Journal of Water Resource and Protection, **2012, 4, 638-647**
http://dx.doi.org/10.4236/jwarp.2012.48074 Published Online August 2012 (http://www.SciRP.org/journal/jwarp)

Influence of Temperature on Mutagenicity in Plants Exposed to Surface Disinfected Drinking Water

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Received May 31, 2012; revised July 2, 2012; accepted July 14, 2012

ABSTRACT

Disinfection of surface drinking water, particularly water chlorination, produces by-products with potential genotoxic and/or carcinogenic activity. A study carried out at a pilot plant for drinking water disinfection of lake water revealed mutagenic activity of three different disinfectants (sodium hypochlorite, chlorine dioxide and peracetic acid) in different seasons using *in situ* mutagenicity assays, both in animal (micronucleus test) and in plant organisms (anaphase chromosomal aberration and micronucleus tests). The effects of the disinfectants appeared to be modulated by the season of exposure. In this study, we tried to understand if (and to what extent) the temperature parameter could actually play an independent role in the registered seasonal variation of mutagenic effects, neglecting the variation of other parameters, e.g. physical conditions and chemical composition of the lake water. Therefore plants (*Allium cepa* for chromosomal aberration test and *Vicia faba* for micronucleus test) were exposed to the same disinfected lake-water samples at different temperatures (10˚C, 20˚C and 30˚C), according the ones registered during the *in situ* experiment. Long-term exposure at the temperatures of 20˚C (both *Vicia faba* and *Allium cepa*) and 30˚C (*Vicia faba* only) to disinfected waters induced clear mutagenic effects. These results show that temperature is an important variable which should be taken into account when *in situ* exposure of plants is planned for mutagenicity testing. Also, different plant systems clearly show specific temperature ranges suitable for their growth, thereby indicating the need for an accurate selection of the test organism for a specific experimental plan.

Keywords: Clastogenicity/Aneugenicity; *Allium cepa* Aberration Test; *Vicia faba* Micronucleus Test; Temperature Exposure; Water Disinfection

1. Introduction

Drinking water disinfection may produce toxic compounds, particularly when water is obtained from surface sources. Such disinfection by-products (DBP) are formed when disinfectants (e.g. chlorine, ozone, chlorine dioxide, or chloramines) react with naturally occurring organic matter, anthropogenic contaminants, bromide, and iodide. It has been demonstrated that chlorination, the most widely used method of disinfecting water, leads to the formation of numerous mutagenic and/or carcinogenic DBPs [1-4].

Despite the numerous laboratory evidences on the mutagenic or carcinogenic properties for many DBPs, epidemiologic studies to date have revealed only a modest association between DBP exposure and cancer in humans [4,5]. Interesting findings for their significant implications for cancer prevention come from a recent case-control study carried out in hospital which showed strong associations between DBP exposure and bladder cancer among individuals carrying inherited variants in three genes (GSTT1, GSTZ1, and CYP2E1) that code for key enzymes that metabolize DBPs [6].

As far as the mutagenic and genotoxic potential of DPBs is concerned, still incomplete information for many of them is available, particularly for the emerging ones, the levels of which are increased by alternative disinfectants that are being employed (primarily ozone or chloramines) compared to chlorination. However, many emerging DBPs are more genotoxic than some of the DBPs that are submitted to regulation [4,7]. Since the growing body of evidence about the adverse effects of DPBs produced by common disinfectants, alternative disinfection practices have been implemented and in some instances results from extracted organic material in

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drinking water showed to be less mutagenic than extracts from chlorinated water [4,8].

Nevertheless, the full toxicological effects of the complex mixtures of DBPs present in drinking water are largely unknown, except through epidemiologic studies [5], because the greatest majority of mutagenicity studies are carried out on drinking-water extracts or concentrates, largely in Salmonella [9]. In general, only a few of them involved drinking waters prepared by alternative disinfection methods, which showed that alternative disinfection methods may be considerably less mutagenic than chlorinated drinking water [10]. For this kind of studies an environmental biomonitoring approach can be adopted, in which *in situ* exposure of bioindicator organisms, principally aquatic animals (fish, mollusks) and plants [9,11,12] is performed.

