ARCHITECTURE CIVIL ENGINEERING ENVIRONMENT
The Silesian University of Technology No. 4/2009

EFFECT OF BIOLOGICAL TREATMENT ON GENOTOXICITY OF INDUSTRIAL WASTEWATERS: ROOT TIPS *VICIA FABA* ASSAY

Jarosław WISZNIOWSKI[a*], Piotr SINDERA[b], Isabelle DUQUESNOY[c], Joanna SURMACZ-GÓRSKA[d]

[a] Dr.; Faculty of Energy and Environmental Engineering, Silesian University of Technology, Akademicka 2, 44-100, Gliwice, Poland
*E-mail address: *jaroslaw.wiszniowski@polsl.pl*

[b] MSc; Faculty of Energy and Environmental Engineering, Silesian University of Technology, Akademicka 2, 44-100, Gliwice, Poland

[c] Dr.; ERTAC-PIAF, UMR INRA-UBP, Tumeurs et Autosurveillance Cellulaire, 24 avenue des Landais, 63177 Aubiere Cedex, France

[d] Prof.; Faculty of Energy and Environmental Engineering, Silesian University of Technology, Akademicka 2, 44-100, Gliwice, Poland

Received: 14.06.2009; Revised: 15.07.2009; Accepted: 27.07.2009

Abstract

Industrial wastewater effluents are potential source of hazardous pollutants that are discarded into the watercourses, hence they pose a serious threat to aquatic organisms as well as human health. Identifying genotoxic substances in industrial effluents can minimize the risk of exposure to these compounds, which are suspected to have carcinogenic properties. In the present studies, the phytotoxic and genotoxic effects of wastewater obtained from fertilizer factory were investigated in *Vicia faba* root cells of hydroponic culture. The toxicity tests of crude wastewaters indicated an important fluctuation (seasonal differences) of mutagenic compounds among the different periods of the chemical operation. Moreover, the mutagenic profile was also assessed for biologically treated wastewaters in laboratory scale models fed with wastewater effluent from the fertilizer factory. The results proved that after biological treatment, the genotoxcity of treated effluent significantly decreased in comparison to untreated wastewaters.

Streszczenie

Ścieki przemysłowe są potencjalnym źródłem szkodliwych zanieczyszczeń odprowadzanych do wód płynących, przez co stanowią zagrożenie dla organizmów wodnych jak również dla ludzi. Monitoring substancji genotoksycznych w ściekach przemysłowych może ograniczyć ryzyko ekspozycji na te związki. W ramach przeprowadzonych badań oceniono fitotoksyczny i genotoksyczny wpływ ścieków przemysłowych pochodzących z zakładów produkujących nawozy sztuczne na korzenie rośliny *Vicia faba* w kulturach hydroponicznych. Testy genotoksycznosci wskazały dużą zmienność potencjalnej mutagenności ścieków surowych. Jednocześnie oceniona została mutagenność ścieków po procesie biologicznego oczyszczania, który był prowadzony w skali laboratoryjne, w modelowym systemie zasilanym ściekami przemysłowymi. Badania wykazały znaczące obniżenie się genotoksyczności ścieków po procesie biologicznego oczyszczania, w stosunku do ścieków nieoczyszczonych.

Keywords: **Mitotic index; Micronuclei; Phytotoxicity; Activated sludge; Industrial effluents.**

1. INTRODUCTION

Composition of the wastewaters generated in the chemical industry sector is not always predictable. As a consequence, many unknown or unidentifiable substances may be released into the environment especially due to their incomplete removal during the wastewater treatment process.

The basic physico-chemical analyses (TOC, DOC, COD AOX, nutrients, pH, conductivity etc.) or even specific approach for priority hazardous substances

listed in Council Directive 76/464/EEC [1] (i.e. PAH, PCB, heavy metals) provide merely an incomplete insight, because it covers only a limited part of the substances in complex effluents.

