

ASSESSMENT OF TOXICITY OF INDUSTRIAL WASTES USING CROP PLANT ASSAYS

Carmen Alice Teacă* and Ruxanda Bodîrlău

Environmental pollution has a harmful action on bioresources, including agricultural crops. It is generated through many industrial activities such as mining, coal burning, chemical technology, cement production, pulp and paper industry, etc. The toxicity of different industrial wastes and heavy metals excess was evaluated using crop plant assays (germination and hydroponics seedlings growth tests). Experimental data regarding the germination process of wheat (from two cultivars) and rye seeds in the presence of industrial wastes (thermal power station ash, effluents from a pre-bleaching stage performed on a Kraft cellulose – chlorinated lignin products or chlorolignin), along with use of an excess of some heavy metals (Zn and Cu) are presented here. Relative seed germination, relative root elongation, and germination index (a factor of relative seed germination and relative root elongation) were determined. Relative root elongation and germination index were more sensitive indicators of toxicity than seed germination. The toxic effects were also evaluated in hydroponics experiments, the sensitivity of three crop plant species, namely *Triticum aestivum* L. (wheat), *Secale cereale* (rye), and *Zea mays* (corn) being compared. Physiological aspects, evidenced both by visual observation and biometric measurements (mean root, aerial part and plant length), as well as the cellulose and lignin content were examined.

Keywords: Crop plant; Industrial wastes; Seed germination; Nutrient solutions; Hydroponics seedling tests; Biometric measurements

Contact information: Romanian Academy, “Petru Poni” Institute of Macromolecular Chemistry, 41 A Gr. Ghica Voda Alley, Iasi, RO-700487, Romania; *Corresponding author: cateaca@icmpp.ro, cateaca14@yahoo.com

INTRODUCTION

Assessment of the environmental hazard and toxicological potential of industrial wastes and effluents has become an acute problem in many countries. The impact of chemicals on organisms is related to leaching, exposure and uptake by organisms, and their effects on critical biological functions, rather than total concentrations in contaminated soil or waste. Methods that employ aquatic organisms (luminescent bacteria, *Daphnia*, algae growth inhibition, etc.) or soil organisms (plant growth, seed germination, soil invertebrates) can be directly, or with minor modifications, applied to leachates, effluents, and solid waste studies (Schultz et al. 2002) to measure the potential biological impact (toxicity).

Higher plants provide suitable systems for a wide range of toxicological assays applicable to the estimation of risks to the environment, to ecosystems and, in some cases, to vertebrates/humans. The material most frequently used in the plant-based tests is

easy to handle. Seeds of cereals and some other crop plants provide a nearly inexhaustible and cheap source of biologically unique material for the production of seedlings.

The culture media for *in vitro* germination and growth of these materials are extremely simple because seeds usually contain enough nutrients for several days. Thus, water is the only necessary constituent of the media. Culture of seedlings can usually be performed in a simple incubator without sterile conditions if distilled water is used and seeds are surface-sterilized with 1-2% H₂O₂ for some minutes prior to germination. The equipment necessary for the determination of growth inhibition and for rapid statistical calculation of toxicity data is usually available in each standard laboratory (Kristen 1997).

Germination tests, biometric measurements, and chemical analysis (Wuncheng 1985; Tiquia et al. 1996; Lau et al. 2001) can be used in order to evaluate the toxic action of industrial wastes on the crops under study. Germination index is the most sensitive parameter, the seeds germination and roots growth depending even on a low level of toxicity (Zucconi et al. 1981). Germination index values of 100 % indicate a stimulation of plants growth and development (Keeling et al. 1994).

Root elongation is an important early developmental event in the growth and survival of plants. Unlike the seed germination test, the root elongation test evaluates only the water soluble constituents of a sample. As a general rule, root elongation is more sensitive than seed germination. This test may be done with a number of economically important species that germinate and grow rapidly (EPA Office of Research and Development Project EPA/540/R-92/003, 1991).

Harvesting of plants species grown on hydroponics media has no commercial purposes. Hydroponics media composition depends on the nutritive demands for each plant species. In hydroponics, the supports for plants have to separate easily from plant roots. For hydroponics media, it is recommended to use a pH in the range 5.5 - 5.8 because the soluble Mn, Cu, Zn, and especially Fe become deficient at high levels of pH. In our experiments, the initial pH for nutritive solutions has variable values. Some experimental data (Bowen and Roveira 1976; Trollemer and Hect-Bucholz 1984) evidenced that both microorganisms and organics have no high levels in hydroponics liquid solutions, root growth being reduced due to a deficient aeration in hydroponics cultures.

Fly ash disposal is a major problem in and around thermal power plants. It is characterized by a high alkalinity and salinity, as well as a high content of toxic trace elements that suppress plant growth and deteriorate soil properties (Tripathy and Sahu 1997). Despite these constraints, some plants are capable of growing in fly ash-amended soils without any injury symptoms. On the other hand, fly ash contains some growth essential elements like K, Ca, Mg, Fe, Zn, B, Mo, and S which promote plant growth (Wong and Wong 1989). Preliminary investigation performed to assess the influence of thermal power station ash in germination and seedling growth tests evidenced a different behavior depending on the plant species and ash concentration value (Popa et al. 2000).