Plants are unique in their ability to serve as *in situ* monitors for environmental genotoxins that exist either as single or complex forms [13] and to date new plant systems are being proposed for assessing the genotoxic potential on living organisms as a sensitive indicator of water quality complex-mixtures [14-16], besides the traditional plant systems routinely employed for cytogenetic damage. Plant bioassays can detect a wide range of genetic damage, including gene mutations and chromosomal aberrations; in particular, mitotic cells in meristems of plant roots are considered excellent experimental tool for assessing the cytogenetic damage induced by clastogenic and aneugenic environmental pollutants [13,17].

Cytogenetic tests have been successfully performed in plant systems for assessing genotoxic contamination of aquatic environments—especially polluted rivers [18,19] and soil [20-23], as well as for detection of drinking-water mutagenic potential [13,15,24-31]. Among plants, *Vicia faba* and *Allium cepa* are routinely used especially because of their sensitivity to a wide variety of mutagenic compounds [32] and to extremely low doses of X-rays [33]. Other factors, such as high percentage of dividing cells in root tips, uniform size of chromosomes, easy culturing in laboratory conditions and growth under *in situ* exposure, are further advantages which encourage the use of these systems [34].

Vicia faba and *Allium cepa* are widely used for the evaluation of genotoxic potential of disinfected wastewater, surface water, and drinking water [12,16,34-40].

A previous study was carried out to evaluate the genotoxic potential induced by surface water disinfected for drinking usage in root cells of plant organisms exposed *in situ* to unconcentrated water samples, using the *Allium cepa* anaphase chromosomal aberration test and the *Vicia faba* micronucleus test [11,29]. This approach allows waters to be tested without requiring extraction processes/concentration methods of water samples, so as to expose test organisms both in the laboratory and *in situ*. That study was performed at a pilot drinking water treatment plant, located near Lake Trasimeno (Central Italy), where lake water was experimentally disinfected using three alternative compounds: sodium hypochlorite (NaClO), chlorine dioxide (ClO_2) and peracetic acid (PAA). Results indicated that all the disinfection treatments, especially ClO_2 and NaClO, induce a clastogenic/aneugenic effect. All the plant tests gave overall similar results, yet a seasonal correlation was also observed, since a noticeable variability of the mutagenic effect was found among the different sampling months (October, February and June): while in *Allium cepa* raw water resulted to be weakly genotoxic in October and February, the *in situ* exposures carried out in October to disinfected waters exerted the strongest mutagenic effects both in *Vicia faba* and in *Allium cepa*, which were induced by all the compounds employed for drinking water treatment [11]. These differences were thought to be due to either temperature or chemical composition of disinfected water, being the two factors closely correlated.

To verify the first hypothesis, ***i.e.***, the influence of temperature on the expression of mutagenic damage, the same samples of raw and NaClO-, ClO_2- and PAA-disinfected water were supplied to the plants (*Allium cepa* for chromosomal aberration test and *Vicia faba* for micronucleus test) at different temperatures (10˚C, 20˚C and 30˚C) in the present laboratory tests following the same protocol as that adopted for *in situ* exposures.

2. Materials and Methods

2.1. Lake Water Sampling and Treatment with Disinfectants

Water taken from Lake Trasimeno underwent sedimentation, filtration and acidification (H_2SO_4, pH 7.0), followed by disinfection with 3 mg/L of sodium hypochlorite (NaClO), chlorine dioxide (ClO_2) and peracetic acid (PAA). Water samples were taken in springtime and used immediately for plant exposures.

The disinfection treatments were as follows:

1) Chlorine dioxide (ClO_2): produced directly in treated water from an 8% $NaClO_2$ solution and a 10% HCl solution using an automatic generator (Tecme S.r.l., Gardolo di Trento, TN, Italy);

2) Sodiumhypochlorite (NaClO), (Solvay S.p.A., Rosignano, LI, Italy): supplied as a 14.5% - 15.5% solution via a membrane pump;

3) Peracetic acid (CH_3COO_2H), (Promox S.r.l., Leggiuno, VA, Italy): supplied as a 15% solution via a membrane pump.

2.2. Water Quality Measurements

Total Organic Carbon (TOC), Adsorbable Organic Halo-

gens (AOX), UV absorbance at 254 nm and UV absorbance at 254 nm after filtration on a metallic filter at 0.45 µm (DUV) were measured in raw and treated water [29,41]. All these parameters indicate the total organic content that can react with disinfectants to produce potentially toxic and carcinogenic compounds.

