effects are likely to be observed frequently. PCBs and DDTs in many marshes in central and south SFB exceeded sediment quality guidelines (Leatherbarrow et al., 2005, Hwang et al., 2008), indicating that organic contaminants in many marshes in SFB are still high enough to be a cause for concerns.

High concentrations of sedimentary contaminants typically show good correlations with adverse biological responses in SFB marshes (Fairey et al., 2001; Hunt et al., 2001, Hwang et al., 2013). In highly degraded marshes, contaminants in sediments were found to be linked to disturbance of benthic macroinvertebrate community. Reduced benthic species diversity and prevalence of opportunistic species were frequently observed in highly contaminated marshes. In many marsh sites, sediment porewater toxicity tests showed very low survival of amphipods and reduced fertilization of sea urchin embryos (Hunt et al., 2001; Hwang et al., 2013).

High levels of contaminants in the SFB system are also considered contributors to the decline of avian species. Hatching success of California Clapper rails in SFB marshes are substantially lower compared to those in other areas (Schwarzbach et al., 2006). In many cases, we speculate possible impacts of contaminants using indirect evidence such as the presence of contaminants in their bodies and eggs at levels higher than toxic thresholds. Current mercury concentrations in marsh birds such as rails breeding in SFB are still higher than threshold levels, which are known to be responsible for impairment of reproduction (Schwarzbach et al., 2006; Tsao et al., 2009). Additional indirect evidence is the level of contaminants in diets. Body burdens of PCBs and DDTs in transplanted fish (mudsucker; *Gillichthys mirabilis*) in Stege Marsh (Hwang et al., 2008) were higher than the wildlife criteria, which were set by USEPA for the protection of fish eating birds. It implies that sedimentary contaminants can eventually be transferred to fish eating birds and possibly affect their health.

Through maternal transfer of contaminants, embryos are exposed to them and may experience hatching problems (Custer and Custer, 1995). Mercury concentrations were much higher in failed-to-hatch eggs than in normally hatched eggs. Cumulative effects of these responses over the long-term can eventually affect the population of birds, especially endangered species such as California Clapper Rails. Among contaminants measured in birds, DDT and its metabolites (DDD and DDE) were the most problematic due to their notorious eggshell thinning effect. Ohlendorf et al. (1988) found that levels of DDE in eggs were negatively correlated with eggshell thickness in night herons and snowy egrets. A negative correlation was found between embryo weight and PCB residues in eggs of night-herons collected from San Francisco National Wildlife Refuge, suggesting a possible impact of PCBs on the growth of embryos (Hoffman et al., 1986). Schwarzbach et al. (2006) found positive correlations between deformities and elevated concentrations of mercury in fail-to-hatch eggs.

Oil spills also cause severe damages in aquatic life and wildlife and degrade habitat quality. In 1998, about 1.5 million L of crude oil leaked from Shell storage tanks was released into northern SFB and caused tremendous ecological damages. The spilled crude oil coated about 100 acre of Peyton Marsh, killed thousands of birds, and ruined valuable habitats for aquatic life and wildlife. Two recent oil spill accidents, *Cosco Busan* and *T/V Dubai Star* oil spills occurred in 2007 and 2009, respectively, poured about 220,000 L and 3,200 L of fuel oil, respectively, into the bay. More than a thousand birds died due to acute toxicity of the spilled oil. Contaminants such as PAHs from oil spill accidents can also damage aquatic life such as crabs and bivalves in marshes.

Discussion

The environmental quality of many marshes of SFB does not fully support the health of the organisms. Some studies provide evidence that persistent organic contaminants and trace metals deposited in sediments could be desorbed and affect the health of aquatic organisms. These contaminants eventually reach higher trophic level organisms such as birds and can provoke adverse effects on them. Most marsh studies, however, have not investigated overall impacts of contaminants,

which warrants that more integrated studies are required to better support marsh management and restoration activities. It is also desirable to further investigate the roles of marsh plants in controlling the fate and transport of contaminants and their possible usability for phytoremediation to clean up sedimentary contaminants. Lack of information regarding integrated impacts of simultaneous exposure to multiple contaminants also hinders accurate assessment of their overall adverse effects. Linking concentrations of individual chemicals in field samples to toxicity data may lead to underestimation of the toxicity potential of environmental contaminants. More studies are also needed for emerging contaminants such as PBDEs, phthalates, and pharmaceuticals.

