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Interference of tributyltin(IV)chloride on the vascular plant cells

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In the recent past years, interest in the environmental impact of organotin(IV) compounds has risen markedly. Considering that most data in the scientific literature refers to the animal kingdom and not to higher plants cells, we tested the effects of organotin compounds on vascular plant cells, by studying the interaction of tributyltin chloride (TBTCl) with *Allium cepa*, *Solanun tuberosum* and *Solanum melongena*, vascular plants that are directly involved in the human food chain. The TBTCl effects on mitotic metaphase plates, on pollen grains and on both microtubers and adult tuber parenchymatic cells were investigated and the concentrations of TBTCl inside the treated parenchymatic cells were determined through ICP-mass spectrometry. Oxygen and chlorophyll productions were also determined. The obtained results showed that TBTCl influenced not only morphology, but also the physiology of the vegetable cells since, despite the low concentrations used, the stress the cells were submitted to was experimentally confirmed. Moreover, the increase in the tin concentration in the cells, with increasing incubation time, showed that TBTCl possesses a very high capacity to be bioaccumulated and, as a consequence, it is able to enter the food chain. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: TBT; *Allium cepa*; *Solanun tuberosum*; *Solanum melongena*; oxygen; chlorophyll

INTRODUCTION

Organotin compounds are strong contaminants in the environment.[1,2] They have been used in industrial and agricultural applications as plastic stabilizers and catalysts, antifouling paints, molluscicides, fungicides, disinfectants, etc.[3] The toxicity spectrum of organotin compounds is very broad, and they may provoke immunotoxicity, hepatotoxicity, teratogenicity and neurotoxicity in animals and humans.[4–6]

The tributyltin chloride (Bu_3SnCl = TBTCl) is a membrane active molecule[7,8] and its action mechanism appears to be dependent on organotin lipophilic behavior.[9] It possess the ability to bind proteins, to act as an ionophore and to produce haemolysis, alteration of phosphatidylserine and histamine levels, and perturbation of membrane enzymes. Oedema formation may be caused by TBT accumulation, or by an increase of water percentage in the brain and in spinal cord; the normal functioning of Golgi apparatus and endoplasmic reticulum may be influenced,[10] together with an alteration of mitochondrial membrane permeability, which may occur in several ways, such as:

- interaction with membranes, causing intumescences and destruction;
- minor effects deriving from their action as ionophores;
- capacity to inhibit basic energy conservation processes involved in the ATP synthesis, while in chloroplasts oxidative phosphorylation is inhibited.[3,11]

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Tributyltin chloride (TBTCl) and tributyltin hydride (TBTH) effects on the willow tree have been investigated, at pH 4 and at pH 7, using transpiration test. The results showed symptoms of poor health at low pH (= 4) with transpiration decreasing at concentrations above or equal to 0.1 mg TBTCl/l and 1 mg TBTH/l. TBT toxicity increased at pH 7. At concentrations as high as 10 mg/l TBTCl or TBTH, the trees survived, but their growth and transpiration was strongly reduced.[12]

It is apparent that the use of the organotin(IV) compounds may influence not only vital functions, but even organoleptic and nutritive characteristics of vegetable organisms. They may be influenced by the use of organotin(IV) compounds, either by sprinkling or by simply contact.

MATERIALS AND METHODS

Materials

Samples of vegetable material were treated with TBTCl solutions at different concentrations (10^{-5}, 10^{-6}, 10^{-7} M), for incubation periods of 24, 48 and 168 h. *Allium cepa*, *Solanun tuberosum* and *Solanum melongena* were used because these vegetable materials are easy to find, inexpensive and furthermore give optimal answers in the laboratory in short times.

Allium cepa was used to observe interferences of TBTCl on metaphase plates,[13] because it is able to emit apexes in a short time, and it has large and regular cells in optimal chromosomal conditions (a low number of large chromosomes, $2n = 16$), which makes it easy to observe chromosomic anomalies during mitosis.[14]

Solanum tuberosum was chosen for the ease of obtaining parenchymatic cell disks from tubers, calculating the TBTCl concentration in parenchymatic disks, calculating oxygen production, calculating the chlorophyll contents, and treating microtubes obtained from *in vitro* culture.