At present, there is a growing interest in Whole Effluent Assessment (WEA) strategy. This strategy is defined as the assessment of effluents by using a range of biological methods in order to reveal an adverse (potential) effects, based on an assessment of persistence, bioaccumulation and toxicity [2]. One of the most important issues of WEA consist in identifying genetic risks of discharged wastewaters.

Mutagenicity testing can be performed on different kinds of organisms like bacteria, invertebrates, mammals, fish and plants [3]. Nevertheless, higher organisms (eucaryotes), both *in vitro* and *in vivo* assays seem to be more relevant for monitoring purposes, human and ecological risk assessment.

The plant *in vivo* bioassays are: easy to handle, of low cost, and in many cases are more sensitive than other available systems [4]. Furthermore, the tests can be used without pretreatment of crude environmental sample such as concentration and purification [5]. This last is of a great importance for getting a realistic estimation of the genotoxicity of tested effluent.

The meristematic mitotic cells of higher plants i.e. *Tradescantia palludosa* [6, 7, 8], onion *Allium cepa* [9, 10, 11] and broad bean *Vicia faba* [12, 13], have been used to evaluate potential genotoxicity of environmental chemical pollutants. Plant bioassays have been validated in international collaborative studies and have been shown to be an efficient way of monitoring the genotoxicity of environmental pollutants [13, 14, 15]. Genotoxicity test on plants system has been reported for surface water polluted with industrial and municipal waste [16, 17]. The test was also applied for monitoring municipal sewage [5] and a broad range of industrial effluents such as paper effluents, petrochemical, dye industry [5, 18] or recently from fertilizer factory [10], landfill leachates [19, 20] and oil mill [21].

However, despite the fact that many studies showed a good correlation of the plant system with the mammalian test system [4,14], the large evolutionary distance to humans is a main drawback of the test and most likely have led to lack of general recognition of plant genotoxicity assays.

The main objectives of work were to asses potential mutagenicity of wastewater from a chemical factory before and after biological treatment. In the studies, two samples were collected and tested at different periods of factory operating, which provided information on toxicity variability in real wastewater samples.

2. MATERIALS AND METHODS

2.1. Origin of industrial wastewater

The samples of crude industrial wastewaters originated from chemical factory, producer of nitrogen fertilizers and other chemical products such as pastifiers. The wastewaters from factory's confines are treated on a mechanical-biological wastewater treatment plant (MBWWTP) before they are discharged into the river, but no toxicity screening of effluents is performed. In the presented studies, the samples were benchmarked from the main collecting pipe which leads primary effluent to MBWWTP at two different periods (series 1 – February 2008 and series 2 – May 2008).

2.2. Biological treatment

The samples were used as a feed for the laboratory scale continuous flow activated sludge system, which consisted of aeration tank (10L) and settling tank (5L). Each time, activated sludge was taken in MBWWTP and about one month acclimation period was ensured in lab conditions before taking the samples for genotoxicity tests. During the experimentation, ammonia and COD loading rate were very similar to those of MBWWTP and amounted on average to 0.015 g NH_4^+-N /g MLVSS*d and 0.18 g COD/g MLVSS*d respectively. Dissolved oxygen (2.0 mgO_2/L), was maintained at the appropriate level to enhance biological activity.

Both crude industrial and biologically treated wastewater samples were centrifuged (at 3000 rpm, within 10°C and supernatant were frozen at -20°C) until exposure experiments and physicochemical analysis. The analysis included COD (Chemical Oxygen Demand- dichromate method), TOC (Total organic carbon – Shimadzu Analyser TOC-V_{CSH} with autosampler ASI-V), and BOD_5 (5 day Biochemical Oxygen Demand – Oxi Top WTW system) research. Ammonium nitrogen, nitrite and nitrate nitrogen were determined according to standard Merck methods using Spectroquant®test. Conductivity and pH were analyzed using METER CC-401 (ELMETRON) and pH-meter (WTW) respectively. The average values of each parameter obtained during the studies are shown in Table 1.