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The Use of Metallic Copper as an Antimicrobial Surface in Clinical Settings

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Abstract

In the present day, Healthcare-acquired infections continue to be a major concern accounting for about 100 thousand deaths annually in the US. The major causes of these infections are antibiotic resistant bacteria which can be found on frequently touched surfaces in hospitals and clinics. Recently the interest in metallic copper has been revived for its antimicrobial abilities. This versatile metal was used in ancient times for medicinal purposes and may even present itself as a solution to the current dilemma posed by the excessive use of antibiotics. This review explores the antimicrobial properties of metallic copper and validates the claims that it may be used as a beneficial and safe alternative to the common metals and materials currently in place in clinical settings.

Introduction

Copper has been used for ages by human civilizations. Even in ancient times the antimicrobial effects of this metal were exploited in medicine. For example, ancient Egyptian medical text written between 2600 and 2200 BC show that copper was used to sterilize chest wounds and drinking water. Greeks, Romans and Aztecs were also reported to have used copper for treatment of headaches, burns, intestinal worms, and ear infections (Grass et al. 2011). In the 19th century the use of copper became widespread and was used to treat ailments like tubercular infections, chorea and facial neuralgia to name a few. The use of copper as an antimicrobial agent in medicine continued until the introduction of commercially available antibiotics in 1932. In present day the interest in this metal is being revived due to the increasing rate of antibiotic resistance in harmful bacteria (Grass et al. 2011). This advent of antibiotic resistant bacteria presents a predicament in the form of Health-care Acquired Infections (HAIs), also known as nosocomial infections.

The CDC describes HAIs as infections that patients acquire during receiving treatment for other conditions. Several strides have been made in recent years to reduce the amount of infections and deaths associated with treatment in a health care facility. In the modern hospitals one can find many mechanisms in place to reduce the transmission of infectious diseases including touch-less systems (automatic soap dispenser, automatic flush), strategic placement of sinks and hand sanitizers and use of gloves. Even with these accommodations health-care acquired infections (HAIs) continue to be a major concern, costing hospitals $45 billion in the US. With an estimated 1.7 million HAIs occurring each year and leading to about 100,000 deaths (Sharpe and Schmidt 2011). It is proposed that the use of antimicrobial copper for frequently touched hospital surfaces such as door handles, furniture hardware, bed rails, IV poles, nurses' call buttons, sinks and work stations along with the use of hygienic practices and proper sanitation can help reduce the rate of HAIs and the spread of drug-resistant bacteria in hospitals. This review explores the functions of antimicrobial copper and the legitimacy to these claims.

Characteristics of Copper

Copper is an essential trace element required for survival in most organisms including bacteria. On one hand, copper is essential for a variety of biochemical reactions involving oxygen, on the other hand, unbound copper cations participate in dangerous Fenton-like reactions which lead to oxidative damage of any cellular macromolecule by the production of hydroxyl ions. Copper ions bind easily to thiol groups of proteins, and may displace other transition metals from their respective complexes. Bacteria have different efflux systems which detoxify copper from the cytoplasm or periplasm, yet they are rapidly killed on a dry metallic copper surface (Mikolay et al. 2010).

The mechanisms by which bacteria are rapidly killed on contact with metallic copper surfaces are still under investigation. Espirito Santo and associates (2011) found that cells exposed to dry copper have damaged membranes and suggest this as a contributor to contact killing. Furthermore, metallic copper has been found to not kill exposed cells by generating lethal mutations in the DNA (Espirito Santo et al. 2011). This further improves the case for copper since it reduces the chance

of bacteria developing resistance to these surfaces.