Solanum melongena was used for pollen analyses. For the *in vitro* culture a solid medium MS (Murashige and Skoog) was used,[14,15] both charged and not charged with vegetable hormones such as NAA (naphthalenacetic acid) and BAP (benzylaminopurine).

In order to use vegetable material of reliable origin, everything was planted in non-chemically treated ground, without any pesticide, to avoid the interference of Bu_3SnCl and other chemicals.

Methods

TBTCl solutions 10^{-5}, 10^{-6} and 10^{-7} M were obtained by diluting a 10^{-4} M solution, containing 1% (v/v) of DMSO. Furthermore, in order to verify the possible influence of DMSO, a 1% solution of DMSO was also prepared as control.

Allium cepa: metaphasic plates

For every investigation 15 samples were used. The basal part of each sample was placed in solution. Several elements were placed in DMSO solution, several in distilled H_2O, and finally several others in Bu_3SnCl solutions at different concentrations (10^{-5}, 10^{-6} and 10^{-7} M), with incubation periods of 24, 48 and 168 h.[16,17]

In particular, the karyologic analysis of *Allium cepa* apexes was carried out using the Fulgen method. As a consequence they were immersed in a colchicine aqueous solution (0.3 g/10 ml) and after 3 h the apexes were rinsed repeatedly with distilled water, followed by immersion fixation with Carnoy solution (ethanol : acetic acid, 6 : 1) for about 30 min and finally dipped in 1 M HCl solution. The apexes were then immersed for 3 min in distilled water at 60 °C to remove HCl and then basic fuchsin added to colour the chromatine. After exposition to sunlight, the apexes, which appeared coloured, were rinsed several times with distilled water, crushed between slides and finally observed under a Reichert-Jung Microstar 110 microscope.

Solanum tuberosum: parenchymatic disk

Parenchymatic disks of about 1 mm diameter were obtained from adult peeled and washed potato tubers with a bored cork; five parenchymatic disks were placed in flasks. For each single investigation, 30 flasks containing five parenchymatic disks each, whose total weight was 1.5 g, were used, of which six flasks contained only distilled water, six flasks contained 1% DMSO solution, six flasks contained 10^{-5} M, six 10^{-6} M and six 10^{-7} M Bu_3SnCl solutions.

The cotton stoppered 250 ml flasks were shaken at 25 °C, in a rotary shaker, in order to keep the disks gently moving.[18] Samples were dipped in $CaSO_4$–containing solutions, which prevented the collapse of the vegetable cell as a consequence of osmotic processes. At the end of the incubation period, samples were mineralized and tin concentration values were determined using an Agilent ICP-MS mod. 4500 instruments, equipped with a CETAC ASX500 autosampler.

Starch grains

Several samples, treated using the previously described method, were not mineralized at the end of the estimated incubation periods, but were included in resin and dissected through microtome, obtaining 4–5 μm sections. The occurrence of cell lysis, and the consequent formation of starch grains, has been evidenced by adding Lugol dye to a drop of the obtained milky suspension. The appearance of blue-violet staining and observation through the microscope confirmed the presence of starch grains.

Respiration of tissue sections

Oxygen production of several samples, at the end of the estimated incubation times, was determined[19,20] using a portable oxygen electrode (Hanna Instruments, mod. HI9142), equipped with a drill, which allowed the desired sizes to be obtained without changing the permanent environment for the same samples.

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Chlorophyll content

From plants grown in a pot, branches 20–25 cm in height were resected and put in 100 ml beakers containing distilled H_2O, or 1% DMSO solution or 10^{-5}, 10^{-6} or 10^{-7} M Bu_3SnCl solutions. After the estimated incubation times, several leaves of 0.5 g medium weight were withdrawn and then the chlorophyll content was extracted using the Arnon method and its content determined at 652 nm, using a Beckman Coulter, model DU800, spectrophotometer.