Table 1.
Composition of the crude (influent) and biologically treated (effluent) wastewaters

Parameters*– Influent	Series 1 (Feb. 08)	Series 2 (May 08)
TOC (mgC/L)	308	320
COD (mgO_2/L)	1676	1899
BOD_5 (mgO_2/L)	360	500
NH_4^+-N(mg/l)	147	149
NO_2^- -N(mg/l)	0.0	0.0
NO_3-N (mg/l)	0.0	0.0
Conductivity (mS/cm^2)	2.27	2.01
pH	7.8	7.5
Parameters*– Effluent	Series 1 (Feb. 08)	Series 2 (May 08)
TOC (mgC/L)	62	62
COD (mgO_2/L)	177	109
BOD_5 (mgO_2/L)	5.5	8.5
NH_4^+-N(mg/l)	47	1
NO_2^- -N(mg/l)	28.2	0.5
NO_3-N (mg/l)	62.1	137.5
Conductivity (mS/cm^2)	2.25	1.90
pH	7.9	8.0

*average values of 2-3 replicates

2.3. Root tip preparations and treatment

The protocol published by Cotelle and co-workers [12], with minor modifications, was adopted for *Vicia faba* seeds (Cultivar: Windsor White were bought in local store). Dry seeds were abundantly rinsed with distilled water, then soaked in distilled water for 24-h. Then they were allowed to germinate on moist gauze for 3 days at 25°C. When newly emerged primary roots were 1.00-2.00 cm in length, their seeds were used in the test. Root tips were exposed for 24 h and for 7 d to different concentrations (5%, 10%, 30%, 60% and 100%) of the industrial crude and biologically treated samples in 1L boxes. During the test, the hydroponic culture of *V. faba* was aerated in order to avoid root hypoxia or anoxia conditions which result in inhibition of leaf expansion, a reduction in root and shoot growth [22]. Plants were cultivated in a controlled chamber with a 16h/8h photoperiod and an irradiance provided by neon lamps (Daylight, PILA). For each experiment from five to seven seeds were used per treatment. Chlorine free tap water was used as a negative control and handled alike for all the experiments. As a positive control maleic hydrazide (MH) at concentration 0.448 g/L ($C_4H_4N_2O_2$ ≥99.0%, Acros Organics) was used. All the experimental groups were kept in at 24±2°C.

2.4. Growth inhibition test and genotoxicity

Root length measurements were performed after 7 days for each plant. Growth inhibition rate expressed in % was determined by comparison of the plant's lengths in different exposure concentration with plants growing in control medium (tap water). The comparison with positive control (MH) was also ensured.

After treatment of 24h or 7d, root tips (20 mm) were cut, fixed in Carnoy's solution (glacial acetic acid/ethanol 1:3) at 4°C within 24h and transferred into 70% ethanol for storage. Before the microscopic observation, roots were hydrolyzed in 1N HCl at 60°C for 5-7 min and corresponding number of slides was prepared. After staining the root tips with 1% aceto-orcein, the interphase cells were scored for micronucleus frequencies at 1000× magnification.

The mitotic index (MI) was determined by examination of 1000 cells in five or seven root tips. MI was expressed as number of dividing cells per 100 scored cells (%). The micronuclei frequency (MN) was defined as a number of cells with micronuclei per 1000 cells scored, resulting from 5000-7000 examined for each treatment.