Copper as a safe alternative

Currently hospitals invest in decontamination agents such as hydrogen peroxide, halide-containing agents and quaternary ammonium compounds which degrade the environment. These agents are hazardous to health and are relatively weak biocides (Cooke, Hughes et al. 2005).Quaternary ammonium compounds found in common disinfectants may even drive antibiotic resistance (Akimitsu et al. 1999).

The Environmental Protection Agency has registered a range of copper alloys for their ability to kill six types of bacteria: *E.coli* O157:H7, *Staphyloccus aureua*, Methicillin-resistant *Staphyloccus aureus* (MRSA), Vancomycin – resistant *Enterococcus faecalis* (VRE), *Enterobacter aerogenes*, and *Pseudomonas aeruginosa.* MRSA, VRE, Gram negative bacteria and spore forming bacteria can survive on inanimate objects for months, endangering susceptible patients and health care workers. Copper is the only solid material that has been found to actively kill bacteria continuously for 24 hours and in between discharge cleanings (Sharpe and Schmidt 2011). In a previous study it was found that there was no reduction in killing efficiency of copper over 30 cycles of bacterial inoculation accompanied with cleaning with a 1% nonionic detergent solution (Grass et al. 2011). Copper surfaces even remain active when soiled (Wheeldon et al. 2008). Cleaning agents that leave a thin film on the copper surfaces do, however, reduce the killing rate (Grass et al. 2011). This issue can be resolved with the implementation of special cleaning products.

In a study by Grant and associates (2007), three copper based formulations - CuAL42, CuPC33 and CuWB50 were considered for their ability to kill microorganisms which cause HAIs and test their toxicity on two human cell lines. The cell lines used were HT-29, an intestinal epithelial cell line, and A431, a squamous epithelial cell line. They found that all three copper-based formulations reduced bacterial numbers in a dose dependent fashion, with control cultures composed of PBS showing no reduction in cfu formation over a 2 hour incubation period. In regards to the cell line they found that the three copper formulations displayed no significant cytotoxicity to either cell line at 1 to 100 ppm. The formulations were found to cause cell damage at 1000 ppm, demonstrating that copper exhibits biocidal toxicity for microorganisms well below those toxic for human tissues (Gant et al. 2007).

Investigation of contact killing in the lab

Endospores formed by Gram positive bacteria resist heat, radiation; desiccation and denaturing chemicals, but there have been reported cases of metallic copper killing these spores (Grass et al. 2011). One study found that viable spores were reduced by 99.8% in 3 hours on solid copper (Wheeldon et al. 2008) and another study showed complete inactivation of spores in 24 to 48 hours (Weaver et al. 2008). This property is of great importance since spores excreted by infected individuals can contaminate hospital surfaces and provide a long term reservoir for transmission.

Furthermore, testing the antimicrobial abilities in the lab setting found that the higher content of copper in alloys (Wilks et al. 2005), along with higher temperature (Elguindi et al. 2009)and higher relative humidity (Michels et al. 2009) increased the efficacy of contact killing.

Contact killing in a health care setting

Currently the predominant metal used in hospitals is stainless steel. This surface has no antimicrobial properties (Kusumaningrum et al. 2003) but is so widespread because of its clean appearance and ability to resist erosion (Grass et al. 2011). Even with constant cleaning and hand washing, frequently touched surfaces are highly contaminated and dangerous bacteria can persist on these surfaces for months. The replacement of these surfaces with metallic copper can reduce surface contamination, in turn reducing the risk of infection. A pilot study was done in an outpatient clinic in Manhasset, New York. The study lasted 15 weeks and focused on the ability of copper to reduce microbial burden (MB) on the tray tables and arms of phlebotomy chairs. In the study the trays and arms on two of 3 phlebotomy chairs, each in separate rooms were replaced with an EPA registered copper-nickel alloy. Every 5 weeks the chairs were rotated between the three rooms to minimize preference for any one location. The study found that the copper significantly lowered the MB found on the

tray and arm surfaces by a median of 88% and total bacteria on the tray tops was reduced by 90%. The majority of the microorganisms found were *Staphylococci* (Sharpe and Schmidt 2011).