Microtubers

Several sterilized buds were explanted from a mother tuber and soaked in 0.03 mM gibberellic acid (GA) in order to break their dormancy and planted on the surface of sterile moist vermiculite in a growth chamber. Shoot tips were harvested when they reached 3–5 cm in length.[21,22]

After about 30 days, when seedlings had developed from the buds, meristems domes were dissected out with one adjacent primordium,[23–26] cultured on the surface of MS agar medium and finally incubated under a 12 h photoperiod at 25 °C, for 30 days. At the end of this time the shootlets were transferred to the surface of MS agar medium containing 0.05 μM NAA in a 250 ml flask, where the axillary shoot was fixed horizontally on the surface of medium, promoting additional axillary branching.[27–29]

When they reached a height of 10 cm, the axillary shoots (with adventitious root) were transferred to soil using the procedures previously described and then to MS agar medium containing 22–44 μm BA and 0.05 μM NAA in a 500 ml flask.[30,31] Thirty to fifty miniature tubers can be harvested at the end of 4 months and stored under aseptic conditions.[32,33] Once microtubers of about 0.8 g of weight were obtained, they were put into a soil–vermiculite mixture, which allowed a humid environment to be maintained and therefore allowed normal growth of the samples, some of which were sprinkled with test solutions (distilled water and DMSO) and others with Bu_3SnCl solutions at different concentrations (10^{-5}, 10^{-6} and 10^{-7} M) and at different incubation times. Finally, after the treatment, microtubers were weighed once again and then dissected, obtaining sections.

Polyphenols

Polyphenols are an expression of the stress which plants may undergo, so that, in order to verify if the treatment with Bu_3SnCl solutions causes stress inside the parenchymatic cells, the cryosections were coloured with Fast Blue BB (4′-Amino-2′, 5′-diethoxybenzanilide) to verify the presence of polyphenols. Sections were finally observed and photographed with polarized light under the Ortoplan microscope.

Solanum melongena: Pollen grains

The TBTCl solution effects on *Solanum melongena* pollen grains were analysed by the germinability test with the 'falling drop' method. Pollen grains were put on a liquid substrate into Van Tieghem's chambers, where the basic solutions were mixed with the 1% DMSO and Bu_3SnCl solutions at different concentrations (10^{-5}, 10^{-6} and 10^{-7} M), at 4th day incubation time, which is the time necessary for the pollen tubes to grow.

RESULTS AND DISCUSSION

The effects of TBTCl solutions on vegetable material were analysed from both a macroscopic and a microscopic point of view. The vegetative apexes of *Allium cepa* developed quickly and in a normal way in both distilled water and 1% DMSO solution, normal in colour and consistency with regular metaphasic plates, as evidenced from karyologic analysis (Figs 1 and 2). The bulbs directly treated with 10^{-5} M Bu_3SnCl solution developed very slowly, generating dark-colored, hardened and lignified apexes, with totally aborted meristem cells.

In 10^{-5} M TBTCl solutions, apexes, previously grown in distilled water, started to decrease their growth velocity and to become thinner and darker after 48 h. The observations at the microscope showed a high number of irregular plates, and in particular the following anomalies were evident: chromosome fragmentation, breakdown of several elements in the same metaphasic plate, chromosome bridges, different levels of chromatin condensation, absence of mitotic spindle and increase of permeability in tissues.