2.3. *Allium cepa* Test

After root germination under controlled laboratory conditions (20˚C in mineral water) young bulbs of *Allium cepa* of equal size (2 - 2.5 cm in diameter) were exposed to disinfected and raw water for 24 and 72 hours at 10˚C, 20˚C and 30˚C. At the end of exposure roots were cut and fixed in 1:3 acetic acid-ethanol solution and stored in 70% ethanol. Clastogenic effects (chromatin bridges, fragments) and spindle disturbance (vagrants, c-mitosis, multipolar anaphases) were studied in root cells after Feulgen staining [42,43]. The mitotic index (MI) was also evaluated as a measure of cell division rate; MI values lower than 10/1000 was not considered as they are indicative of toxicity. At least 800 anaphase cells per experimental point (40 for each root, 20 slides) for anaphase aberrations and 5000 cells per experimental point (1000 for each root, 5 slides) for the mitotic index were analyzed. Root length was used as an index of toxicity, and modifications in root form (formation of tumours, hook roots, twisted roots) and root consistency were observed [44]. Statistical data analysis was performed by means of the χ^2 test. Raw and treated waters were compared to a negative control (mineral water stored in glass bottles). Maleic hydrazide (10 mg/L) was used as a positive control.

2.4. *Vicia faba* Micronucleus Test

The micronucleus test was performed in secondary root tips of *Vicia faba* [32] which have been exposed to raw and disinfected lake waters for 6 and 72 hours at 10˚C, 20˚C and 30˚C. After the initial 6-hour exposure to the disinfected waters, part of the seedlings were transferred in Hoagland's solution for the next 66 hours (recovery time) and maintained at the corresponding temperatures, according to the experimental protocol (10˚C, 20˚C and 30˚C). Hoagland's solution was also used for roots of the negative control group. After exposure, roots were fixed in 1:3 acetic acid/ethanol mixture and Feulgen stained. Root tips were cut and squashed onto microscope slides. Micronucleus frequency was studied in the proliferating tissue of each root, analysing 5×10^3 cells/root tip, 10 tips/experimental point of secondary roots (5×10^4 proliferating cells). This analysis was carried out after checking the mitotic index, which allowed the study of cell populations with comparable proliferation activity (data not shown). Statistical analysis of data was carried out through the Mann-Whitney test; pair-wise comparesons of micronucleus frequencies were carried out between root cells exposed to the differently-disinfected waters and either raw waters or Hoagland's solution. In addition, a comparison was made between data from 6-hour and 72-hour exposure to the same samples of water. Regression analyses were also performed between temperatures and micronucleus frequencies for each treatment and time of exposure. Maleic hydrazide (10 mg/L) was used as a positive control.

3. Results

3.1. Water Quality Measurements

The results of the physical and chemical analyses are set out in **Table 1**. A high concentration of TOC was observed in raw lake water (7.8 mg/L), similar to that for water disinfected with NaClO and ClO_2. The light increase registered in PAA treated water may be a conesquence of the carbon content of the compound itself. Organic carbon in water is composed of organic compounds in various oxidation states and TOC is the direct expression of total organic content. For disinfected waters, organic compounds may react with disinfectants to produce potentially toxic and carcinogenic compounds.

UV-absorbing organic constituents in a sample absorb UV light in proportion to their concentration. Samples are filtered to control variations in UV absorption caused by particles. UV_{254} absorbance is 0.074 abs/cm and 0.069 abs/cm in raw water and filtered raw water (DUV), respectively. The absorbance value of ClO_2-disinfected water was similar to that of raw water. NaClO- and

Table 1. Physical and chemical analyses of disinfected and raw lake water.

Parameters	Water samples			
	Raw water	ClO_2-treated	NaClO-treated	PAA-treated
$UV_{254\ nm}$ (abs/cm)	0.074	0.072	0.083	0.090
$DUV_{254\ nm}$ (abs/cm)	0.069	0.048	0.052	0.056
TOC (mg/L)	7.8	7.6	7.6	9.2
AOX (µg/L)	nd	22	166	16

nd = not detected.

