adnate, golden yellow; quickly cyanescent; pores angular, concolorous, cyanescent; spores olive brown. ellipsoid, weakly striate, guttulate, 14–16×5.5–7 μm; basidia pyriform; cystidia fairly common on the tube surface and edge, hyaline, ventricose, thin walled, 45–90×12–17 μm; hyphae from pileal surface 5–10 μm broad with brown contents.

*Chemical reactions*: KOH–context light pink; $NH_4OH$–context light pinkish brown; $FeCl_3$–no reaction; $H_2SO_4$–context yellow brown; HCl–context light pink; $FeSO_4$–context light bluish green, becoming gelatinous; Melzer's reagent–context dark brown.

*Habit and habitat*: Solitary to gregarious, collected from Nalaina, Nainital, N 421, 22 July, 1980 under mixed forests.

3. *Tylopilus indecisus* (Peck) Murr., Mycologia **1**:15. 1909.

Pileus 5–15 cm broad, convex to plano-convex becoming shallowly depressed, surface moist to dry; margin entire, dull with age; context 1.5–3 cm, white near the cuticle, tan elsewhere, changing slowly to light brown on bruising; stipe 7–10×2–4.5 cm, subclavate, distinctly reticulated, solid, pinkish-buff to cinnamon; flesh white changing to tan on exposure; tubes 0.5–1 cm long, depressed to decurrent, pink to pale to dark; pores angular, concolourous with the tubes, changing to brown on bruising; spores flesh coloured, thin-walled, smooth, cylindric to ellipsoid, 10–13×3–5 μm basidia hyaline clavate 16–22×5–7 μm hymenial cystidia inconspicuous, rare, clavate, somewhat incrusted, 18–26×6–10 μm; hyphae from pileal surface 5–8 μm broad.

*Chemical reactions*: KOH–cuticle black; $NH_4OH$–cuticle red; HCl–cuticle yellow brown; $FeSO_4$–cuticle black, stipe and context grey.

*Habit and habitat*: Scattered on soil under oak forest, collected from Chaubatia Garden, Ranikhet, Almora, N 479, 2, August 1980.

The authors are thankful to Professor B. S. Mehrotra, Botany Department, D.S.B.C. College, Nainital for facilities.

4 September 1982; Revised 29 November 1982

## CELLULAR DAMAGE INDUCED BY FOOD PROCESSING INDUSTRY WASTE WATERS IN *ALLIUM CEPA*

R. K. SOMASHEKAR AND M. T. G. GOWDA
P. G. Department of Botany, University of Mysore, Mysore-560 006, India.

CELLULAR damage caused by waste waters of industrial origin are not very well understood. Mudd and Kozlowski[1] have shown that industrial effluents can induce cellular damage among plant and animal cells. Fishbein[2] who supported this view opined that industrial effluents are responsible for genetic damage among plants and animals. Cellular damage caused by waste waters of industrial origin have been worked out in India[3-5]. However, such studies have been limited to the actual damage caused and no reports are available on the factors responsible for inducing such changes. The present communication is a report on the somatic cell abnormalities induced by food processing industry waste waters in the case of *Allium cepa*.

The industry is located about 4.5 km away from Mysore City, Karnataka. It generates about 50,000 litres of waste water per day. The effluens run in the form of a stream for about 1.5 km and join one of the nearby sewage channels. Effluent samples were collected at 5 different sampling points from January 1979 to December 1980, and analysed using standard methods[6]. The combined effluents were used to study the cellular damage. Fast growing onion root tip (*Allium cepa*) cells were treated for 6, 24 and 48 hr with 25, 50, 75 and 100% effluent samples and recovery experiments were carried out. Control was maintained in all the cases. The control as well as treated root tips were fixed in acetic-alcohol for 24 hr and preserved in 70% alcohol. Root tips were examined by