Analogous anomalies were seen in 10^{-6} and 10^{-7} M TBTCl solutions, with the exception of the irregular plate number, which decreased along with the TBTCl concentration. Similarly, these observed results, as breakages and chromosome bridging, are comparable with the chromosomal anomalies described by Vitturi *et al*.[34] on *Truncatella subcylindrica* (Mollusca), following exposure to TBTCl. The structural alterations of the chromosomes and/or

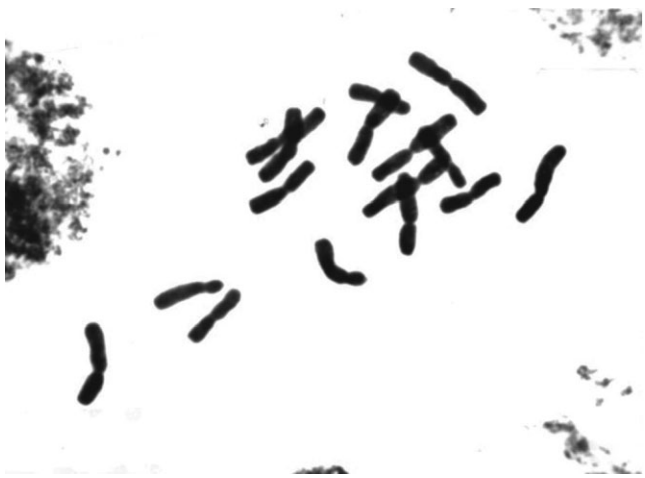

Figure 1. Metaphase chromosomes in apex controls of *Allium cepa* (magnification ×1000).

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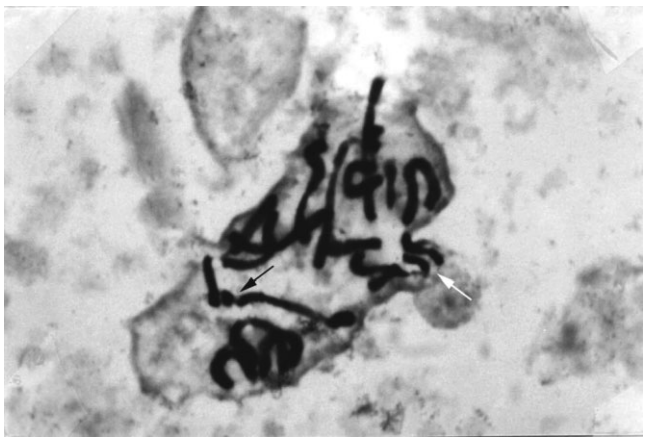

Figure 2. Anomalous metaphase chromosomes in apexes of *Allium cepa* treated with 10^{-7} M of TBTCl solution after 168 h (black arrow indicates a bridging and white arrow indicates a breakage; magnification ×1000).

inhibition of mitotic spindle are provoked presumably through modification of the chemical structure of the citoskeleton. As reported by Cima *et al.*,[35] TBTCl altered the intracellular calcium homoeostasis through inhibition of the membrane Ca^{2+}-ATPase activity, which first causes internal disassembly of cytoskeletal proteins and consequently inhibition of mitosis.

Parenchymatic disks became darker with the decreasing incubation period when placed both in distilled water and in 1% DMSO solution, increasing their weight, which reached the higher values after 24 h.

Samples treated with 10^{-5} M TBTCl solutions did not darken, showing an evident loss of consistency and weight; the increase of the incubation period provoked an increase of the consistency loss and a decrease of their weight. The same results have been obtained in samples treated with 10^{-6} and 10^{-7} M TBTCl solutions. In all the cases, the tin content into the parenchymatic disks increased with the increase of the solution concentration and the increase of the incubation period (Fig. 3). Upon treatment with TBTCl solutions, the grain cells released starch, as evidenced from the milky aspect of the solution, the black-violet color assumed after addition of the Lugol reagent, and from observation of the sections at the light microscope. Such a release increased with increasing the incubation time and the concentration of TBTCl solutions. In the samples treated with 10^{-5} M TBTCl solutions, several cells were empty, in contrast with distilled water and 1% DMSO solutions. However, the decrease of the starch grains number is not attributable to a possible death of the cells of the treated samples, as confirmed by the oxygen production observed through analysis carried out with an oxymeter and the chlorophyll content. Both oxygen and chlorophyll, produced by the cells, decreased with the increase in the TBTCl solution concentration and the increase of the incubation period (Figs 4 and 5). The inhibitive effect of organotin compounds on growth, respiration rate and chlorophyll content of the green freshwater alga *Scenedesmus quadricauda* have been demonstrated by Fargašová.[36] In agreement with our results, seagrass *Ruppia maritima*, was negatively affected by TBTCl, with the most severe effects seen in net photosynthetic activity, with reductions up to 60% in activity, in comparison to reference plants. Respiration and growth were also reduced by exposing plants to TBTCl.[37]

