MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITY OF DRINKING WATER

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Abstract

This study was conducted on the water samples collected before and after filtration treatment was given. Five types of filtered drinking water (A1, B1, C1, D1 and E1) and five types of unfiltered tap water (A2, B2, C2, D2 and E2) were chosen randomly from houses in Klang Valley for analyses. The purpose of this study was to determine the quality of filtered drinking water by looking into the microbiological aspect and several physicochemical analyses such as turbidity, pH and total suspended solid (TSS). The microbiological analyses were performed to trace the presence of indicator organisms and pathogens such as *Escherichia coli*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*. All of the water did not comply with the regulations of Food Act as consisted of more than 10^3-10^4 cfu/mL for total plate count. However, the total coliforms and *E. coli* were detected lower than 4 cfu/mL and not exceeding the maximum limit of Food Act. While the presence of *S. faecalis* and *P. aeruginosa* were negative in all samples. The pH value was slightly acidic (pH<6.5) compared to the range in the regulations. The TSS for the samples were low (1.0×10^{-4} - 2.2×10^{-3} mg/L) and the turbidity for all the samples were recorded below 1 Nephelometric Turbidity Units (NTU) thus, complying with the regulations. All the water samples that undergo the filtration system were fit to be consumed.

Keywords: quality, drinking water, filters, microbiology, physicochemical

Abstrak

Kajian ini melibatkan pengumpulan sampel air sebelum dan selepas diberi perlakuan penapisan. Lima jenis sampel air minuman yang telah ditapis (A1, B1, C1, D1 dan E1) dan lima jenis sampel air paip yang belum ditapis (A2, B2, C2, D2 dan E2) telah dipilih secara rawak daripada perumahan di sekitar Lembah Klang untuk kajian ini. Analisis yang dijalankan bertujuan menentukan kualiti air minuman bertapis dari segi mikrobiologi dan fizikokimia seperti kekeruhan, pH dan penentuan Jumlah Pepejal Terampai (JPT). Analisis mikrobiologi yang dijalankan bertujuan untuk mengesan kehadiran organisma penunjuk pencemaran air dan patogen seperti *Escherichia coli*, *Streptococcus faecalis* dan *Pseudomonas aeruginosa* telah diuji dalam analisis mikrobiologi. Keputusan analisis mendapati semua sampel mempunyai hitungan piring jumlah 10^3-10^4 cfu/mL dan tidak mematuhi kriteria keselamatan yang ditetapkan oleh Akta Makanan. Walau bagaimanapun, jumlah koliform dan *E. coli* dalam semua sampel adalah kurang daripada 4 cfu/mL dan mematuhi Akta Makanan. Keputusan adalah negatif bagi kesemua sampel air dalam penentuan kehadiran *S. faecalis* dan *P. aeruginosa*. Nilai pH bagi kebanyakan sampel adalah sedikit berasid (pH<6.5) seperti yang ditetapkan oleh Akta Makanan. Nilai jumlah pepejal terampai adalah sangat rendah iaitu 1.0×10^{-4} - 2.2×10^{-3} mg/L. Nilai kekeruhan bagi semua sampel adalah kurang daripada 1 *Nephelometric Turbidity Unit* (NTU) dan mematuhi nilai yang ditetapkan oleh Akta Makanan. Sampel-sampel yang diberi perlakuan penapisan mempunyai kualiti yang lebih baik berbanding tidak ditapis (air paip). Semua sampel air yang telah melalui sistem penapisan sesuai dijadikan air minuman.

Katakunci: kualiti, air minuman, penapisan, mikrobiologi, fizikokimia

Introduction

The key to increase human productivity and long life is good quality water [1]. The provision of good quality household drinking water is often regarded as an important means of improving health [2]. According to World Health Organization (WHO) [3], there were estimated 4 billion cases of diarrhoea and 2.2 million deaths annually. The consumption of unsafe water has been implicated as one of the major causes of this disease. Most gradual deterioration of water quality was resulted by the increase in human populations and urbanization [4]. As water pollution is getting serious, houses especially in the urban area started to equip with a water filter system. People are concern with the presence of pollutants such as heavy metals and toxic chemical in their daily drinking water. Filtered water is the main source of safe and reliable drinking water. However there is still a debate on the efficiency of filtration system to comply with the regulations as water that physically looks