PAA-treated waters showed higher values. Some organic compounds commonly found in surface water, such as lignin, tannin, humic and fulvic substances and various aromatic compounds, strongly absorb UV radiation; UV absorption is a useful surrogate measure of selected organic constituents in waters because a strong correlation may exist between UV absorption and organic carbon content and precursors of trihalomethanes and other disinfection by-products. The highest values of UV absorbance were found in NaClO and PAA disinfected waters suggesting these disinfectants induced DBPs. DUV values, *i.e*. measured in filtered water, were lower than UV ones in all samples, particularly in the treated waters suggesting the presence of compounds on particles in water that can absorbe UV. Analyses of detectable AOX concentrations were carried out only in disinfected samples. AOX is a measurement used to estimate the total quantity of dissolved halogenated organic material in a water sample. The presence of halogenated organic molecules is indicative of disinfection by-products. AOX concentration was significantly increased in NaClO-disinfected water (166 µg/L), whereas low values were found in ClO_2- and PAA-treated water (22 µg/L and 16 µg/L, respectively).

3.2. *Allium cepa* Test

The results of the *Allium cepa* root anaphase aberration test are set out in **Table 2** and in **Figure 1**.

The mutagenic potential was evaluated in proliferating cells in the meristematic tissue of Allium roots, analyzing both the chromosomal damage and the mitotic disturbance (*i.e*., clastogenic and aneugenic effects, respectively) induced in mitotic cells, through the chromosomal aberration test in anaphase, which are the first manifestation of an induced mutagenic damage and represent the "generating events" that give rise to micronucleus formation.

No data were obtained after long-term exposure at 10˚C because low temperature negatively influenced root growth. On the contrary, data obtained after the short-term exposure at the same temperature did not significantly differ from the raw water and negative control.

Table 2. Percent frequencies of anaphase aberrations (AA) and of mitotic index (MI) in *Allium cepa* root cells exposed to samples of raw and treated lake water at different temperatures for 24 or 72 hours; 800 anaphases (40 anaphases, 20 roots) and 5000 cells per experimental point were analyzed for AA and MI, respectively.

Exposure temperature	Water samples	Exposure time			
		24 hours		72 hours	
		AA (%)	MI (%)	AA (%)	MI (%)
10˚C	Negative control	2.8	9.2	nd	nd
	Raw water	2.7	8.0	nd	nd
	ClO_2-treated	3.5	9.7	nd	nd
	NaClO-treated	2.6	8.7	nd	nd
	PAA-treated	2.1	9.3	nd	nd
20˚C	Negative control	1.6	6.5	2.1	9.1
	Raw water	2.5	7.7	2.5	8.4
	ClO_2-treated	3.6**	7.7	4.7°**	8.2
	NaClO-treated	3.5*	7.4	4.3°*	8.2
	PAA-treated	2.7	8.2	3.7*	8.0
30˚C	Negative control	4.0	7.9	4.0	7.9
	Raw water	4.3	9.1	5.1	9.8
	ClO_2-treated	5.6	8.1	3.4	7.8
	NaClO-treated	3.5	9.5	5.4	8.3
	PAA-treated	5.6	8.9	1.7	8.9

nd = not detectable (necrotic tissue); °Statistically significant vs raw water according to χ^2 test ($p < 0.05$); *Statistically significant vs negative control according to χ^2 test ($p < 0.05$); **Statistically significant vs negative control according to χ^2 test ($p < 0.01$); Positive control: maleic hydrazide (10 mg/L) for 6 hours, AA 11.4 (%).

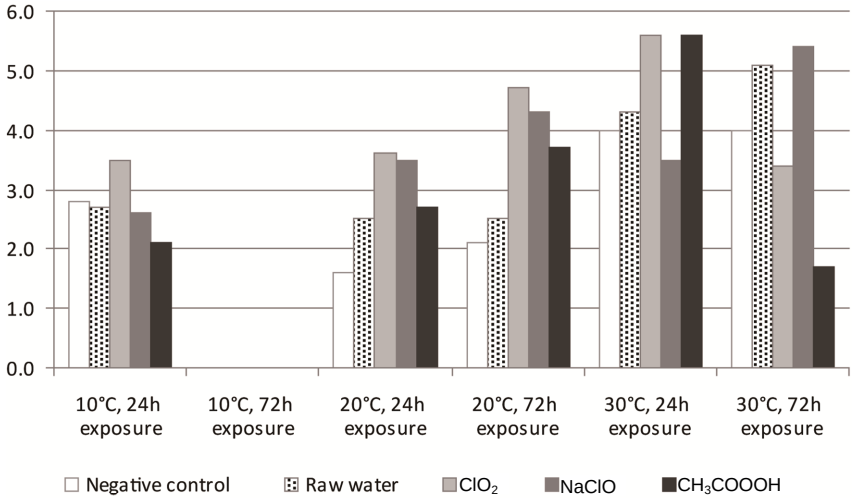

Figure 1. Percent frequencies of anaphase aberrations (AA) in *Allium cepa* root cells exposed to samples of raw and treated lake water at different temperatures for 24 or 72 hours.