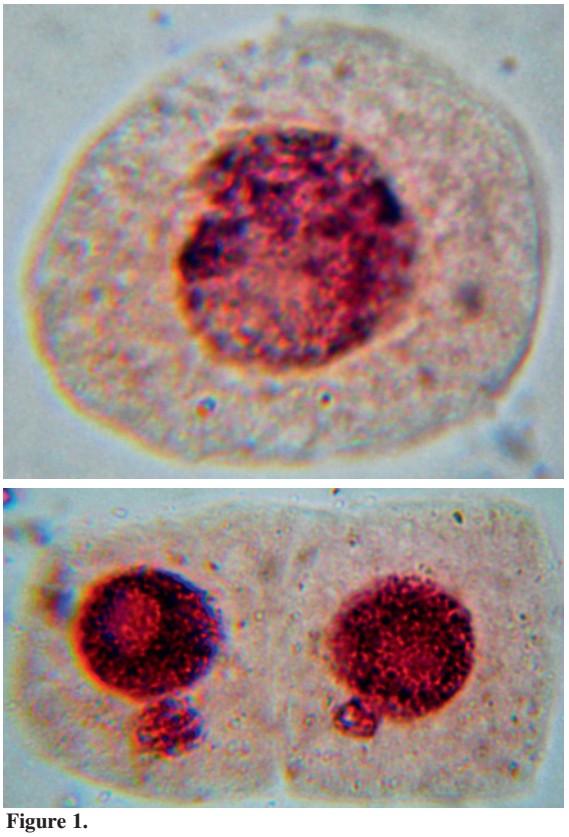

Figure 1.
The natural cell a) and the two micronucleated cells b) in *V. faba* root tips

Statistical analysis of the data

Results are presented as mean ±S.D. and the statistical significance of the differences between the means of control and treated groups were determined using the oneway method: ANOVA test and Microsoft™ Excel Statistic ToolPack. Least significant difference was used to determine the difference between samples and negative control or between samples and positive control. Significant from positive control at $p < 0.05$, 0.01 and 0.001 was marked as "+", "++" and "+++" respectively. Significant from negative control at $p < 0.05$, 0.01 and 0.001 was marked as "*", "**" and"***" respectively.

3. RESULTS AND DISCUSSION

3.1. Biological treatment of industrial wastewaters

The biological treatment in lab scale was carried out in two periods (series 1 and series 2), but using the same technological parameters (ammonia and COD loading rate, HRT, biomass concentration). Chemical analysis of parameters COD, TOC, BOD5 indicated that organic contents in crude industrial samples in series 1 and series 2 were on similar level (Table 1). Biodegradability (expressed as BOD_5/COD ratio) was below 0.3, which is a value lower than usually observed for municipal wastewaters 0.4-0.6 [23, 24]. However, removal efficiency of organics in laboratory activated sludge system amounted to 80, 89 and 98% for TOC, COD and BOD_5 in series 1 and 81, 94 and 98 % for TOC, COD and BOD5 in series 2 respectively. Despite the fact that ammonia contents in crude wastewater samples (series 1 and series 2) were within the same range, undoubtedly higher removal efficiency of ammonia nitrogen in the system was observed for series 2. For this last wastewater, almost complete ammonia oxidation was achieved which resulted in very low concentration of ammonia (1 mg NH_4^+-N /L) and presence of nitrate as main oxidation form of nitrogen in the effluent. Ammonia oxidation for series 1 was much lower and amounted to 70%. Similar periodical failure of nitrification performance had been noted during last years in MBWWTP operation (data not shown) within the winter time (between December and March). The possible causes of recurring failures of nitrification is seasonal decrease of temperature of treated wastewater and/or variable composition of wastewaters (appearance of toxins in the treated wastewater). The presence of xenobiotics and their primary degradation products in the influents of STPs may inhibit irreversibly sensitive biological processes, such as nitrification [25].

3.2. Mitotic index and root length in *V. faba*

Plant response to the presence of mutagens can be considered on different levels of organization: from DNA, chromosome, and genome to the whole organism (plant physiology) [26]. Mitotic Index (MI) and root growth lengths in V. faba are often used parameters for tracking cytotoxicity and phytotoxicity respectively of tested industrial effluent substances [5, 27]. Decrease of both parameters in comparison to the negative control implies increase of toxicity.