Chlorine in pulp bleaching has been used to complete the delignification of pulp after cooking and facilitate lignin removal in subsequent bleaching stages. Issues of bleach plant effluent toxicity have been raised through the years, and chlorine is strongly

linked to this toxicity. Nevertheless, chlorine and now chlorine-based chemicals continue to be principal bleaching agents.

Concerns about the toxic effect of chlorinated organics and other chemical discharges have caused the industry to review pulping and bleaching processes and make changes. Bleaching process changes have included the use of various amounts of chlorine dioxide as a substitute for elemental chlorine. These efforts were directed toward "environmentally compatible" production processes. The next modifications eliminated the use of any elemental chlorine. This step further reduces, but does not eliminate, the discharge of toxic compounds. Many bleached kraft pulp manufacturers are responding to these demands by experimenting with and using ozone, enzymes, and other chemicals to further reduce or eliminate the use of chlorine-based chemicals.

Regarding total chlorine free (TCF) pulp production, there has been continuous growth, and the production statistics change rapidly (Albert 1994). Sweden and Finland, with pulp industries sensitive to European market demand, are converting rapidly to TCF production. Europe is close behind in production of TCF pulp. Approximately 30% of all TCF mills are located in Sweden. Finland has effluent discharge regulations similar to those in Sweden. Europe and especially the German speaking countries are providing the greatest demand for chlorine-free pulp and paper products.

U.S. and Canada have both national and regional regulations. In 1996, the first "cycle" of Environment Canada's Environmental Effects Monitoring (EEM) (<http://www.ec.gc.ca/eem/english/AboutEEM.cfm>) program was completed, in which algae, daphnia, and trout embryos were exposed to effluents from Canadian pulp mills. Bailey and Young (1997) reported on the sensitivity, variability and inter-correlation of these toxicity tests for various pulping methods including kraft, sulfite, and thermo-mechanical pulps.

Many recent pollution prevention efforts in the pulp and paper industry have focused on reducing the releases of toxics, in particular, chlorinated compounds. Pollution prevention techniques found to be effective at pulp and paper facilities are represented by extended delignification, oxygen delignification, and ozone delignification, which reduce the lignin content in the pulp, having the potential to eliminate the need for chlorine-based bleaching agents (EPA Office of Compliance Sector Notebook Project EPA/310-R-02-002).

It is usually assumed that chlorinated compounds have high molecular weight and are probably biologically inactive, because they cannot penetrate cell membranes of living organisms. In the last decade research was based on this argument and an appreciable effort has been made to find microorganisms able to degrade these compounds (Cammarota and Sant'Anna 1992; Esposito et al. 1991). Previous experimental studies performed to investigate the chlorolignin toxicity in seed germination and seedling growth tests (Popa and Teacă 1998; Teacă and Popa 2000) gave evidence of its negative influence by inhibiting the process, mainly at high concentration values.

Heavy metal contamination is prevalent in soil environments. Copper and zinc are frequently related to soil pollution, and are potentially toxic to living organisms. Heavy metals in higher doses may cause metabolic disorders for most of plant species (Carlson et al. 1991). Many studies have demonstrated that heavy metals influence the seed

germination and early seedling growth of plants (Lin et al. 2003; Fuentes et al. 2004; An 2006).

Three higher plant species from the Monocotyledonae Family – Gramineae (Poaceae), namely *Triticum aestivum* L. (wheat - two cultivars: *Gabriela base* type and *Fundulea 4* type), *Secale cereale* (rye), and *Zea mays* (corn) were selected as test plant species due to their sensitivity to a wide range of contaminants (OECD 1984; Wang et al. 1990; Wang 1991; Gorsuch et al. 1991; ASTM 2002). Contaminants are toxic to plants at elevated levels, acting directly (causing adverse effects on growth, survival, and reproduction, even death) or indirectly (through damaging food sources or habitat).

For assessing the toxicity of industrial wastes, germination and hydroponics seedling growth bioassays were performed by testing influence of thermal power station ash (as aqueous extract), chlorinated lignin products (effluents from pulp and paper industry), and metals in excess (Cu and Zn).

Relative seed germination, relative root elongation, germination index, as well as biomass production (as dry matter) for each experiment have been evaluated in order to assess the pollutants toxicity as a consequence of their presence in the germination media. For hydroponics experiments, physiological aspects through visual observation and biometric measurements, as well as the main chemical components (cellulose and lignin content) were investigated.

EXPERIMENTAL

Seed Germination Experiments

The germinative material, provided by AGROSEM Seed Center, Iasi, Romania, included seeds of wheat (*Triticum aestivum* L.) – Gabriela base and Fundulea 4 types, and rye (*Secale cereale*).