Another 10 week study was carried out at the Selly Oak Hospital in Birmingham, UK. In this study both copper and control surfaces were used in the same ward. After 5 weeks the copper and non-copper surfaces were interchanged for the same reason as in the previous study. In this instance the copper items were installed 6 months before the beginning of the study to allow workers to get used to the surfaces and to test whether antimicrobial ability was reduced over time. Bacterial contamination of a copper coated composite toilet seat, brass tap handles and brass push plate was compared with the equivalent items composed of plastic, chrome-plate or aluminum surfaces. The median numbers of bacteria isolated from the copper-containing items were between 90% and 100% lower than the control surfaces. MRSA, VRE and *E. coli* were found on the control surfaces but not on the copper surfaces (Casey et al. 2010).

An additional 32 week study in Germany featured the replacement of touch surfaces in patient rooms, rest rooms, and staff rooms in an oncological/pneumological and geriatric ward with brass (a copper/zinc alloy). The control rooms maintained the aluminum door handles and push plates as well as plastic light switches (Mikolay et al. 2010). All surfaces were sampled each morning and cleaned with a disinfectant. More samples were taken immediately after cleaning and followed by additional sampling at 3, 6 and 9 hours later. There was an average of 63% reduction in microbial load on the copper surfaces compared to the controls. The rate of surface repopulation by bacteria was less than half on the copper surfaces than on the controls.

Conclusion

The hospital trials have proven that metallic copper surfaces cause a reduction in bacterial counts. Laboratory experiments have found that metallic copper is even effective against disease causing spores that usually resist killing by several aseptic techniques. Further and more large-scale trials need to be conducted to determine whether the use of these surfaces will have a direct impact on the occurrence of health-care associated infections. Since contact killing is so rapid and bacteria do not divide on these surfaces this may prevent the acquisition of resistance, further enhancing the attractiveness of this metal as the new universal standard. Also the lack of toxicity to humans makes it an exceptional alternative to other harsh chemicals used to clean surfaces on a regular basis. It should be noted that the use of antimicrobial copper surfaces should not be a replacement of, but rather a supplement to proper aseptic and hygienic procedures.

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Treating Community-Acquired Pneumonia using Anti Inflammatory Drugs

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Abstract

Community-acquired pneumonia exhibits symptoms from mild to extreme severity such as death. Excessive inflammation is capable of leading to fragmentary local and systemic damage while microorganisms need a tolerable inflammatory response for clearance. Different treatment options have been suggested that are worth investigating such as macrolides, Toll-like receptors, and corticosteroids. The aim of this mini-review is to summarize the immune system response, anti-inflammatory therapies, and possible future roles of these therapies in CAP patients.

Introduction

Community-acquired pneumonia (CAP) is one of the most commonly contagious diseases addressed by physicians worldwide (Meijvis et al. 2012). Presently, the statistical rate for pnuemonia-assocaited deaths is as high as 40% or as low as 5% with age and co-morbidities being influential factors. Effective treatment requires diagnosis in a timely manner with the start of specific and suitable antibiotic therapy within a period of 4 hours (Meijvis et al. 2012).

Due to their immunosuppressive properties, adjunctive anti-inflammatory drugs therapies have been minimally recommended by physicians (Meijvis et al. 2012). Anti-inflammatory drugs, such as corticosteroids and macrolide antibiotics, have been tested on patients with various infections. The anti-inflammatory drugs suppress systemic inflammation; the response is typically immense and, as a result, its clinical consequences are severe and undesirable (Meijvis et al. 2012).