MI values for series 2 were similar to negative control, which suggested lack of cytotoxic pollution in the crude wastewaters in series 2 (Table 2).

Table 2.
The MI (%) average values in the *Vicia faba* after exposure to wastewaters within 7d and (n=7)

Treatment	Mitotic Index, % (x±S.D.)		Sign. P<0.05
Neg. control (tap water)	7.22	±0.8	
Positive control (MH)	0.35	±0.12	
Feb.2008 (Influent)	3.93	±1.08	*/+
Feb.2008 (Effluent)	3.94	±0.54	*/+
May 2008 (Influent)	6.85	±0.78	+
May 2008 (Effluent)	7.80	±1.78	+

Significant difference from negative (*) and positive control (+)

Decrease of the mitotic index MI in V. faba root meristems in comparison to the negative control (tap water) reached statistical significance only for wastewaters in the series 1 both for crude and bio-treated. Average values of MI obtained for *V. faba*, in all tested wastewaters were much higher and significantly different from MI value noted for positive control (MH).

Inhibition of mitotic activity above 78% caused lethal effect on the whole root of tested *Allium cepa* [28], while the inhibition above 50% usually has sublethal effect [29]. Reduction in cells division at level witch lead to lethal effect was noted only for positive control (MH). The maleic hydrazide (MH) herbicide induced cell death and inhibition of mitosis in *A. cepa* root tips [30], which increased the percentage of non-dividing cells leading to a decline in mitotic indices (MI). Del Campo and Coletto [31] showed that MH exerted a genotoxic effect on root tips of *A. cepa L. via* the inhibition of DNA replication. MH, which is highly mutagenic and clastogenic [32] was used as a

positive control in the genotoxic study conducted by these authors.

Positive effect of the biological treatment was confirmed by root elongation test (Table 3).

Table 3.
Root lengths (cm) average values in the *Vicia faba* after exposure to undiluted wastewaters within 7d and (n=7)

Treatment	Mitotic Index, % (x±S.D.)		Sign. P<0.05
Neg. control (tap water)	7.22	±0.8	
Positive control (MH)	0.35	±0.12	
Feb.2008 (Influent)	3.93	±1.08	*/+
Feb.2008 (Effluent)	3.94	±0.54	*/+
May 2008 (Influent)	6.85	±0.78	+
May 2008 (Effluent)	7.80	±1.78	+

Significant difference from negative (*) and positive control (+)

Apparently, *V. faba* roots exposed to treated wastewater both in series 1 and series 2 reached similar lengths in comparison to negative control (Table 3). Moreover, some stimulation effect of biologically treated wastewaters on plant physiology noted for series 2 most likely can be explained by the presence of micro- and macro- elements in the wastewaters indispensable for plants growths [27]. Relatively low acute phytotoxicity of different industrial wastewater effluents [5, 27] does not exclude their genotoxic activity.

The root lengths (irrespectively wastewaters) were always statistically different form positive control (maleic hydrazide), which is a herbicide used to inhibit plant growth, to stop sprouting of vegetables in storage [33]. Growth inhibition of *V. faba* roots exposed to the maleic hydrazide was 78% on average.

3.3. Micronucleus test

Micronuclei is small nucleus, separate from and additional to the main nucleus, produced during telophase of mitosis by lagging chromosome fragments or whole chromosomes [2] and micronucleus assays are considered as a clastogenic endpoint, as an indicator of exposure to mutagenic/carcinogenic agents [14, 21]. The increase of micronuclei frequency in comparison to negative control indicates increase of genotoxic potential of the tested sample towards tested organism.

The *V. faba* micronucleus test showed that the crude wastewaters collected on the premises of the factory was significantly genotoxic (at concentration 60 and 100%) as compared to the tap water used as the negative control (Fig. 2 and Fig. 3). However, the wastewaters from the series 1 induced considerably stronger genetic damage towards *V. faba* cells than the wastewater collected in the series 2. The wastewater from series 1 produced micronuclei frequency similar to the positive control, which is a potent, well-known mutagenic/clastogenic agent in plan system [14, 21]. The results imply a seasonal difference in genotoxicity induced by wastewaters.