ClO_2-, NaClO- and PAA-disinfected lake water showed mutagenic activity at 20˚C. This effect was detected for ClO_2- and NaClO-disinfected waters in comparison with the negative control treatment after exposures of 24 and 72 hours; such an effect was also shown in compareson to raw water after 72 hours of exposure. After 72 hours of exposure, PAA-disinfected lake water also showed a mutagenic effect in comparison to the negative control. The experiment carried out at 30˚C gave negative results for all the disinfectants and for all the exposure times.

3.3. *Vicia faba* Micronucleus Test

Unlike *Allium* roots, *Vicia faba* seedlings maintained at the temperature of 10˚C did not exhibit a considerable growth inhibition of secondary roots, even if a slight reduction of the mitotic indexes was observed in both control and treated root samples maintained at the low temperature for 72 hours.

The results of the micronucleus test in *Vicia faba* are set out in **Table 3** and visualized in **Figure 2**.

Significant increases of micronucleus frequencies appear to be induced by all disinfected-water under any experimental-exposure condition. Namely, NaClO- and ClO_2-disinfected lake waters showed a strong clastogenic/aneugenic activity at all the temperatures, whereas raw lake water showed no effect. This result is in agreement with the data from *in situ* exposure carried out in previous experiments at the disinfection treatment pilot plant [11]. In addition, the mutagenic effect increased with exposure time, mainly at 20˚C and 30˚C; a positive significant correlation between micronucleus frequentcies and temperature was also found after 72-hour exposure to NaClO- and ClO_2-disinfected lake waters (**Figure 3**).

4. Discussion

The present experimental design has been established taking into account the exposure conditions and positive response of the two plant test systems obtained from our previous *in situ* study, as well as their specific characteristics [11]. Namely, the temperatures were chosen so as to cover the whole range of those registered in the different seasons at which these plants had been exposed and responded, showing no growth inhibition of roots (data not shown). Different exposure protocols were established for the two plant systems: the 24 and 72 hours of continuous exposure were chosen for *Allium cepa*, on the basis of its relatively limited responsiveness observed after a short time exposure to disinfectants, especially if compared to that of *Vicia faba* which, on the contrary, showed consistent increases of micronucleus frequencies already after an exposure time of 6 hours. The short exposure times were long enough to hit the asynchronously proliferating cells in every phase of their cell cycle, so as to allow the possible induction of DNA damage (clastogenic mutagen) and/or impairment of mitotic process (aneugenic mutagen) in both test organisms. Also, the 72-hour fixation time was scheduled in order to allow a sufficiently large recovery time for the detection of possible S-dependent clastogenic effects, as well as to allow the occurrence of two or more mitotic rounds in order to detect micronuclei possibly deriving from mitotic disturbance.

Data from chemical analysis suggest the presence of DBP and their precursors in disinfected water, and in particular in NaClO-treated water where the AOX value is higher than water treated with other disinfectants.

The used bioindicators are sensitive to DBP but their response in terms of mutagenicity results to be modulated by the temperature of exposure.

Table 3. Mean frequency (±SE) of micronuclei/1000 cells in root tip cells of *Vicia faba* exposed to the same samples of raw and treated lake water at different temperatures for 6 hours (plus 66 h recovery) or 72 hours, evaluated in 10 root tips, 5000 cells per tip.