Fly ash is a useful ameliorant that may improve the physical, chemical, and biological properties of problem soils and can enhance plant biomass production from degraded soils, being a source of readily available plant macro and micronutrients (Jala and Goyal 2006). Thermal power station ash, resulted from coal burning, was provided by thermal power station CET Holboca, Iasi, Romania. An aqueous extract, obtained by mixing the thermal power station ash and distilled water (1:10 w/w), which were further periodically stirred during 48 hours, at room temperature of $23 \pm 2^\circ\text{C}$, was used in experiments. The filtrate had a pH of 6.71 and a dry matter content of 0.98 g/L.

Considerable research effort has shown effluents from pulp and paper bleach mills using chlorine to be extraordinarily complex (Suntio et al. 1988; Sodergren 1993; Martin et al. 1995). Comparison of low level, long term effluent toxicity between mills using a variety of bleach processes has shown that effluents derived from elemental chlorine bleaching are indeed the most toxic. Toxic properties of kraft mill effluents were evaluated in previous studies using Microtox and *Daphnia magna* bioassays (Rao et al. 1994), or fish biomarker tests (Tana et al. 1994).

A chlorinated lignin effluent (pH of 8.62 and a dry matter content of 3.86 g/L) from the pre-bleaching stage applied on the Kraft cellulose (provided by SOMES SA Dej, Romania) was also tested in germination experiments.

Copper and zinc (essential micronutrients) are frequently related to soil pollution, and are potentially toxic to living organisms. Copper (Cu) is necessary for plant growth in low concentrations, a structural part of enzymes, and is uptaken as divalent cation (Cu^{2+}) or Cu chelate. Copper, however, is often present in high concentrations that are toxic enough to biota. Copper is necessary for carbohydrate and nitrogen metabolism and, inadequate copper results in stunting of plants. Copper also is required for lignin synthesis, which is needed for cell wall strength and prevention of wilting.

Zinc is an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation. Zinc deficient plants exhibit delayed maturity. Zinc is not mobile in plants, so zinc-deficiency symptoms occur mainly in new growth. Poor mobility in plants suggests the need for a constant supply of available zinc for optimum growth (http://www.ecochem.com/t_micronutrients.html).

Algae and daphnia are the most sensitive test organisms for heavy metals, followed in order of decreasing sensitivity by Microtox (*Photobacterium fisherii*), seed germination, and earthworms (Miller et al. 1985). The *Arabidopsis thaliana* plant bioassay involving growth in vermiculite in a hydroponic system with test chemicals added to the nutrient solution reservoir (Ratsch et al. 1986) evidenced a reduction in total biomass following different patterns with the chemicals tested, the sensitivity increasing in the order: fly ash < trichloroacetic acid (TCA) < copper sulfate.

Our last experiment has used salt solutions having different metal ions, namely ZnSO_4 (40 μM), and CuSO_4 (10 μM) with high values of concentration comparatively with those usually used in hydroponic media for wheat plants (Utah State University, 1996).

Conditions for the germination experiments are presented in Table 1.

Table 1. Experimental Conditions for Seed Germination

Test type	Static
Temperature	23 \pm 2°C
Test vessel	20×20 mm Petri dish plus Whatman Number 1 filter paper
Test solutions	thermal power station ash (water extract 0.25 g/L dry matter content) - I _G
	chlorinated lignin (1.158 g/L dry matter content) - II _G
	Zn ²⁺ solution (ZnSO_4) - III _G
	Cu ²⁺ solution (CuSO_4) - IV _G
Test volume	5 mL per dish
Number of seeds	10 per dish
Number of Petri dishes per test	10
Number of experiments per test	3
Reference test (control)	distilled water
Test duration	4 days
End point	Germination, primary root \geq 5 mm

Relative seed germination percentage, root elongation, and germination index were determined using the following relationships:

Relative seed germination, % = $\frac{\text{number of seeds germinated in test solution}}{\text{number of seeds germinated in control}} \times 100$

Relative root elongation, % = $\left\{ \frac{\text{mean root length in test solution}}{\text{mean root length in control}} \right\} \times 100$

Germination index = $(\% \text{ seed germination} \times \% \text{ root growth}) / 100$

Hydroponics Seedling Tests

Normal healthy seeds of different crop plant species, provided by AGROSEM Seed Center Iasi, namely wheat *Gabriela base* type (*Triticum aestivum* L.), rye (*Secale cereale*), and corn *Santiago* type (*Zea mays*) were chosen for germination in Petri dishes with addition of 5 mL distilled water to each dish, at temperature of $23 \pm 2^\circ\text{C}$, for 96 hours (4 days).

Preliminarily, the seeds of cultivars were surface sterilized with hydrogen peroxide for 30 minutes. Seeds with a visible shoot or root were counted as germinated.

Young seedlings, presenting a normal development, were further introduced in special vessels designed for hydroponics experiments (black cups with mesh bottoms and covered with black beads), with addition of nutrient solutions as can be seen in Table 2. Cups were positioned above nutrient solution in holes of pots fitted with aeration tubes. A daily aeration of hydroponics media was performed, maintaining also constant both the solution volume and temperature ($23 \pm 2^\circ\text{C}$). The experiment was randomly arranged with each treatment in triplicate. Plants were grown under glasshouse conditions with natural light, day/night temperature of $30/25^\circ\text{C}$, and day/night humidity of 70/90%. Nutrient solution pH was adjusted to 5.5 with 0.1M NaOH or 0.1M HCl and was renewed at every fourth day during the experiment (28 days).