colourless, odourless and even tasteless is not sufficient to determine that the water is safe for consumption. In fact, the drinking water should be examined on microbiological and physicochemical quality. The WHO [3] in its 2002 report, recommended that increased emphasis be placed on home water treatment and storage, and that more research should be conducted to assess the health benefits of such interventions. Contaminants can be in the form of microorganism that barely visible in unaided eyes. A number of authors have reported a statistically significant deterioration in the microbiological quality of water between the source and point of use in the home [5, 6, 7]. According to WHO [8], the physical parameters that are likely to give rise to complaints from consumers are colour, taste, odour, and turbidity while low pH causes corrosion and high pH results in taste complaints. Therefore the objective of this study was to determine the microbiological and physicochemical quality of water samples collected before and after the filtration treatment given. This study however only measured water quality at point of use in houses around Klang Valley.

Experimental

Pre-Survey
The purpose of this pre-survey was to record about the consumer's installment of water filter system. This is to ensure that all water filter systems were picked with the approximately similar duration of usage to avoid biasness. The project information was explained to each household and their consent for participation obtained.

Sampling
A total of ten samples were collected aseptically at room temperature from the housing area in Klang Valley. The samples were collected using the sampling and storage procedures according to Benjamin and Brown [9]. All samples were collected in July 2004 and samples were kept in 1000 mL sterile autoclavable plastics (polypropylene) bottles, labelled with different codes and immediately stored at 4°C when transported back to the laboratory from sample collecting site. The same alphabet in the code represented the water from the same tap and the numbering that follows differentiates between filtered and unfiltered water. The source of collection can be refered to Table 1.

Table 1 Coding and Sources of Sampling Study

Code	Sample	Source
A1	Filtered Water	Kajang
A2	Unfiltered Tap Water	Kajang
B1	Filtered Water	Serdang
B2	Unfiltered Tap Water	Serdang
C1	Filtered Water	Cyberjaya
C2	Unfiltered Tap Water	Cyberjaya
D1	Filtered Water	Puchong
D2	Unfiltered Tap Water	Puchong
E1	Filtered Water	Bangi
E2	Unfiltered Tap Water	Bangi

Gram Staining
The chemicals and media used for this study were from OXOID and MERCK. The microbiological analyses performed on the samples were total viable count using the method from Roberts et al. [10], bacteria were identified using Gram staining. A loopful of bacteria grown on R2A agar was placed on a clean slide and air dry by slight heating. The slide was coloured using crystal violet for 1 min and rinsed off with water. A few drops of alcohol were used to discolour the crystal violet and immediately rinsed off the alcohol with flowing water. The remaining stain was then washed by safranin and the slide was examined under a microscope.

Coliforms, Faecal coliforms and Escherichia coli (E. coli) (Preliminary and Confirmation test)
Enumeration of viable cell was done according to Roberts et al. [10]. A serial decimal dilution was prepared using Maximum Recovery Diluents (MRD). The original water samples were diluted (10^{-1}-10^{-4}) and pipetted on R2A using spread plate method and incubated at 20-22°C for three days. Gram staining suggested by

Roberts and his colleagues [10], were used to identify bacteria present on the R2A agar. One hundred mL samples were filtered through 0.45µm pore size cellulose nitrate membrane filters (Sartorius). Membrane filters were subsequently transferred face upwards onto Endo Agar using a sterile forceps. The agar was incubated at 35°C for 18-24 hours. A confirmation test on the presence of coliforms, faecal coliforms and *E. coli*, were performed by inoculating culture into two Brilliant Green 2% Bile Broth (BGBB) test tubes with each having a Durham test tube and test tube containing 1% tryptone water (TW). One BGBB test tubes were incubated at 37°C for 48 hours while the other BGBB and TW tubes were incubated at 44°C for 24 hours. About 0.2-0.3 mL Kovac's Reagent were added to TW to detect indole formation, (a red colour layer on surface). Gas released in Durham test tubes indicated presence of coliforms, faecal coliforms and *E.coli*. The comparison between coliforms, faecal coliforms and *E.coli* were shown in Table 2.