A few generations ago, antibiotics were nonexistent. Depending on the person's immune system, those with a weaker system were least likely to endure an occurrence of pneumonia than those with a stronger response (Meijvis et al. 2012). Generally, the body's inflammatory response, if overactive, can cause detrimental collateral damage when fighting pneumonia. However, introducing antibiotics has allowed for anti-inflammatory drug usage as a means of adjunctive therapy (Meijvis et al. 2012). There is the risk of putting the patient into an unsolicited state of immunosuppression, but with all medicines, there is always the risk of minor to serious side effects. In order for an anti-inflammatory drug to be ideal, it would need to sustain the effect of local inflammation and minimize the unnecessary overall difficulties that occur from inflammatory response (Meijvis et al. 2012). This mini-review will summarize immune system response, anti-inflammatory therapies, and possible future roles of these therapies in CAP patients.

Response of the Immune System

Reade et al (2009) evaluated whether or not sex-influenced survival after acquiring CAP. Their result showed that women do, indeed, have a greater life expectancy then men, and men had a higher risk of death than women in a one-year span of statistical analysis; the average age for both sexes was 64.9 years. Their analysis also suggested that males have an anti-inflammatory immune profile while women have a pro-inflammatory profile; the results may not hold certainty for seniors because the danger of their developing infections is greater (Reade et al. 2009).

Corticosteroids and antibiotic treatment of CAP

In the last decade or so, numerous studies have looked at patients with severe cases of community-acquired pneumonia with emphasis on their pulmonary and inflammatory responses as well as data on the adrenal function of these patients (Annane and Meduri 2008). In addition, the administration of systemic corticosteroids is associated with reduced pulmonary inflammation in patients with bacterial pneumonia and acute lung injury, as well as improvement in oxygenation and outcomes in patients with *Pneumocystis jirovecii* pneumonia. Recent guidelines for the management of CAP suggest the benefit of systemic corticosteroids for patients with a severe presentation. Yet, at the time of their publication, only data from a single small randomized controlled trial (RCT) demonstrating improved survival were available.

Presently, few studies have addressed the use of

corticosteroids in the treatment of patients with CAP and their role is still unclear in this setting (Reade et al. 2009). Macrolides have been and continue to be the acclaimed therapeutic application for patients who have community-acquired pneumonia in the United States and Canada. Individuals with health factors that include, but are not limited to, consumption of alcohol, tendencies to smoke, malnutrition, chronic lung disease, and of ages 65 or older are all at a heightened risk to contract CAP (Caballero and Rello 2011). In 2007, an article was published that cited the use of antibiotics for outpatient therapy of CAP (Mandell et al. 2007; Caballero and Rello 2011). Macrolide or doxycycline was suggested for any adult in healthy condition who had not endured antibiotic therapy within the last 90 days. Those individuals who were once healthy but have gone through the therapy in the previous 3 months were suggested a dosages of azithromycin or clarithromycin, including a heavy dose of either amoxicillin-clavulanate or amoxillin alone. The condition becomes somewhat difficult for patients who have any comorbidities such as diabetes. Individuals without therapy are recommended to begin dosages of respiratory fluoroquinolone, or clarithromycin. Individuals having undergone recent therapy should be administered a combination of the two mentioned dosages. Macrolides were stated to remian efficiently persistent for outpatients without risk factors and with moderately severe to mild cases of community-acquired pneumonia (Mandell et al. 2007; Caballero and Rello 2011).

Effect of toll-like receptors in CAP

Toll-like receptors are of crucial importance in defending the host from microorganisms that try to invade during sepsis (Meijvis et al. 2012). TLR-4 is the receptor primarily involved with the inflammatory responses to bacteria containing lipopolysaccharide, which are not common observed in community-acquired pneumonia (Yuan et al. 2008; Meijvis et al. 2012). Also, TLR-2, another toll-like receptor, has been identified as of key importance in the immune system reaction to components of the cell wall of bacteria that are Gram-positive (i.e. peptidoglycan) and may serve as a better objective for inhibition to occur. In a previous study it has been shown the blockade of toll-like receptor 2 (TLR-2) has led to an adequate, yet insignificant, inhibition of cytokine release based on a human lung tissue culture model. As far as the treatment of community-acquired pneumonia is concerned, toll-like receptors do not have a part in treatment (Yuan et al. 2008, Meijvis et al. 2012).