Table 2. Nutritive Solution Composition in Hydroponics Media*

Salt	Solution concentration	mL/50 L nutritive solution	Final concentration of nutritive solution
Ca(NO ₃) ₂	1 M	50.00	1 mM
KNO ₃	2M	25.00	1 mM
KHPO ₄	0.5 M	50.00	0.5 mM
MnSO ₄ · 7H ₂ O	0.25 M	100.00	0.5 mM
ZnSO ₄ · 7H ₂ O	20 mM	10.00	4 μM
H ₃ BO ₃	20 mM	5.00	2 μM
CuSO ₄	20 mM	2.50	1 μM
(NH ₄) ₆ Mo ₇ O ₃₄ · 4H ₂ O	0.6 mM	7.50	0.09 μM
FeSO ₄ · 7H ₂ O	50 mM	100.00	10 μM
KCl	100 mM	5.00	10 μM
MnSO ₄	60 mM	2.50	3 μM

* Utah State University, Crop Physiology Laboratory, Current Nutrient Solution Wheat-January 1996-modified

The first variant (I) is the control application, with no addition of other substances. Extract of thermal power station ash was introduced in hydroponics media (variant II), while chlorinated lignin products solution was added for variant III. Mineral elements in excess were added in order to assess their effects on plant growth (variants IV and V). The plants dry weight was determined upon harvesting after 28 days of vegetation. Plants were harvested and subsequently dried at 70°C to a constant weight.

The cellulose and lignin content were determined in accordance with the literature as follows: cellulose (by gravimetry), after reaction with a 1:4 v/v mixture of concentrated nitric acid and ethyl alcohol (Pettersen 1984); lignin (by gravimetry) after 72% sulfuric acid hydrolysis (by TAPPI norm T 222 om-88).

Cellulose and lignin are the building blocks of all plants. Their chemical properties make them a substrate of enormous biotechnological value. The basic chemistry of cellulose and lignin has a profound effect on lignocellulose tertiary architecture. These associations act like physical and chemical barriers and may have a real significance in testing the action of different harmful stress factors on plants' growth.

For variants IV and V, the amount of Zn, respectively Cu, added to the nutritive solution was greater than that used in the control application, being multiplied with 10. A daily aeration of hydroponics media was performed, maintaining also constant both the solution volume and temperature ($23 \pm 2^\circ\text{C}$). Each hydroponics seedling test was replicated three times for statistical purposes.

RESULTS AND DISCUSSION

Germination Experiments

A seed germination test has been used as a rapid, simple, reliable and reproducible technique to indicate the damaging effects of different industrial wastes on plant growth. Inhibition of root elongation was a valid and sensitive indicator of toxicity. Germination index (GI) which combines germination and root growth, has proved to be a very sensitive parameter, being able to account both for low toxicity, which affects root growth and increased toxicity which affects germination.

Experimental data on germination tests performed by using the seeds of the wheat (two cultivars - *Gabriela base* and *Fundulea 4*) and rye crop plants and different solutions mentioned in the first section of Experimental, are presented in Table 3.

The relative seed germination of wheat (*Fundulea 4*) was retarded in the presence of thermal power station ash, the effect being more pronounced for rye. However, trace elements like Cu, Co, Ni, Se, Al, and Cr present in the fly ash might impair seed germination process and so, either delayed or inhibited the process (Wong and Wong 1989; Singh et al. 1997). The toxic properties of coal fly ash samples obtained from various coal combustion power plants were evaluated, for example, in other bioassays (Tsiridis et al. 2006), including *Daphnia magna*, which was the most sensitive test organism.

Table 3. Germination Indicators for the Crop Plants in Study

Germination indicator /Crop species	Culture variant			
	I _G	II _G	III _G	IV _G
<i>Relative germination, %</i>				
wheat (Gabriela base)	113	100	110	118
wheat (Fundulea 4)	96	100	94	94
rye	85	100	95	105
<i>Relative root elongation, %</i>				
wheat (Gabriela base)	117	24	88	87
wheat (Fundulea 4)	111	17	73	56
rye	119	25	66	67
<i>Germination index</i>				
wheat (Gabriela base)	132	24	97	103
wheat (Fundulea 4)	107	17	69	53
rye	101	25	63	70

Zn excess induces decrease of this test for the same plant species, while Cu affects only germination of wheat (Fundulea 4). The effects of metals can be plant-specific. The toxicity of the metals, including Ag, Cu, Fe, Mn, and Zn, was evaluated during the Toxicity Identification Evaluation (TIE) procedure performed with *Daphnia magna* (48-h immobility) and *Lactuca sativa* (lettuce) seeds (96-h root elongation), and this can be ordered as Ag>Cu>Zn>Fe>Mn for *Daphnia* and Ag=Zn=Fe=Cu>Mn for lettuce seeds (Fjällborg et al. 2006).