Streptococcus faecalis and Pseudomonas aeruginosa
A detection test on the presence of *Streptococcus faecalis* was done. One hundred mL samples were filtered through cellulose nitrate membrane filters. Membrane filters were subsequently transferred face upwards onto KF Agar using a sterile forcep. The agar was then incubated at 35°C for 48 hours. Pink colour colonies with a diameter of 0.5-2.0mm showed the presence of *S. faecalis*. A further confirmation test was done by inoculating the colony onto Kanamycin Esculin Azide Agar and incubated at 35°C for 72 hours. A catalyst test was used to identify *S. faecalis*. The detection of *P. aeruginosa* was done by placing filtered cellulose nitrate membrane filters onto basic *Pseudomonas* Agar. The media was incubated at 42°C for 40-44 hours. Later the agar was checked under ultra violet light to detect pigment thought to be *P. aeruginosa.*

Table 2 Comparisons Between Coliform, Faecal Coliform and *E. coli*

	Gas formation in BGBB 37°C (48hours)	Gas formation in BGBB 44°C (24hours)
Coliform	+	-
Faeces Coliform	+	+
E.coli	+	+

Source: Roberts et al. [10]

Determination of Water Quality Parameters
The samples were also evaluated on the physicochemical aspects to determine turbidity, total suspended solid (TSS) and pH.

Turbidity: Turbidity in Nephelometric Turbidity Units (NTU), was measured using a HACH ratio turbidometer (Model 2100 AN, Co., USA). A total of 15mL sample was placed in a special container and inserted into the turbidometer for reading.

pH : WTW pH 420 meters were used.

TSS : The Total Suspended Solid (TSS) was according to Mazlin et al. [11]. A filter paper was weighed and later wet with sterile distilled water. The sample was stirred homogenously to ensure no precipitation. The sample was measured accurately and poured through the filter paper. The filtering was done using a suction pump. Next the filter paper was transfer into the oven for drying at 103°C for 24 hours. The filter paper was left to cool before being weighed. The TSS was counted according to the formula below:

$$\text{mg of total suspended particles/ L} = \frac{(A-B) \times 1000}{\text{mL sample}}$$

where, A = filter paper weight + residue after filtering and drying (mg), and
B = filter paper weight (mg)

Statistical Analyses

The entire tests were done in replicates. The data yield were analysed by Statistical Package Social Science (SPSS) programme version 11.0. Analyses of variance (ANOVA) at 95% confidence level were used to determine the significance difference.

Results and Discussion

Total Viable Count

The nature and quantity of microbes in the water samples will determine the health risk of consumer after consuming the water. Bacterial analysis at 22°C is indicative of the bacteria found within the environment [12]. The total viable count showed that sample B1 had the lowest count 3.5 log cfu/mL followed by D1 having 3.6 log cfu/mL count (Figure 1). However this does not necessarily determine that B1 is the most effective water filter as when comparison were done on water before and after filtration, D1 was proved to be most effective. This was due to the decrease of 0.4 cfu/mL of bacterial population after filtering process. All filtered samples were still not able to comply with the standard for microbial quality of water for human consumption given by European Council (EC) as shown in Table 3 that only allowed not more than 10 cfu/ mL in potable drinking water. As Food Act [13] and most countries do not state the limit of total viable count for drinking water, EC criteria was used to make comparison in this study. All samples recorded were not significant different (p>0.05) although the result in Figure 1 showed a decrease of total viable count in all samples after filtering. There was likely that most water were still exposed to the contamination of environmental bacteria even though after filtering treatment was provided.

Gram Staining Test

Gram staining showed that 60% of the samples (A1, A2, B2, C2, D2 and E2) were identified as gram negative, where 80% of them were rod shaped and 20% were in coccus form. The other 40% samples (B1, C1, D1 and E1) were bacteria gram positive. This is in agreement with previous studies where most bacteria found in the drinking water were the Gram negative [14, 15]. These bacteria were naturally present in the water [16].
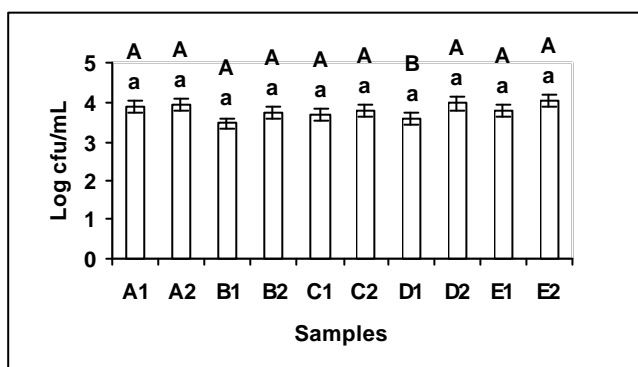