Exposure temperature	Disinfection treatments	MCN (‰)	
		Exposure time	
		6 hours	72 hours
10˚C	Negative control		0.22 ± 0.06
	Raw water	0.28 ± 0.05	0.26 ± 0.08
	ClO_2-treated	0.42 ± 0.09	0.34 ± 0.09
	NaClO-treated	0.58 ± 0.11°**	0.66 ± 0.09°°**
	PAA-treated	0.50 ± 0.16	0.34 ± 0.07
20˚C	Negative control		0.16 ± 0.05
	Raw water	0.28 ± 0.10	0.44 ± 0.11
	ClO_2-treated	0.26 ± 0.08	0.62 ± 0.14**^
	NaClO-treated	0.40 ± 0.09	1.22 ± 0.41***^
	PAA-treated	0.32 ± 0.06	1.14 ± 0.36**^
30˚C	Negative control		0.24 ± 0.08
	Raw water	0.50 ± 0.13	0.46 ± 0.12
	ClO_2-treated	0.56 ± 0.14	1.48 ± 0.27°°***^^
	NaClO-treated	0.76 ± 0.11**	1.90 ± 0.37°°***^^
	PAA-treated	0.46 ± 0.09	0.72 ± 0.16*

°Statistically significant vs raw water, with the same exposure time, according to Mann-Whitney Test ($p < 0.05$); °°Statistically significant vs raw water, with the same exposure time, according to Mann-Whitney Test ($p < 0.01$); *Statistically significant vs negative control according to Mann-Whitney Test ($p < 0.05$); **Statistically significant vs negative control according to Mann-Whitney Test ($p < 0.01$); ***Statistically significant vs negative control according to Mann-Whitney Test ($p < 0.001$); ^Statistically significant vs the same treatment, 6-hour exposure, according to Mann-Whitney Test ($p < 0.05$); ^^Statistically significant vs the same treatment, 6-hour exposure, according to Mann-Whitney Test ($p < 0.01$); Positive control: maleic hydrazide (10 mg/L) for 6 hours, 31.7 ± 11.5 (‰).

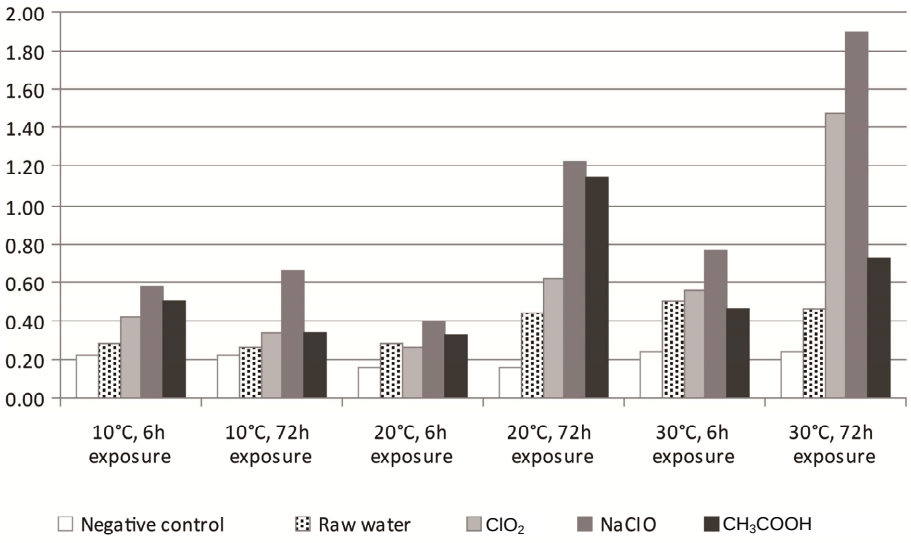

Figure 2. Graphical representation of mean micronucleus (MCN) frequency (‰) data from *Vicia faba* root tip cells (see Table 3) exposed to disinfected lake-water at different temperatures (10˚C, 20˚C and 30˚C) for either 6 h plus a 66 h recovery time (6 h exposure) or 72 h (72 h exposure).