Conclusion

The use of adjunctive anti-inflammatory medication in addition to patient treatment with antibiotics has, increasingly, become of interest in the medical and scientific communities. The drugs mentioned previously are all capable of decreasing inflammatory response; although, not every adult with differences in factors such as age and comorbidities will have the same response (Meijvis et al. 2012). The amounts of dosage will vary from person to person with individuals either benefiting from the treatment or not benefiting from it at all.

The treatment of CAP using anti-inflammatory medication, such as corticosteroids, may be beneficial when assessing certain patients who have higher than normal responses from their immune systems (Meijvis et al. 2012). Research could be furthered if the focus was on those individuals who are in greater need of anti-inflammatory agents because they have higher-intensity of immune responses. Randomized controlled trials would help in better determining the usefulness and effectiveness of anti-inflammatory medication among different subdivisions of outpatients.

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Pathogenic Shiga Toxin Producing Bacteria

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Abstract

Worldwide major outbreaks of bloody diarrhea and Hemolytic Uremic Syndrome (HUS) have brought about much focus on Shiga-toxin (Stx) producing bacteria. Collective data and reviews have shown that *Shigella dysenteriae* type I (SD1) and *Enterohemorrhagic Escherichia coli (EHEC*) 0157:H7 are two major Shiga-toxin producing bacteria that are the causative agents of these worldwide outbreaks. Currently, there is no treatment available to combat diseases caused by these toxin producing bacteria. The mechanisms used by Stx producing bacteria adhere and translocate inside target cells will be discussed. In addition, current vaccination studies that aim to inhibit receptor binding and quorum sensing of the bacterial cell will be illustrated. Finally, future avenues of treatment methods will be addressed in this review.

Introduction

Enterohemorrhagic Escherichia coli (EHEC) 0157:H7 is a lethal pathogen that causes gastrointestinal infections in humans. It is responsible for 73,000 illnesses, 1800-3600 hospitalizations and accounts for 61-541 deaths each year in the United States alone (Pacheco and Sperandio 2012) (www.cdc.gov). This pathogen is typically ingested with contaminated ground beef, steak, salami, dairy products (raw milk, cheese, butter, and cookie dough) and vegetables (spinach, lettuce, sprouts). Even in healthy individuals, EHEC has a remarkable ability of effectively causing disease at very low doses (Pacheco and Sperandio 2012). Most EHEC infections involve bloody diarrhea eventually clear up on its own. However, about 5-7% of cases develop into HUS (Pacheco and Sperandio 2012). One of the two major virulence factors of EHEC is its ability to produce Shiga toxin (Stx).

Shigella dysenteriae type I (SD1) infection causes shigellosis in dysenteric patients. HUS can develop three to eight days after contamination with SD1. Individuals with HUS experience abdominal pain with watery and bloody diarrhea followed by acute renal failure, thrombocyptopenia and hemolytic anemia (Westra et al. 2012). SD1 outbreaks are prevalent in developing countries and are usually more severe in children between two and six (Westra, et al. 2012), and elder people (Butler 2012). Stx levels have been measured in stool specimens of infected patients, but it is unknown of how much blood absorption is needed to cause HUS.

Shiga Toxin

Shiga toxin was first identified by Kiyoshi Shiga from serotype I of *S. dysenteriae*. It was later determined that a group of *E. coli* strains also produced Stx and they are now collectively identified as Stx producing *E. coli* (STEC). There are two main groups in the Stx family which are Stx1 and Stx2. Stx1 consists of Stx1, Stx1a, Stx1c and Stx1d. Stx2 consists of Stx2, Stx2c, Stx2c2, Stx2d, $Stx2d_{activatable}$, Stx2e, Stx2f and Stx2g (Bergan et al. 2012). Stx1 and Stx2 are structurally similar but they share about 55% similarities in their amino acid sequence (Pacheco and Sperandio 2012). Stx2 vary in their receptor binding capabilities which allow them to bind to a wider variety of target cell types; making them more potent to effect humans than Stx1. A comparative epidemiological study revealed EHEC that encoded Stx2 were more likely to cause disease than strains encoding Stx1 or a combination of both (Pacheco and Sperandio 2012).