The responses of the three plant species to the toxicity of industrial wastes in terms of relative root elongation were different. With respect to root development, the root elongation of all plant species was significantly retarded by chlorolignin products throughout the experiment. The root lengths of wheat and rye seedlings in chlorolignin effluent were only 17-25% of the control. By contrast, the fly ash extract stimulated root elongation of all plant species; their root lengths were even longer than the control. An insignificant reduction on root lengths for wheat (Gabriela base) in the presence of heavy metals excess was recorded. This reduction was more significant for wheat (Fundulea 4) and rye. Root development was found to be more susceptible to stresses such as metal toxicity than was seed germination (Carlson et al. 1991).

Although the relative root elongation was to a large extent inhibited, the relative seed germination was not affected by chlorolignin (for all plant species) and heavy metals (for wheat Gabriela base) presence in germination media. These results suggested that relative root elongation was a more sensitive test than relative seed germination.

The germination index has proven to be a very sensitive parameter, since it combines germination and root growth (Tam and Tiquia 1994). Its values greater than 97% indicated the disappearance of toxicity. The most sensitive crop plant species in the presence of heavy metals excess are represented by wheat (Fundulea 4) and rye. Chlorolignin poses significant toxicity on the all plant species, a fact evidenced by the calculated percentages of relative root elongation and germination index.

The germination test revealed that fly ash extract had low toxicity on plant species. It may have stimulatory effects on plant growth due to the presence of mineral

nutrients (Singh et al. 1997). The sensitivity to toxicity increases in the order: fly ash<heavy metals<chlorolignin.

Hydroponics Seedling Tests

A decrease in length of the aerial part for two plant species, rye and corn, in comparison with the control application, was evidenced for the culture variants IV (Zn in excess) and V (Cu in excess) – see Table 4. An opposite effect was observed for wheat plants.

Table 4. Growth Intensity for Plant Species Evaluated in Different Hydroponics Media

Culture variant	Growth intensity for plant species under study from visual observation performed each 48 hours during 28 days of vegetation		
	Plant species		
	wheat	rye	corn
I	++++	++++	++++
II	++++	++++	++++
III	++++	++++	++++
IV	+++++	++	++
V	+++	++	++

Biometric measurements (mean root, aerial part, and plant length) have evidenced a different behavior of crop seedlings, depending on the applied treatments (Figures 1-3).

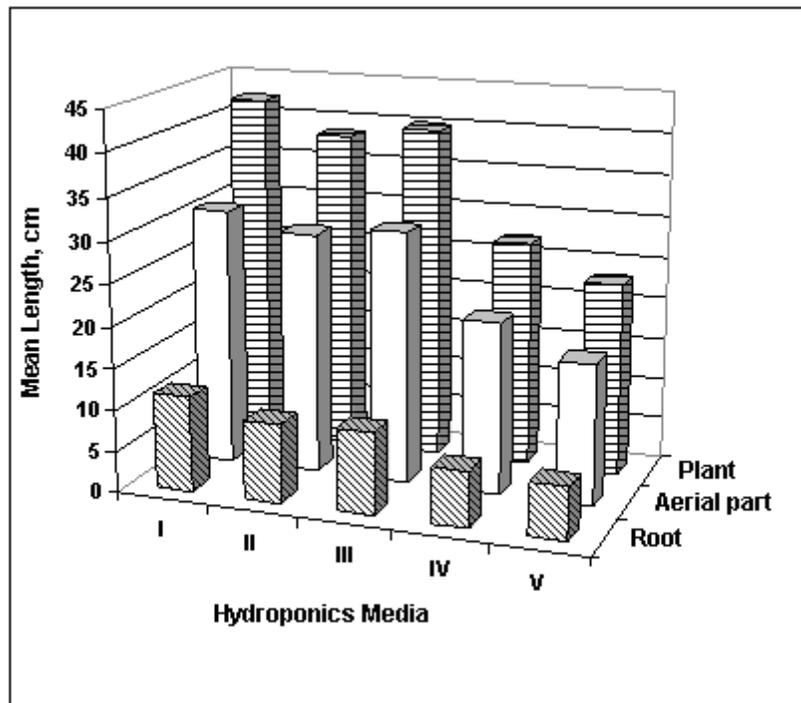


Fig.1. Mean length of *Triticum aestivum* L. (wheat) seedlings in hydroponics media.