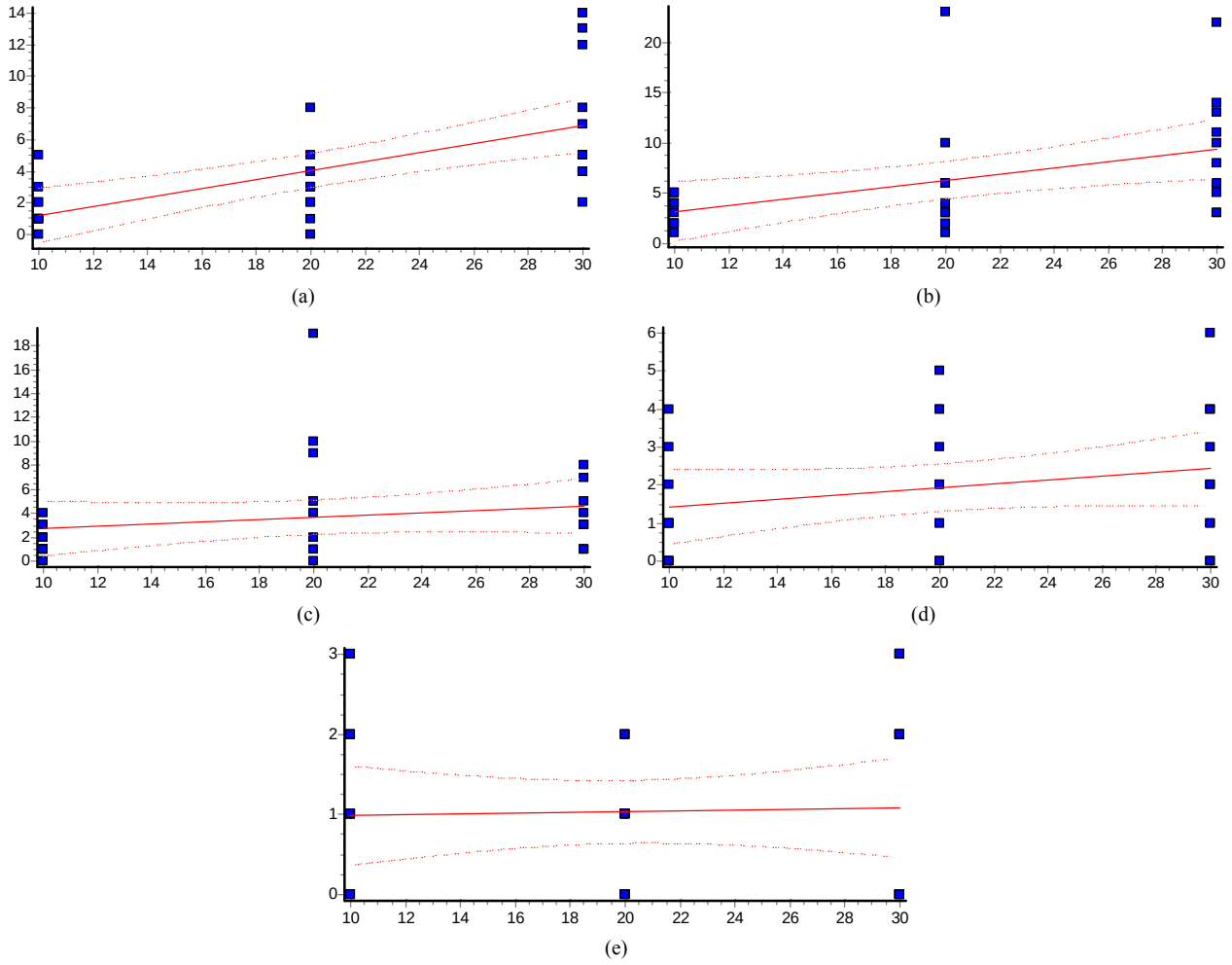

Figure 3. Regression analysis curves between temperature values (˚C, abscissa) and micronucleus frequencies (ordinate) obtained from 72 h exposure of secondary root tips of *Vicia faba* to samples of disinfected lake water (a)-(c), raw water (d) and negative control water (e). (a) ClO_2; (b) NaClO; (c) PAA; (d) Lake raw water; (e) Hoagland's solution.

The results of this study indicate that temperature may play an important role in mitotic cell cycle progression, at least in *Allium cepa* and *Vicia faba* root cells, and in modulating the expression of clastogenic/aneugenic damage.

Despite the positive results obtained in *Allium cepa* from the field study carried out at the Trasimeno water-treatment plant, where roots not only survived the 72-hour exposure at temperature as low as 10˚C, but also gave the most powerful mutagenicity data, in the present experiment the same temperature showed to be not permissive for root growth.