Molecular Pathogenesis

Stx targets the 60S ribosomal subunit of gastrointestinal cells by inhibiting protein synthesis and inducing apoptosis. Stx is an AB5 toxin which consists of a catalytic subunit A bound non-covalently to a pentamer of B subunits (Stein et al. 1992). The five B subunits collectively bind the globotriaosylceramide (Gb3) receptor on the surface of eukaryotic cells; particularly Paneth cells of the intestinal epithlelium and kidney epithelial cells (Pacheco and Sperandio 2012); through this interaction, Stx is absorbed into epithelium and enters the systemic circulation, which allows full access to the kidneys. Once bound to receptors of the cell, Stx depends on the cell in order to be transported inside the cell. Stx can then avoid lysosome degradation

through the endocytic pathway and can be conveyed from the trans-Golgi network to the endoplasmic reticulum. Once here, the Stx can attack its ribosomal target by allowing the A subunit to cleave an adenosine residue from 60S ribosomal subunit which ceases protein synthesis and ultimately results in cell death (Pacheco and Sperandio 2012).

With full access to the target cells, the bacterium utilizes the resources of the cells to support its pathogenic lifestyle. In EHEC O157:H7, the stx encoding genes are located in intact or partial genomes of prophages from the lambda family, which are integrated in the bacterial chromosome (Pacheco and Sperandio 2012). When the lytic cycle is activated, new phages are produced as well as Stx. Once the bacterial cell is lysed, Stx is released into the environment. The gene encoding Stx1 and Stx2 are located within the late phage genes, where expression of the *stx*AB is under control of the phage cycle. To inhibit the phage from entering the lytic cycle, a repressor, cI, binds to the right (O_R) and left (O_L) operator sites to repress the activity of the P_R and P_L promoters (Pacheco and Sperandio 2012). When the SOS response of the bacterial cell is initiated, RecA, which is produced as a result of the response, cleaves the cI repressor. This frees the operator sites and activates the promoters P_R and P_L. In addition, without the cI repressor, anti-terminaters N & Q are expressed and the Q protein then binds to P_R to activate *stx*AB expression. Finally, Stx is released into the environment when the bacterium is lysed.

Inhibition of Quorum Sensing

One major avenue for the development of vaccines against Stx-mediated diseases would be to inhibit quorum sensing. Bacterial cells use quorum sensing to communicate from cell-to-cell for coordinating gene expression. Pathogenic bacteria typically use it for producing and signaling the release of virulence factors. The quorum sensing system of EHEC O157:H7 is a two component system (TCS) called the QseBC TCS. TCSs comprise of a sensor histidine kinase and a response regulator to regulate quorum sensing. The sensor kinase QseC is the only sensor of the auto-inducer (AI) AI-3 (which are signaling compounds involved in adaptive responses) in EHEC. QseC has been found to be involved in the expression of *stx2* when recA is activated in the SOS response of bacteria. This illustrates that virulence production is co-regulated with bacterial stress.

EHEC O157:H7 can hijack mammalian stress response hormones epinephrine (epi) and norepinephrine (NE) to regulate expression of its major pathogenic traits: attaching and effacing lesion formation, flagellar motility and Stx production (Pacheco and Sperandio 2012). EHEC uses QseBC TCS to sense epi/NE production by the host cell to regulate virulence production. When the signal is sensed, the histidine kinase transfers its conserved aspartate residue to the response regulator. The binding of the phosphate causes a conformational change that allows the response regulator to bind to the DNA and promote the expression of virulent genes such as *stx2*. In an attempt to disrupt the major pathogenic traits of EHEC, it would be difficult to down regulate the expression of epi and NE because they are vital in human stress response pathways. Therefore, it is more feasible to use the immune system to target EHEC.