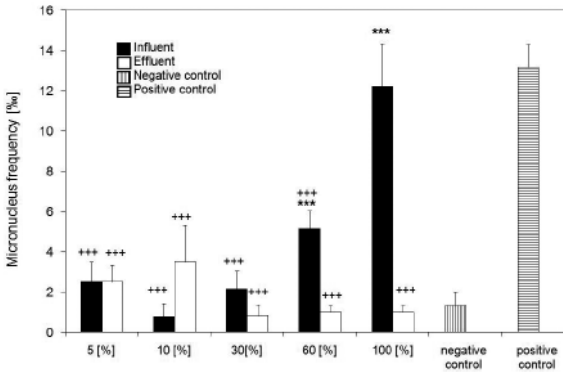

Figure 2.

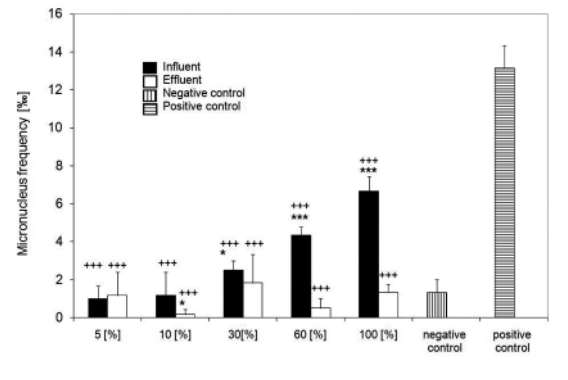

Figure 3.

MN assays proved to be a reproducible and sensitive indicator of exposure for mutagenic risk. MN frequencies significantly increased with increasing concentrations of untreated wastewater (Fig. 2 and Fig. 3) and the concentration–response curve fitted well with following equations: $y = 0.109x + 0.088$, ($r^2 = 0.90$) for series 1 and $y = 0.060x + 0.654$, ($r^2 = 0.999$) for series 2.

For biologically treated wastewaters, both in series 1 and series 2, MN frequencies decreased to the level of the negative control and no relationships between dose (concentration) and response were noted. The results showed a predominated influence of biological treatment in decreasing mutagenic potential of wastewater. It is possible that most of genotoxins were degraded via biochemical reactions or/and removed from wastewater body by sorption to sludge particles (like some xenobiotics with strong hydrophobic organics properties i.e PCB, PAH, phtalates or heavy metals) and transfer to the sludge processing systems [34]. It is becoming difficult to disentangle/predicate which particular contaminants are actually responsible for genotoxicity of wastewaters. In complex mixtures, environmental samples a co-exposure to different substances can cause genotoxic effect. Thus, even analysis of suspected heavy metals or xenobiotics (PCB, PAH, etc.) contents cannot be correlated to genotoxic reations in different environmental samples [20].

4. CONCLUSION

Untreated wastewaters in series 1 have greater potential genotoxicity than wastewaters in series 2. It indicates an important fluctuation (seasonal differences) of mutagenic compounds among the different periods of chemical factory operation.

Variability in toxicity cannot be correlated to general parameters that characterize wastewaters such as BOD_5, COD, nitrogen…etc.

The results of studies on plants (i.e. MN assay on *V. faba* root tips) cannot be directly extrapolated to animal systems, but bioassays might be a valuable tool for monitoring genetic risks from part of complex wastewater mixtures and might be also introduced as a part of test battery in industrial laboratories.

Although numerous convincing studies have been published, no internationally accepted guideline for waste water assessment currently exists. Genotoxicity and mutagenicity tests with mammalian cells could better predict human risks, but are highly time and cost intensive. Thus, more cost effective and less time consuming *in vivo* plant assays (i.e. using *V. faba* root tips) can be an interesting alternative, especially for assessment of mutagenic substance in environmental complex mixtures.