Indeed, mutagenic effects were only observed at 20˚C, which shows to be the optimal temperature for cell proliferation of this plant roots. In this organism, mitotic cycle duration has been determined by several authors, who showed that the optimal temperature for root growth is 20˚C ± 2˚C; under such conditions mitotic cycle duration is about 24 hours [42]. The observed variations reported in the present study could largely be explained by the different exposure temperatures, which appear to influence root growth [45].

Treatments of *Allium cepa* with NaClO- and ClO_2-disinfected lake waters performed at 20˚C induced a clear aneugenic/clastogenic effect, detectable at both exposure times. This effect seemed to be more pronounced at the long-term exposure (72 hours), at which PAA-disinfected lake water also appears to exert a mutagenic effect. Short-term exposure in the *Allium cepa* test gave generally negative results for all disinfectants, although NaClO- and ClO_2-disinfected lake water produced mutagenic effects compared to the negative control. On the other hand, when the exposure time was increased (72 hours, about two mitotic cycles), mutagenic effects were detected for the NaClO- and ClO_2-disinfected lake water compared to raw water.

Low temperature (10˚C) is likely to cause a delay in cell proliferation in *Allium cepa* roots, which may ex-

plain the long-term exposure inhibition of root germination and growth. At the same temperature, short-term exposure produced no genotoxic effects since mitosis rate was reduced, and possibly aberration frequency as well.

The highest temperature (30˚C) did not cause any remarkable mutagenic effects, despite the increase in frequency in some samples. The negative control also gave a high frequency of aberrations at the highest temperature. High temperature is likely to impair root proliferating cells, inducing a sort of stress that may be the cause of most mutagenic damage. In this study the *Allium cepa* test was less sensitive to mutagenic effects at high temperature conditions, which is in agreement with the data reported in a previous study on *in situ* exposure in June, which corresponded to a very warm period [11]. In *Allium cepa* the highest temperature did not seem to influence the mitotic index, and hence the cell cycle was not influenced. This means that cell division rate was not changed, but root length was on average shorter than the negative control. Moreover, other signs of toxicity were observed in Allium roots (form and consistency of roots) and the toxicity may have concealed the genotoxic effects.

In *Vicia faba* temperature also plays an important role in modulating the expression of clastogenic/aneugenic damage. Low temperature may slow down the progressive reduction in micronucleus frequency after short-term treatments, which is expected from their dilution/destruction [32,46], as a consequence of the lengthening of the cell cycle. This could explain why 72-hour exposure produced higher clastogenic/aneugenic effects than 6-hour exposure at a higher temperature (**Figure 2**). Increasing clastogenic/aneugenic activity of NaClO- and ClO_2-disinfected lake waters at increasing temperatures is suggested by the increase in micronucleus frequencies after 72 hours of exposure. Equilibrium value of micronucleus frequency is reached after long-term treatments (72 hours) for the rise of new micronuclei and the dilution/destruction of the old ones [32,46]. An increase in the equilibrium frequency of micronuclei therefore suggests a higher rate of new micronucleus formation. A comparison of these findings with those from *in situ* exposure [11] confirmed that NaClO- and ClO_2-disinfected lake waters have stronger clastogenic/aneugenic effects than PAA-disinfected waters; the lack of a difference in micronucleus frequency after 6-hour and 72-hour exposure in the cold season is also confirmed. The hypothesis that low temperatures slow down micronucleus dilution/destruction with time relenting cell cycle progression is therefore supported.

The importance of cell cycle duration in evaluating genotoxic damage has been seen for other genotoxicity plant tests as well. In the *Tradescantia*/micronuclei test, exposure to some toxic compounds or to overdoses causes a delay in the meiotic cycle, and the induced damage may be not recognized using the standard time of test protocol [47].

The temperature affecting cell cycle duration may influence sensitivity and the possibility of revealing low levels of environmental genotoxins. The results of this study show that temperature plays an important role in mitotic cell cycle progression in *Allium cepa* and *Vicia faba* root tips. *Vicia faba* micronucleus test proved to be more sensitive than *Allium cepa* chromosomal aberration test with regard to temperature influence on genotoxic damage expression. The different temperatures at which the plant tests were carried out could have impaired the plausibility of the experimental data obtained.

In conclusion, temperature is an important variable to be taken into account when planning *in situ* exposure of plants for mutagenicity tests. This poses the question of selecting appropriate test organisms, taking into account their tolerance and viability under relatively wide thermal ranges among environmental variables.

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