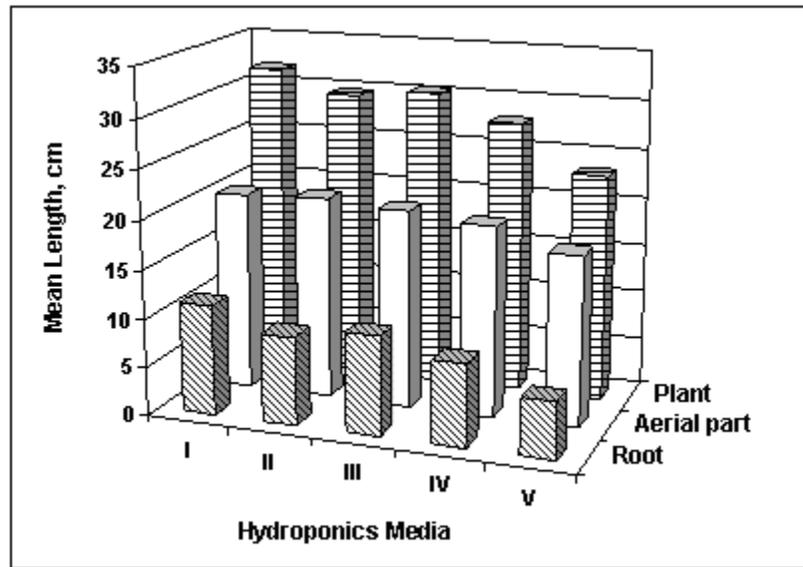


Fig. 2. Mean length of *Secale cereale* (rye) seedlings in hydroponics media.

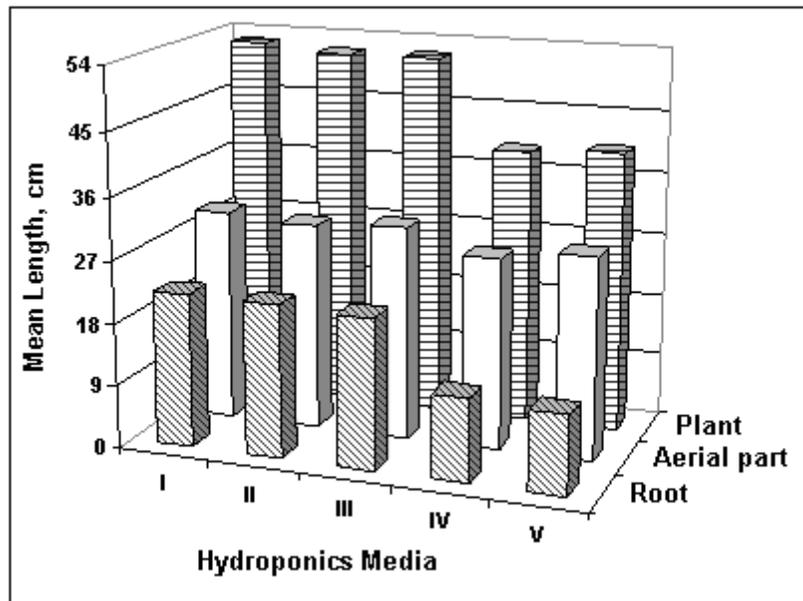


Fig. 3. Mean length of *Zea mays* (corn) seedlings in hydroponics media.

Biomass production (as dry weight) after 28 days of seedling growth in hydroponics tests presented some differences between crop plants, depending on the applied treatment (Figure 4).

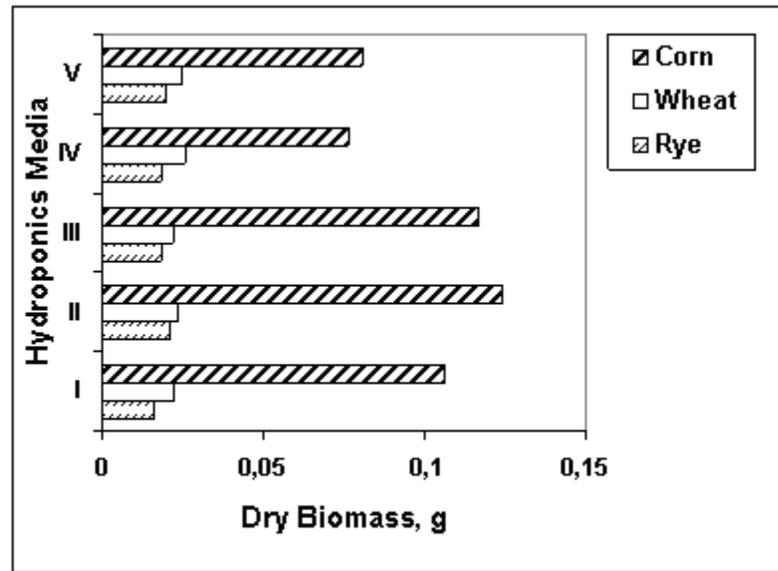


Fig. 4. Effects of different hydroponics media on the growth of crop seedlings (dry weight).

Figure 5 shows the effect of different hydroponics media on the cellulose content in crop seedlings. As was expected, the toxicity of chlorinated lignin products was the most pronounced in rye and corn, while wheat did not show any toxic effect of treatment under this experimental condition. There can be noticed higher values for cellulose content in the corn seedlings in Cu-enriched media and rye seedlings in Zn-enriched media. A positive effect was given by thermal power station ash treatment, especially on the rye seedlings.