TABLE 1

*Physico-chemical factors of the effluents (100% concentration). All values except pH are in mg/l.*

| Characteristics | Average values |
|---|---|
| pH | 9.8 |
| Total dissolved solids | 3048 |
| Dissolved oxygen | 0.85 |
| Biochemical oxygen demand for 5 days at 20° C | 6000 |
| Chemical oxygen demand | 6146 |
| Chlorides as Cl | 1421.6 |
| Sulphates as $SO_4$ | 212.5 |
| Silicates as $SiO_2$ | 64.83 |
| Ammonical Nitrogen | 89.4 |
| Nitrate Nitrogen | 0.032 |
| Nitrite Nitrogen | 0.130 |
| Calcium salt as Ca | 277.5 |
| Magnesium as Mg | 19.2 |
| Nickel as Ni | 0.90 |
| Cobalt as Co | 0.90 |
| Zinc as Zn | 0.70 |
| Boron as B | 0.295 |

TABLE 2

*Mitotic index, mito-depression and % abnormal mitosis observed after treatment and 24 hr recovery*

| Concentration and treatment period | Mitotic index | Mitodepression | Disturbed metaphase | Chromosome thinning | Anaphase bridge | Unequal distribution | Micronuclei | Diagonal pole & spindle | Binucleate cells | Sticky metaphase | Metaphase with uncondensed chromosome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25% 6 hr | 2.3 | 90.8 | — | — | — | — | — | 0.2 | — | — | 0.1 |
| 50% 6 hr | 2.0 | 92.0 | — | — | — | — | — | 0.4 | — | — | 0.3 |
| 75% 6 hr | 1.5 | 94.0 | 0.2 | — | — | — | — | 0.8 | — | — | 0.8 |
| 100% 6 hr | 0.9 | 96.4 | 0.6 | — | — | 0.2 | — | 1.4 | — | 0.6 | 1.0 |
| 25% 24 hr | 1.7 | 93.2 | 0.8 | 0.4 | — | 0.2 | 0.8 | 1.8 | 0.2 | 0.6 | 1.1 |
| 50% 24 hr | 3.7 | 85.2 | 1.0 | 0.6 | 0.6 | 0.6 | 0.8 | 1.9 | 0.8 | 0.6 | 1.0 |
| 75% 24 hr | 2.7 | 88.8 | 1.4 | 0.6 | 0.8 | 0.8 | 1.6 | 1.9 | 0.8 | 1.0 | 1.4 |
| 100% 24 hr | 2.8 | 92.8 | 2.6 | 1.0 | 0.8 | 0.8 | 1.8 | 2.7 | 1.0 | 2.1 | 1.0 |
| 25% 48 hr | 1.0 | 96.0 | 1.8 | — | 1.3 | 0.9 | 2.4 | 2.9 | 1.2 | 2.4 | — |
| 50% 48 hr | 2.1 | 91.6 | 2.1 | 0.9 | 1.8 | 1.2 | 2.6 | 3.4 | 1.6 | — | — |
| 75% 48 hr | 3.4 | 86.4 | 3.0 | 2.3 | 1.9 | 1.3 | — | 3.9 | 2.6 | — | — |
| 100% 48 hr | 3.8 | 84.8 | 3.6 | 2.6 | 3.4 | 2.8 | — | 3.9 | — | — | — |
| Control | 25 | — | — | 0.11 | — | — | — | 0.1 | — | — | — |

Haematoxylin squash method. The mitotic index and abnormalities have been recorded after scoring nearly 2000 cells in each case.

The average values of physico-chemical chracteristics of the effluents analysed are given in table 1 (100% concentration). The effluents are alkaline and contain high amounts of dissolved salts, BOD, COD, chlorides and considerable amounts of sulphates, silicates and calcium salts. The biological effects of the effluents have been described elsewhere [7,8].