Inhibition of Receptor Binding

Another target for possible drug development would be to disrupt the efficacy of receptor binding. Stx utilize the globotriaosylceramide (Gb3) receptor of the host cells to bind and cause organ failure (Bergan et al. 2012). Gb3 receptors are mostly found in kidney epithelium and endothelium, intestinal lamina propia, platelets, B lymphocytes and peripheral and central nervous tissue. Cellular lipid bilayers consist of cholesterol and membranes proteins that are referred to as detergent-resistant membranes (DRMs). Gb3 associated-DRMs play a role in extent of toxicity. When Stx binds to Gb3 receptors in detergent resistant glomerular cells, it is highly toxic and can lead to HUS in glomerular tissue (Bergan et al. 2012). However, when bound to Gb3 receptors in tubular cells that are detergent-sensitive, pathogenicity is reduced. This was proven in a cell culture and renal tissue studies where cholesterol was extracted from the membrane. It was determined that excess cholesterol disguises Gb3 from binding to toxins; which decreases pathogenicity of Stx (Bergan et al. 2012).

Drug Therapy

As of now, there is no cure for STEC infection. One hopeful strategy is to work on neutralizing Stx by developing receptor mimics (Bergan et al. 2012). The use of antibiotics to treat STEC infections is also being investigated. In a recent study that included treatment of children infected with EHEC O157:H7, trimethoprimsulfamethoxazole (STX) and quinolones worsened the conditions of the patient. Several antibiotics have been reviewed on their effectiveness against Stx production. Azithromycin (AZM), doxycycline (DOX), phosphomycin (FOS) and gentamycin (GEN) were found to decrease Stx production (Pacheco and Sperandio 2012). All of these antibiotics target protein and cell wall synthesis; which indicates that Stx production is intertwined with DNA synthesis somehow.

Antibody treatment

STEC infections do not typically lead to bacteremia. However, when complications arise as in the case of HUS, Stx are translocated from the intestine into the circulatory system. With this being considered, a logical target against Stx is from a passive immune stand-point. In one study, a group selected a human monoclonal antibody against the A subunit of Stx2 to treat piglets that were infected with STEC O157:H7. All of the piglets that were not treated developed diarrhea and fatal neurological symptoms, while 85% of those treated with the antibody survived (Muniesa et al. 2012). In another study, piglets that were treated with Stx1-specific antibodies all died while those treated with Stx2-specific antibodies had a survival rate of 100% (Muniesa et al. 2012). These results indicated that this method of using monoclonal antibodies against the A subunit could be effective for treating Stx2 strain infections. Further clinical trials need to be conducted.

Inhibitors

Glucosylceramide synthase is an enzyme involved in the synthesis of Gb3 receptors in kidneys. A pyrrolidin derivative can be used to inhibit the activity of glucosylceramide synthase and thus decrease the amount of Gb3 receptors that are synthesized. A group of researchers used this compound and found that the toxin-produced mortality rate of rats was decreased by 50% (Muniesa et al. 2012). Furthermore, the levels of toxins in the kidney and gut of the rats was significantly lower. With further clinical studies, glycosylceramide synthase could possibly be used as a preventative method.

Conclusion

STEC can spread easily via contaminated food. It is evident that STEC take full advantage of the host cells by utilizing the resources of the cell to survive and continue a pathogenic lifestyle. Understanding the pathway of bacterial infection and the mechanisms by which Stx are produced and released are imperative in developing drugs for prevention and treatment. Research that focuses on regulating the production of Stx within the bacteria seems logical. Therefore, present research that aim to inhibit receptor binding and quorum sensing could both be beneficial in prevention and treatment therapies. In addition, by understanding STEC, researchers would be better capable of understanding and treating SD1.

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