The microtubers grew normally both in distilled water and in 1% DMSO and their apexes developed regularly; after a 48 h treatment it was possible to observe buds. The

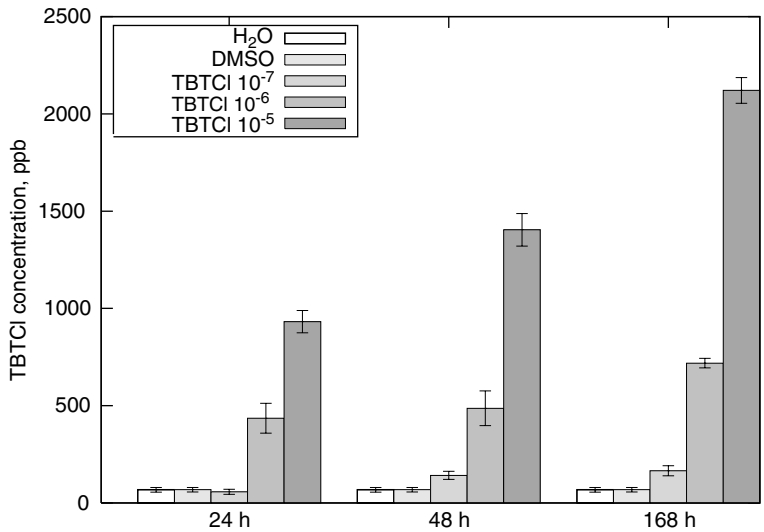

Figure 3. TBTCl concentration in *Solanum tuberosum* parenchymatic disk. Values represent the mean ± standard deviation (SD) obtained in four separate determinations.[38]

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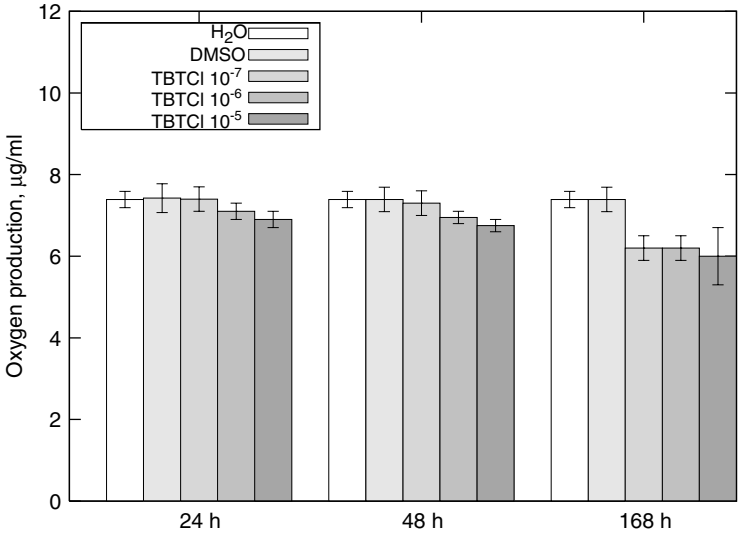

Figure 4. Oxygen production in *Solanum tuberosum* sample. Values represent the mean ± SD obtained in four separate determinations.[38]