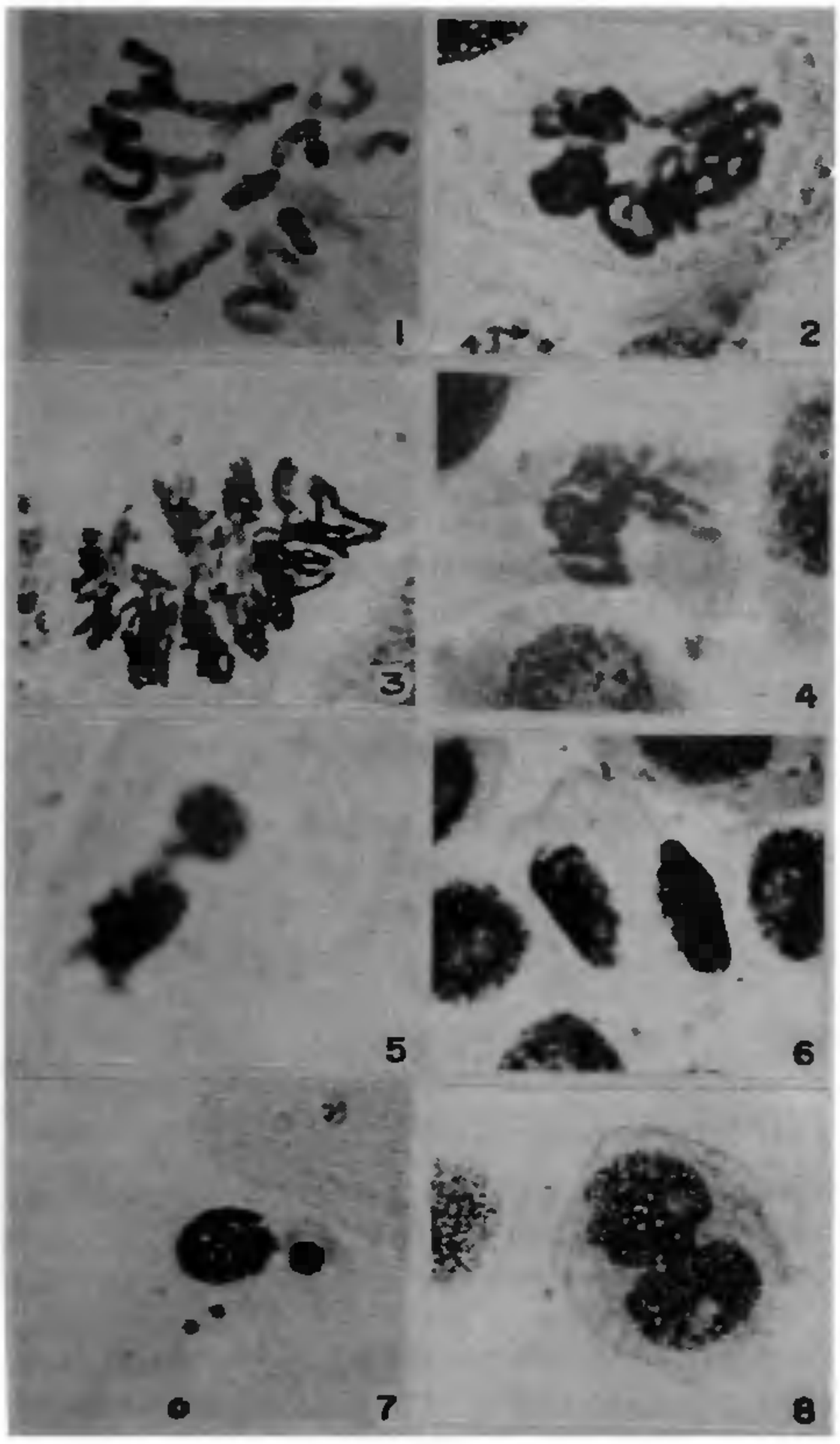
The mitotic index, mitotic depression and the percentage abnormalities observed are given in table 2. The effluents produce delayed effect; the mitotic index and % of abnormalities observed remained dose-dependent. Anomalies such as micronuclei formation (figure 7), disturbed metaphase, diagonal pole and spindle (figure 6) and the anaphase bridge (figure 5) dominated the other types.

Some of the morphological changes observed are contraction and condensation of chromosomes and chromatin erosion leading to fragmentation. In many cells the chromatin material was found spreading all over the cell and ring formation of chromatin threads was also observed. Unequal segregation of chromatin material was observed in number of cells (figure 2).

The effluents also effected the spindle and cell plate formation giving C-metaphases (figure 1). Most of the cells showed binucleate condition (figure 8). formed on account of the lack of cell wall formation. The clastogenic effect of chemicals such as breaks (figure 4), gaps (figure 3) and exchanges were observed at lower concentration.