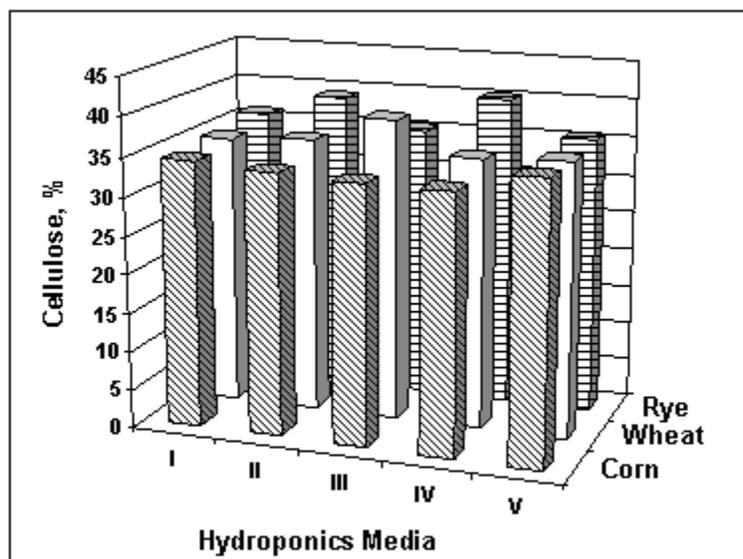


Fig. 5. Effects of different hydroponics media on the cellulose content in crop seedlings.

Figure 6 shows the effect of different treatments toward the lignin content in crop seedlings grown in hydroponics media. The lignin content presented lower values for rye seedlings, in comparison with the control application. An increase of this component was noticed for wheat plants treated with the ash extract, as well as for the corn seedlings in the presence of chlorolignin products, and excess of Zn and Cu.

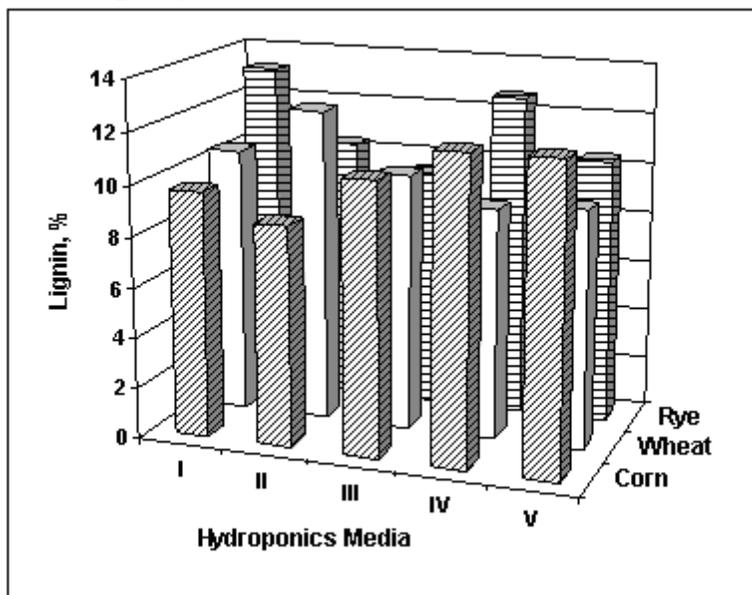


Fig. 6. Effects of different hydroponics media on the lignin content in crop seedlings.

There were significant differences between the culture variants, depending on the crop plant species and solutions tested in germination experiments. Significant dependencies of the relative seed germination were found for the wheat Gabriela base type, for all tested solutions. Lower values both for relative root elongation and germination index were observed for all crop plants, in the presence of the chlorolignin, all these data showing an inhibitory effect of it.

As it can be observed from Table 2, the growth of all seedlings in the presence of thermal power station ash (II), and chlorinated lignin products (III) was almost the same with that for the control seedlings in hydroponics experiments.

The lowest values for aerial part length were observed for all plant seedlings (variants IV and V) in the presence of a metal excess (Zn, respectively Cu), as well as in the presence of thermal power station ash (variant I). An excess of Cu had a significant inhibitory effect on the wheat and rye seedlings, while Zn was more effective as an inhibitor on the corn seedlings. Zinc treatment was effective for the wheat seedlings, both for root and aerial part growth. A significant decrease of dry biomass accumulation was observed for the corn seedlings treated with an excess of Zn and Cu, in comparison with the control application.

Cellulose content of the seedlings grown in hydroponics media was effectively influenced by the treatments with excess of Zn and Cu (see the corn, respectively rye seedlings). The lignin content was reduced in the rye seedlings for all culture variants in comparison with the control application. The positive effect of thermal power station ash

was shown especially for lignin in wheat seedlings, while for the corn ones this had greater values for hydroponics media containing chlorinated lignin products and heavy metals excess.