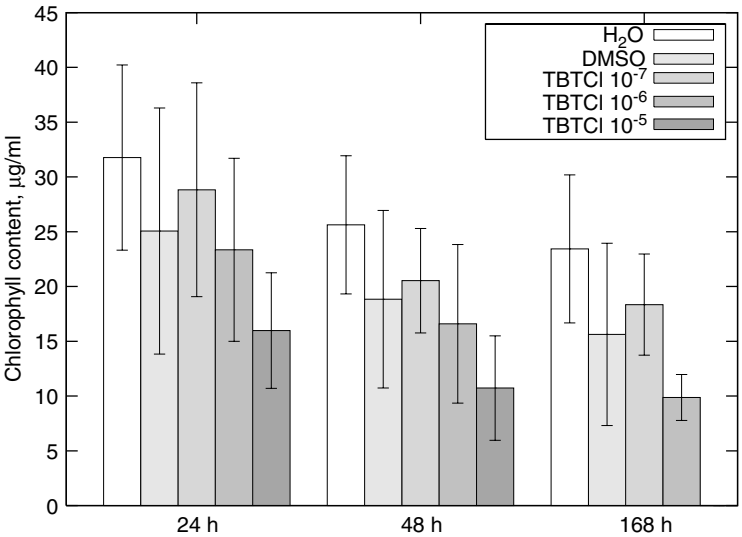

Figure 5. The chlorophyll content in *Solanum tuberosum* sample. Values represent the mean ± SD obtained in four separate determinations.[38]

microtubers treated with 10^{-5} M TBTCl solutions, even if vegetative apexes and buds were present, grew slower. After a 7 day treatment, the samples showed partial rottenness, which did not cause their death, since they still produced buds. The parenchyma looked almost vitreous and the colour of the suberin, outside the layer, was darker than usual. It was evident that the vitreous aspect of the cells was due to the decrease in starch grain number at the increased the TBTCl solution concentration, while the suberized walls were a consequence of permeability increase.

As far as the samples treated with 10^{-6} and 10^{-7} M TBTCl solutions are concerned, the only difference in behavior observed was that the number of apexes and buds increased with the decrease of the TBTCl concentration.

The hyperproduction of polyphenols near the suberin layers was confirmed by dark-red colored layers appearing in the cryosection with the use of Fast Blue; these layers were more evident with increased the TBTCl concentration, which caused increasing stress inside the cells (Figs 6 and 7). The production of polyphenols in the tuber surface layers constitutes a chemical barrier which prevents the entry of foreign matter into the parenchymatic cells. Nevertheless, the increase in tin content inside the cells with the increase in TBTCl concentration demonstrated that the permeability

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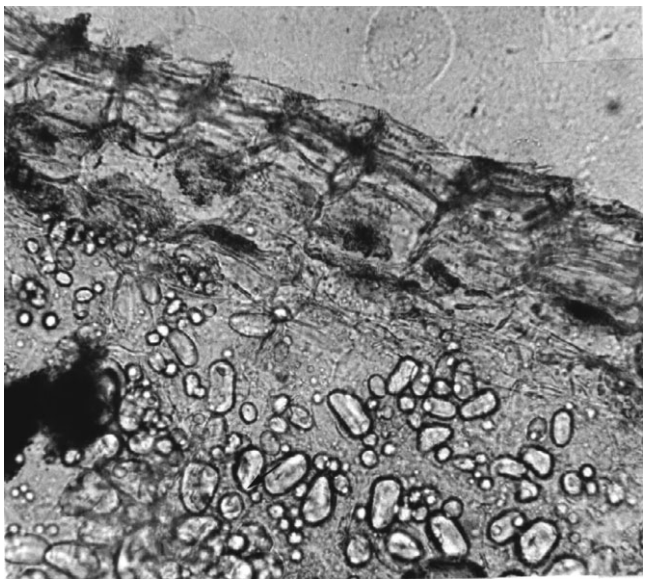

Figure 6. Cryosections of microtuber of *Solanum tuberosum* treated with distilled water and coloured with Fast Blue. The arrow indicates the presence of polyphenols (magnification ×250).