Figure 1 Histogram for Total Viable Count (Log cfu/mL) for Ten Samples

a : The same alphabet on different sample shows no
significant difference (p>0.05)
A-B: The same alphabet on different sample shows no
significant difference (p>0.05)
The comparison were done on two samples before (A2-E2) and after filtration (A1-E1
Error bars = standard deviation for min logcfu/mL (n=2)

Table 3 Standards for Microbial Quality of Water for Human Consumption

Characteristics	UK	EC	WHO	Canada	US
Faecal coliforms	0/100 mL	0/100 mL	0/100 mL	0/100 mL	0/100 mL
Coliforms	n/s	n/s	0/100 mL	10/100 mL	1/100 mL
Total bacteria count	NSI	10/ mL	n/s	n/s	n/s
Total coliforms	0/100 mL	0/100 mL	-	-	-
Faecalis Streptococcus	0/100 mL	0/100 mL	-	-	-

n/s : not stated, NSI : non-significant different
Source: Gray [17]
Vigneswaran & Visvanathan [18]

The Presence of Coliforms, Faecal Coliforms and E. coli
Most coliforms are present in large numbers among intestinal flora of humans and other warm-blooded animals, and are thus found in fecal wastes [19]. As a consequence, coliforms, detected in higher concentrations than pathogenic bacteria, are used as an index of the potential presence of entero-pathogens in water environments [20]. Coliforms are also routinely found in diversified natural environments, as some of them are of telluric origin, but drinking water is not a natural environment for them. As a result, their presence in drinking water must be considered as harm to human health. Positive presence of coliforms in treated water which is usually coliform-free may indicate treatment ineffectiveness. The result obtained for the preliminary test of coliforms, faecal coliforms and *E. coli* showed that all samples of unfiltered water (A2, B2, C2, D2, and E2) and A1 having the growth of red colonies. While other filtered water resulted negative for the presence of coliforms, faecal coliforms and *E. coli*. However, sample A1 only had 2 cfu/mL of coliforms and according to the Food Act [13], the colony count should not result more than 4 cfu/ 100 mL in two following samples. Therefore all the filtered water except A1 was fit for consumptions while all the unfiltered water were not safe to be consumed.

Streptococcus faecalis and Pseudomonas aeruginosa
All samples showed negative results for the presence of *S. faecalis* and *P. aeruginosa*. This indicates that the water was all free from faecal contamination as *Streptococcus* is one of the indicators for faecal contamination in drinking water [21]. The absence of *S. faecalis* was mainly due to the pore sizes (0.3-0.7 micron) of the ceramic filters in all the water filter systems, which enable to filter *streptococcus* with the size of 1.0-1.5 micron from the water. Furthermore, the chlorination by the Water Department (PUAS) was able to kill and injured most of the *S. faecalis* that were present. *Pseudomonas* spp. is the normal microflora in human and animals [22]. According to Hunter [23], *Pseudomonas* does not harm a healthy individual but cause problem in individual with weak immune system. However, it is more reliable and safe if the drinking water does not show the presence of *Pseudomonas* spp. Rosenberg [24], suggested that the source of the presence of *Pseudomonas* spp. in the water is due to contamination by human themselves.

Total Suspended Solid (TSS)
The increase of total suspended solid is constant with the soluble solid content. According to the U.S. Environmental Protection Agency [25], the higher the mineral content in the water, more total suspended solid will be formed. The analyses done on the samples proved that E1 contained less TSS compared before filter treatment (E2) and more effective than other filter system. Most of the filter system only able to filter TSS up to 0.0001 mg/L.

pH Value
The pH values of most water (filtered and unfiltered) were slightly acidic (pH 6.0-6.5) as shown in Table 4 and according to the standard range gazetted by International Bottled Water Association, BWA [26] which permitted water to be within pH 5-7. However the guideline provided by Food Act [13] of pH 6.5-7.0 was not complied by all water samples except A1 which read pH 6.5. The lowest of pH 6.0 was recorded in samples A2, C2 and E1. Low pH tends to make the water corrosive while high pH will result in taste complaints. Considering no significant difference (p>0.05) between all samples the taste perceptions of the water points with the maximum and minimum pH were all deemed satisfactory among the consumers.