REFERENCES

[1] The Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community [complemented by Directive 2006/118/EC]

[2] OSPAR Commission, 2005: Whole Effluent Assessment Report, ISBN 1-904426-45-X

[3] OSPAR Commission, 2002: Survey on Genotoxicity Test Methods for the Evaluation of Waste Water within Whole Effluent Assessment ISBN 1-904426-02-6

[4] *Fiskesjo G.*; The *Allium* test in wastewater monitoring. Environmental Toxicology and Water Quality, Vol.8, 1993, p.291 -298

[5] *Nielsen M. H. and Rank J.*; Screening of toxicity and genotoxicity in waste water by the use of the Allium test. Hereditas Vol.121, 1994, p.249-254

[6] *Majer B.J., Tscherko D., Paschke A., Wennrich R., Kundi M., Kandeler E. and Knasmüller S.*; Effects of heavy metal contamination of soils on micronucleus induction in Tradescantia and on microbial enzyme activities: a comparative investigation. Mutation Research Vol.515, 2002, p.111-124

[7] *Carreras H.A., Pignata M.L. and Saldiva P.H.N.*; In situ monitoring of urban air in Córdoba, Argentina using the Tradescantia-micronucleus (Trad-MCN) bioassay. Atmospheric Environment Vol. 40, 2006, p.7824-7830

[8] *Klumpp A., Ansel W., Klumpp G., Calatayud V., Garrec J.P., He S., Peñuelas J., Ribas A., Ro-Poulsen H., Sanz M.J. and Rasmussen P.V.S.*; Tradescantia micronucleus test indicates genotoxic potential of traffic emissions in European cities. Environmental Pollution, Vol.139, 2006, p.515-522

[9] *Fatima R.A. and Ahmad M.*; Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater. Science of the Total Environment, Vol. 346, 2005, p. 256-273

[10] *Migid H. M. A., Azab Y.A, Ibrahim W. M.*; Use of plant genotoxicity bioassay for the evaluation of efficiency of algal biofilters in bioremediation of toxic industrial effluent Ecotoxicology and Environmental Safety, Vol. 66, 2007, p.57–64

[11] *Yi H., Wu L. and Jiang L.*,; Genotoxicity of arsenic evaluated by Allium-root micronucleus assay. Science of the Total Environment, Vol.383, 2007, p.232-236

[12] Cotelle S., Masfaraud J.F., Ferard J.F.; Assessment of the genotoxicity of contaminated soil with the Allium/Vicia micronucleus and the Tradescantia-micronucleus assays. Mutation Research, Vol. 426, 1999, p. 167–171

[13] *Yi H. and Si L.*; Vicia root-mirconucleus and sister chromatid exchange assays on the genotoxicity of selenium compounds. Mutation Research, Vol.630, 2007, p.92-96.

[14] *Grant W.*; The present status of higher plant bioassay for the detection of environmental mutagens. Mutation Research,Vol. 310, 1994, p.175-185

[15] *Yi H. and Meng Z.*; Genotoxicity of hydrated sulfur dioxide on root tips of Allium sativum and Vicia faba. Mutation Research, Vol. 537, 2003, p.109-114

[16] *Miao M., Fu R., Yang D., and Zheng L.*; Vicia root micronucleus assay on the clastogenicity of water samples from the Xiaoqing River in Shandong Province of the People's Republic of China. Mutation Research,Vol.426, 1999, p.143-145

[17] *Zhong, Y., Feng S. L., Luo Y., Zhang G. D., and Kong Z. M.*' Evaluating the genotoxicity of surface water of Yangzhong city using the Vicia faba micronucleus test and the comet assay. Bulletin of Environmental Contamination and Toxicology, Vol.67, 2001, p.217- 224