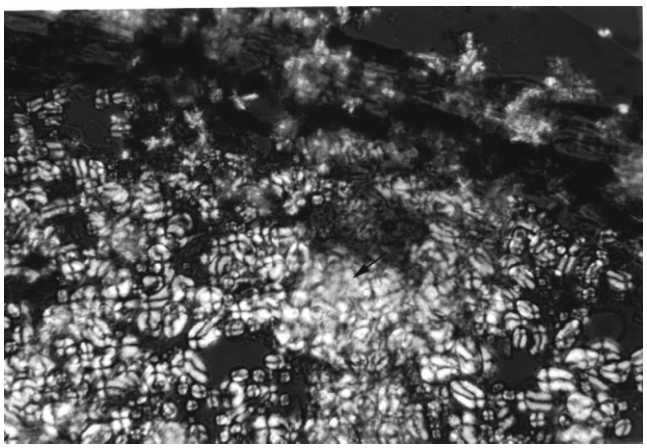

Figure 7. Cryosections of microtuber of *Solanum tuberosum* treated with TBTCl 10^{-7} M solution after 168 h and coloured with Fast Blue. Arrow indicates hyperproduction of polyphenols (magnification ×250).

of the cell increased despite the increased production of polyphenols.

Finally, while the *Solanum melongena* pollen grains developed a high number of pollen tubes both in distilled water and in 1% DMSO, the grains treated in 10^{-5} and 10^{-6} M TBTCl solutions did not form pollen tubes, which were in a very low number in the 10^{-7} M solution.

In conclusion, it is evident that TBTCl influenced not only the morphology, but even physiology of the vegetable cells since, despite the low concentrations used, the stress to which the cells were submitted was experimentally confirmed. Moreover, the increase in tin concentration in the cells at increasing incubation periods demonstrated the TBTCl bioaccumulating capacity and as a consequence the ability of TBTCl to enter the food chain.

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The authors mourn the death of Professor Enrico Bellini, a serious science man.

REFERENCES

1. Attar KM. *Appl. Organometal. Chem.* 1996; **5**: 317.
2. Harbone JB. *Introduction to Ecological Biochemistry*. Academic Press: London, 1997.
3. Evans CJ. Industrial uses of tin chemicals. In *Chemistry of Tin*, 2nd edn, Smith PJ (ed.). Blackie Academic & Professional: Glasgow, 1998; 442–479.
4. Arakawa Y. Biological Actions of di- and trialkyltin compounds. In *Chemistry of Tin*, 2nd edn, Smith PJ (ed.). Blackie Academic & Professional: Glasgow, 1998; 388–428.
5. Smith PJ. Health and safety aspects of tin chemicals. In *Chemistry of Tin*, 2nd edn, Smith PJ (ed.). Blackie Academic & Professional: Glasgow, 1998; 429–441.
6. Cima F, Craig PJ, Harrington CF. Organotin compounds in the environment. In *Organometallic Compounds in the Environment*, 2nd edn, Craig PJ (ed.). Wiley: Chichester, 2003; 101–149.
7. Ambrosini A, Bertoli E, Zolese G. *Appl. Organometal. Chem.* 1996; **10**: 53.
8. Chicano JJ, Ortis A, Teruel JA, Aranda FJ. *Biochim. Biophys. Acta* 2001; **1510**: 330.
9. Kuczera J, Gabrielska J, Kral TE, Przstalski S. *Appl. Organometal. Chem.* 1997; **11**: 591.
10. Davies AG, Smith PG. Tin. In *Comprehensive Organometallic Chemistry*, Wilkinson G, Gordon F, Stone FGA, Abel EW (eds). Pergamon Press: Oxford, 1982; LVol.II, 519–627.
11. Newman WP. *The Organic Chemistry of Tin*. Wiley: Chichester, 1982.
12. Trapp S, Ciucani G, Sismilich M. *Environ. Sci. Pollut. R.* 2004; **11**: 327.
13. Fukui K, Nakayama S (eds). *Plant Chromosomes. Laboratory Methods*. CRC Press: Boca Raton, FL, 1996.
14. Erickson SM, Hillman WS. *Can. J. Genet. Cytol.* 1976; **18**: 189.
15. Murashige TV. *Ann. Rev. Plant Physiol.* 1974; **25**: 135.
16. Skoog F, Miller CO. *Symp. Soc. Exp. Biol.* 1957; **11**: 118.
17. Fiskejo G. The Allium test—a apotential standard of assessment of enviromental toxicity. In *Enviromental Toxicology and Risk Assessment*, Gorsuch JW, Dwyer FW, Inghersoll CG, La Point TW (eds), Vol. 2. ASTM STP 1216. American Society for Testing and Materials: Philadelphia, PA, 1993; 331–345.
18. Liu D, Jiang W, Wang W, Zhai L. *Israel J. Plant Sci.* 1995; **43**: 125.
19. Baker CJ, Mock NM, Deahl K, Domek J. *Plant Cell Tiss. Org.* 1997; **51**: 111.
20. Laties GG. The respiration of plant storage organ tissue sections. In *Experimental Plant Physiology*, San Pietro A (ed.). Mosby: Saint Louis, MO, 1974; 118–124.
21. Dixon RA. *Plant Cell Culture*. IRL Press: Oxford, 1985.
22. Reinart J, Yeoman MM. *Plant Cell and Tissue Culture: a Laboratory Manual*. Springer: Berlin, 1982; 4–17.
23. Hoque MI, Mila NB, Sharker RH. *Bangladesh J. Botany* 1996; **25**: 87.
24. Levy D, Searbrook JEA, Colman S. *J. Exp. Bot.* 1993; **44**: 381.
25. Tisserat B. *Plant Cell Culture; a Practical Approach*, Dixon RA (ed.). Oxford University Press: Oxford, 1984.