Turbidity

The turbidity measured on all the samples were less than 1 Nephelometric turbidity unit (NTU). According to the Food Act [13], the maximum level of turbidity permitted for drinking water is 5 NTU. While World Health Organization (WHO) [27] stated drinking water is best consumed with NTU less than 1 for health purpose. All samples are likely to show no significant difference (p>0.05) except for sample E2 (unfiltered tap water) that was slightly cloudy (1.68×10^{-1} NTU) but after filtration resulted, E1 with (3.52×10^{-2} NTU). The results for these turbidity analyses were shown in Table 4. WHO [27] suggest that the appearance of water with a turbidity of less than 5 NTU is usually acceptable to consumers, although may vary with local circumstances. The consumption of highly turbid water may constitute a health risk as excessive turbidity can protect pathogenic microorganisms from the effects of disinfectants, and also stimulate the growth of bacteria during storage [28].

Table 4 The Total Suspended Solid (TSS), pH and Turbidity Value Measured on the Water Samples

Sample	TSS Value (mg/L)	pH Value	Turbidity Value
A1	7.00×10^{-4} [b]	6.5 [a]	1.18×10^{-1} [a]
A2	1.20×10^{-3} [a]	6.0 [a]	1.08×10^{-1} [a]
B1	6.00×10^{-4} [b]	6.2 [a]	1.00×10^{-1} [a]
B2	1.80×10^{-3} [a]	6.1 [a]	1.40×10^{-1} [a]
C1	6.00×10^{-4} [b]	6.3 [a]	1.20×10^{-1} [a]
C2	1.70×10^{-3} [a]	6.0 [a]	1.33×10^{-1} [a]
D1	1.20×10^{-3} [a]	6.1 [a]	6.75×10^{-2} [a]
D2	2.10×10^{-3} [a]	6.2 [a]	1.10×10^{-1} [a]
E1	1.00×10^{-4} [b]	6.0 [a]	3.52×10^{-2} [a]
E2	2.20×10^{-3} [a]	6.2 [a]	1.68×10^{-1} [b]

a-b: The same alphabet on different sample shows no significant difference (p>0.05).

Conclusion

The study conducted showed that the total viable count for all samples were in the range of 10^{3}-10^{4} cfu/mL. All samples did not comply with the safety regulation from EC for drinking water. About 60% of the samples were Gram negative bacteria. Coliforms, faecal coliforms and *E. coli* were less than the permitted colony count regulated by the Food Act [13]. All the samples were absent from *S. faecalis* and *P. aeruginosa*. The pH values were identified slightly acidic (pH 6.0-6.5), although the pH values permitted by the Food Act [13] is 6.5-8.5 but this range according to IBWA [26] is still acceptable. All samples have the turbidity values within 0.0352-0.1680 NTU and this was less than 1 NTU as limited by Food Act [13]. The total suspended solid for all the samples are low (1.0×10^{-4} – 2.2×10^{-3} mg/L). In conclusion, it is safer to consume filtered drinking water after filtration system and boiled then tap source water.

References
[1] Urbansky, E.T. & Magnuson, M.L. 2002. *Anaytical Chemistry*. **74**: 261A
[2] Moyo, S., Wright, J., Ndamba, J. & Gundry, S. 2004. Realising the maximum health benefits from water quality improvements in the home: a case from Zaka district, Zimbabwe. *Physics and Chemistry of the Earth*. **29**: 1295-1299.
[3] WHO (World Health Organization). 2002. Report.
[4] Ho, K.C. & Hui, C.C. 2001. Chemical contamination of the East River (Dongjiang) and its implication on sustainable development in the Pearl River Delta. *Environmental International*. **26**: 303–308.
[5] Simango, C., Dindiwe, J. & Rukure, G. 1992. Bacterial contamination of food and household stored drinking water in a farmworker community in Zimbabwe. *Central African Journal of Medicine*. **38**(4): 143–149.
[6] Genthe, B.N., Strauss, et al. 1997. The effect of type of water supply quality in a developing community in South Africa. *Water Science Technology*. **35**(11–12): 35–40.
[7] Welch, P., David, J., Clarke, W., Trinidade, A., Penner, D., Berstein, S., McDougall, L. & Adesiyun, A.A. 2000. Microbial quality of water in rural communities of Trinidad. *Review of Panam Salud Publications*. **8**(3): 172–180.