[18] *Grant W. F., Lee H. G, Logan D. M. and Salamone M. F.*; The use of *Tradescantia* and *Vicia faba* bioassays for the in situ detection of mutagens in an aquatic environment. Mutation Research, Vol.270, 1992, p.53-64

[19] *Sang N. and Li G.*; Genotoxicity of municipal landfill leachate on root tips of *Vicia faba* Mutation Research, Vol.560, 2004, p.159-165

[20] *Feng Sh., Wang X., Wei G., Peng P., Yang Y., Cao Z.*; Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity Chemosphere, Vol.67, 2007, p.1133–1137

[21] *El Hajjouji H., Pinelli E., Guiresse M., Merlina G., Revel J.-C., Hafidi M.*; Assessment of the genotoxicity of olive mill waste water (OMWW) with the Vicia faba micronucleus test. Mutation Research, Vol. 634, 2007, p.25-31

[22] *Schaffer B., Whiley, A.W. and B.N. Wolstenholme.*; Avocado: Botany Production and Uses. CAB International Inc., London, 2002, p.416

[23] *Metcalf and Eddy*, Inc. Wastewater Engineering: Treatment, Disposal, and Reuse McGraw-Hill, Inc., New York, NY. ISBN 0-07-100824-1, 1991

[24] *Verstraete W. and van Vaerenbergh E.*; Aerobic Activated Sludge, Chapter 2 in "Biotechnology" Vol.8 ed. Schönborn W. Microbial degradation ed Rehm H.-J. and Reed G., Weinheim; New York. VCH., 1986

[25] *Dokianakis S. N. , Kornaros M. and Lyberatos G.*; Impact of five selected xenobiotics on isolated ammonium oxidizers and on nitrifying activated sludge. Environmental Toxicology, Vol.21, No 4, 2006, p.310-316

[26] *Maluszyńska J. and Juchimowicz J.*; Review. Plant genotoxicity: a molecular cytogenetic approach in plant bioassays, plant genotoxicity. Arh Hig Rada Toksikol, Vol. 56, 2005, p.177-184

[27] *Dane V., Ekici F. and Aktaç Y.K.*; The Effect of Waste Water on Root Growth and Mitosis in Onion (*Allium cepa*) Root Apical Meristem. Asian Journal of Plant Sciences. Vol.5, No 2, 2006, p.331-334

[28] *Antosiewicz D.*; Analysis of the cell cycle in the root meristem of Allium cepa under the influence of ledakrin. Folia Histochem Cytobiol, Vol.28, No.1-2, 1990, p.79-95

[29] *Sharma, C.B.S.R.*; Plant meristems as monitors of genetic toxicity of environmental chemicals. Current Science Association, Vol.52, 1983, p.1000-1002

[30] *Marcano L., Carruyo I., Del Campo A. and Montiel X.*; Cytotoxicity and mode of action of maleic hydrazide in root tips of Allium cepa L. Environmental Research, Vol.94, 2004, p.221-226

[31] *Del Campo A. and Coletto R.*; Capacidad genotoxica de la hidracida maleica (MH) detectada por intercambio de cromatidas hermanas (SCE) y por otros bioensayos en *Allium cepa*. Ciencia 6, 1998, p.7-21 (Genototic capacity of the maleic hydrazide acid (MH) detected by the exchange of sister chromatides (SCE) and by the other bioassays to Allium cepa), (in Spanish)

[32] *Gichner, T., Menke, M., Stavreva, D., Schubert, I.*; Maleic hydrazide induces genotoxic effects but no DNA damage detectable by the Comet assay in tobacco and field beans. Mutagenesis, Vol.15, 2000, p.385-389

[33] Royal Society of Chemistry. The Agrochemicals Handbook, 2nd ed.; Royal Society of Chemistry Information Services: England, 1987; p.259

[34] *Byrns G.*;The fate of xenobiotic organic compounds in wastewater treatment plants. Water Research, Vol.35, No 10, 2001, p.2523-2533