DOI: 10.1002/aoc

26. Tovar P, Estrada R, Schilde-Rentscheler L, Dodds JH. *CIP Circular* 1985; **13**(4): 1.
27. Djurdjina R, Milinkovic M, Milosevic D. *Acta Hort.* 1997; **462**: 959.
28. Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds). *Handbook of Plant Cell Culture, Techniques for Propagation and Breeding*, Vols 1–6. Macmillan: New York, 1983; 1–970.
29. George EF, Sherrington PD. *Plant Propagation by Tissue Culture.* Eastern Press: Eversley, 1984; 29–38.
30. Vreugdenhil D, Boogaard Y, Visser RGF, de Bruijn SM. *Plant Cell Tiss. Org.* 1998; **53**: 197.
31. Wang PJ, Hu C. *Potato Tissue Culture and its Applications in Agricolture. Potato Physiology*. Academic Press: New York, 1985; 504–599.
32. Debergh PC, Zimmerman RH. *Micropropagation Technology and Application*. Kluwer Academic: Dordrecht, 1991; 363–388.
33. Ewing EE. Induction of tuberization in potato. In *The Molecular and Cellular Biology of the Potato*, Vayda ME, Park WD (eds). CAB International: Wallingford, 1990; 25–41.
34. Vitturi R, Mansueto C, Catalano E, Pellerito L, Girasolo MA. *Appl. Organometal. Chem.* 1992; **6**: 525.
35. Cima F, Ballarin L, Bressa G, Burighel P. *Ecotoxicol. Environ. Saf.* 1998; **40**: 160.
36. Fargašová A. *Ecotoxicol. Environ. Saf.* 1997; **37**: 193.
37. Jensen HF, Holmer M, Dahllöf I. *Mar. Pollut. Bull.* 2004; **49**: 564.
38. Kenney JF, Keeping ES. The standard deviation and calculation of the standard deviation. In *Mathematics of Statistics, Pt 1*, 3rd edn. Van Nostrand: Princeton, NJ, 1962; 77–80.

DOI: 10.1002/aoc