[8]   WHO (World Health Organization). 1996. *Guidelines for Drinking-Water Quality, 2nd ed. Health Criteria and Other Supporting Information*. WHO, Geneva, Switzerland.

[9]   Benjamin, A.P. & Brown, R. 2003. *Encyclopedia of Food Sciences and Nutrition*. 2nd Ed. volume 10. United Kingdom: Academic Press.

[10] Roberts, D., Hooper, W. & Greenwood, M. 1995. *Practical Food Microbiology*. London: Public Health Laboratory Service.

[11] Mazlin, M., Mohd Talib, L. & Lee, Y. H. 2003. *Water Chemistry*. Kuala Lumpur: Utusan Publications and Distributions Sdn. Bhd.

[12] Parsons, S.A. 2000. The effect of domestic ion-exchange water softeners on the microbiological quality of drinking water. *Water Research*. **34**: 2369-2375.

[13] Food Act 1983 (Act 281) & Regulations. 2003. Regulations 394 (1) and 360_B (3), Twenty-Fifth Schedule Standard for Water and Packaged Drinking Water. pp.316. Malaysia: International Law Book Services.

[14] Morais, P.V. & da Costa, M.S. 1990. Alterations in the major heterotrophic bacterial populations isolated from a still bottled mineral water. *Journal of Applied Bacteriology*. **61**: 365-372.

[15] Mavridou, A. 1992. Study of the bacterial flora of a non-carbonated natural mineral water. *Journal of Applied Microbiology*. **73**: 335-361.

[16] Armas, A.B. & Sutherland, J.P. 1999. A survey of the microbiological quality of bottled water sold in the UK and changes occuring during storage. *International Journal of Food Microbiology*. **11**: 51-72.

[17] Gray, N.F. 1994. *Drinking Water Quality: Problems and Solutions*. John Wiley and Sons. Inc. Chichester.

[18] Vigneswaran, S. & Visvanathan, C. 1995. *Water Treatment Processes: Simple Options*. London: CRC Press.

[19] Dorothy, A.G. & Philip, R.A. 1998. *Technology of Bottled Water*. USA: Sheffield Academic Press.

[20] Rompré, A., Servais, P., Baudart, J., de-Roubin, M. & Laurent, P. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of Microbiological Method*. **49**: 31-54.

[21] Warburton, D., McCornwick, J.K. & Bowen, B. 1992. Survival and recovery of *Aeromonas hydrophila* in water: development of methodology for testing bottled water in Canada. *Canada Journal of Microbiology*. **40**: 145-148.

[22] Shimeld, L.A. 1999. *Essentials of diagnostic microbiology*. United Kingdom: DelmarPublishing. pp. 207-214.

[23] Hunter, P.R. 1993. The microbiology of bottled natural mineral waters. *Journal of Applied Bacteriology*. **74**: 345-352.

[24] Rosenberg, F. 2003. The microbiology of bottled water. *Journal of Clinical Microbiology* **25**: 41-44.

[25] U.S. Environmental Protection Agency. 2000. *Drinking water microbiology*. Committee on the Challenges of Modern Society. Nato/ CCMS drinking water pilot project series. Edited by D.A. Cliver & R.A. Newman. Environmental Protection Agency, Washington, D.C.

[26] International Bottled Water Association (IBWA). 2003. Water quality and the fitness for drinking (online) http://www.ibwa.com (29 Sept 2004).

[27] WHO. 1998. *Guidelines for drinking water quality. Health criteria and other supporting information*. Geneva: World Health Organization. **Vol. 2**.

[28] Zvikomborero, H. 2005. An assessment of the water quality of drinking water in rural districts in Zimbabwe. The case of Gokwe South, Nkayi, Lupane, and Mwenezi districts. *Physics and Chemistry of the Earth*. **30**: 859-866.