Among cells with agglomeration of chromosomes a characteristic feature observed was that in such cells, the chromosomes appeared fused or bridged together (figure 2). Occasionally fragments were also observed in cells showing agglomeration of chromosomes. Chromosomes in many cells never showed condensation, as prophase advanced and remained as such even at metaphase.

Morphological changes in chromosomes observed in the present study have also been recorded by earlier workers[2-5,9] but they have not analysed the effluents for their physico-chemical characteristics. This limits our knowledge on the actual factor responsible for cellular damage. The present findings indicate that effluents with high organic matter and other dissolved salts such as chlorides, sulphates, silicates and calcium together with trace elements such as nickel, cobalt and zinc are possibly the factors responsible for the damage (see table 1). Hence the evaluation of the cytological damage induced by industrial effluents should be made along with usual physicochemical and biological analysis which may throw more light, not only on the mechanism of cellular damage, but also on the behaviour of environmental toxicants in the polluted habitats.




**Figures 1–8. 1.** C-metaphase × 1300 **2.** Sticky metaphase showing unequal segregation × 1200 **3.** A polyploid cell with gaps × 1350 **4.** Metaphase with breaks × 1000 **5.** Sticky anaphase bridge × 950 **6.** Diagonal anaphase × 850 **7.** Cell with micronuclei × 1000 **8.** A binucleate cell × 1050.

The authors thank the authorities of University of Mysore for providing facilities.

27 April 1982; Revised 31 December 1982

1. Mudd, J. B. and Kozlowski, T. T., *Response of plants to Air Pollution.*, Academic Press, London, 1975.
2. Fishbein, L. *Mutat Rev.*, 1976, **32**, 267.
3. Oblisami, G. and Rajannan, G. *Proc. Int. Symp. on Environmental Agents and their biological effects.*, Hyderabad, 1978.
4. Ravindran, J. N. and Ravindran, S. *Cytologia.*, 1978, **43**, 565.
5. Rangaswamy, V., Shanthamurthy, K. B. and Govindappa, D.A. In *Perspectives in cytology and genetics.*, (Eds) G. K. Manna and U. Sinha, Hindsania Publishers, New Delhi, 1980, 306.
6. APHA, AWWA and WPCF. *Standard methods for the examination of water, sewage and industrial wastes*, APHA Inc., New York 1976.
7. Somashekar, R. K., Ramaswamy, S. N. and Govindappa D. A., *Indian J. Environ. Hlth.*, 1982 ( in press).
8. Somashekar, R. K., Ramaswamy, S. N. and Govindappa D. A., *Proc. Indian Natl. Sci. Acad.*, 1982 (in press).
9. Shanthamurthy, K. B. and Rangaswamy, V. *Cytologia.*, 1979, **44**, 921.

## THIOACETAMIDE CAUSES INCREASE IN LEUCOCYTES IN *CHANNA PUNCTATUS* (BL.).

O. P. Saxena and Aditya Parashari
Ichthyology Research Laboratory, Zoology Research Laboratories, M.M. College, Modinagar 201 204, India.

Thioacetamide is a known carcinogen. Its main target organs are thyroid and in some cases liver[1–3]. Its carcinogenic effect on blood has not been reported so far.

The fish *Channa punctatus* collected from local resources, after acclimatization in the laboratory for ten days, were transferred to aquaria. A sub-lethal concentration of thioacetamide 50 mg/l was dissolved in unchlorinated water (pH 7.5; total solids 14.7 mg/l; alkalinity as $CO_3$ 57 mg/l; alkalinity as $OH^-$ 4.5 mg/l; hardness 60–70 mg/l; dissolved oxygen 6–7 mg/l). The blood parameters were studied after the interval of 15, 30 and 45 days.

The observations (Table 1) reveal that thioacetamide sub-lethal concentration 50 mg/l causes decrease in the total erythrocytes count and haemoglobin percentage. On the other hand a rapid increase in the total number of leucocytes count was observed. The increase in leucocytes count is upto approximately 40 times in 45 days. A considerable increase in the percentage of immature erythrocytes and in erythrocytes sedimentation rate (ESR) also suggest leukaemogenic effects in *Channa punctatus*.

The present observations reveal that thioacetamide probably interferes with the development of erythro-