CONCLUSIONS

1. There are clear differences in the sensitivity of the test methods presented here. The results of the hydroponics tests appear to be much less conclusive in comparison with those observed in germination tests. In the context of our work, the germination index, as a quantitative indicator, is more suitable for assessing the toxicity of industrial wastes tested in experiments. Hydroponics tests offer no advantage regarding sensitivity, but are useful to obtain information. A combination of several test methods, using organisms with different levels of complexity, provides reliable results for the evaluation of toxicity.
2. The influence of crop plant characteristics and test solutions on the germination capacity has been shown, with seeds from different crops presenting a different germination potential. A better relative germination was shown in the case of wheat seeds of Gabriela base type, for all tested solutions.
3. The chlorolignin products have an inhibitory effect on all crop plants in germination experiments, a fact shown by the lower values observed both for relative root elongation and germination index.
4. Hydroponics tests provide evidence of the different behavior of crop seedlings depending on the growth media composition, a fact revealed by visual observation and biometric measurements.
5. A decreasing of the aerial part length for rye and corn seedlings is observed for Zn and Cu treatments, in comparison with the control application. Zinc treatment has a positive effect on the wheat seedlings, both for root and aerial part length.
6. A significant decrease of dry matter accumulation is observed for corn seedlings treated with an excess of Zn and Cu, in comparison with the control application.
7. Different treatments applied to seedlings in hydroponics media have a real influence on the biomass accumulation, expressed also by cellulose and lignin contents.
8. An increase of cellulose content in the rye seedlings treated both with thermal power station ash and excess of Zn, in comparison with the control application is observed, while the lignin value decreases. For corn seedlings, the cellulose content is higher in the presence of Cu in excess, while the lignin content is lower, especially in the presence of thermal power station ash. It seems that an excess of Zn has a stimulating action on lignin biosynthesis in all crop plant seedlings.

REFERENCES CITED

- Albert, R. J. (1994). "Survey shows worldwide growth in mills using TCF processes," *Pulp & Paper* Jun 1994, 1-10.

- An, Y.-J. (2006). "Assessment of comparative toxicities of lead and copper using plant assay," *Chemosphere* 62(8), 1359-1365.
- ASTM (2002). *Standard Guide for Conducting Terrestrial Plant Toxicity Tests*, Method E 1963-98, ASTM, Philadelphia.
- Bailey, H. C., and Young, L. (1997). "A comparison of the results of freshwater aquatic toxicity testing of pulp and paper mill effluents," *Water Sci. Technol.* 35(2-3), 305-313.
- Bowen, G., and Roveria, A. (1976). "Microbial colonization of plant roots," *Ann. Rev. Plant Phytopathology* 14, 121-144.
- Cammarota, M. C., and Sant'Anna, G. L., Jr. (1992). "Decolourization of kraft bleach plant stage effluent in a fungal bioreactor," *Environ. Technol.* 13, 65-71.
- Carlson, C. L., Adriano, D. C., Sajwan, K. S., Abels, S. L., Thomas, D. P., and Driver, J. T. (1991). "Effects of selected trace metals on germinating seeds of six plant species," *Water, Air & Soil Pollution* 59, 231-240.
- EPA Office of Research and Development Project EPA/540/R-92/003, 1991, *Ecological Assessments of Hazardous Waste Sites: A Field and Laboratory Reference Document*, edited by William Warren-Hicks, Benjamin R. Parkhurst, Samuel S. Baker, Jr, p. 6-32.
- EPA Office of Compliance Sector Notebook Project, *Profile of the Pulp and Paper Industry*, 2nd Edition, EPA/310-R-02-002
<http://www.epa.gov/compliance/resources/publications/assistance/sectors/notebooks/>
- Esposito, E., Canhos, V. P., and Duran, N. (1991). "Screening of lignin degrading fungi for removal of colour from kraft mill wastewater with no additional extra carbon-source," *Biotechnology Letters* 13(8), 571-576.
- Fjällborg, B., Li, B., Nilsson, E., and Dave, G. (2006). "Toxicity identification evaluation of five metals performed with two organisms (*Daphnia magna* and *Lactuca sativa*)," *Arch. Env. Contam. Toxicol.* 50(2), 196-204.
- Fuentes, A, Lloréns, M., Sáez, J, Aguilar, M. I., Ortuño, J. F., and Meseguer, V. F. (2004). "Phytotoxicity and heavy metals speciation of stabilized sewage sludges," *J. Hazard. Mater.* A108, 161-169.
- Gorsuch, J. W., Lower, W. R., Lewis, M. A., and Wang, W. (1991). *Plants for Toxicity Assessment*, volume 2, ASTM STP 1115, ASTM, Philadelphia.
- Jala, S., and Goyal, D. (2006). "Fly ash as a soil ameliorant for improving crop Production - A review," *Bioresource Technol.* 97, 1136-1147.
- Keeling, A.A., Paton, I.K., and Mullett, J.A.J. (1994). "Germination and growth of plants in media containing unstable refuse-derived compost", *Soil Biol. Biochem.* 26, 767-772.
- Kristen, U. (1997). "Use of higher plants as screens for toxicity assessment," *Toxicology in Vitro* 11, 181-191.
- Lau, S. S., and Wong, J. W. C. (2001). "Toxicity evaluation of weathered fly-ash amended mature compost," *Water, Air & Soil Pollution* 128, 243-254.
- Lin, J., Jiang, W., and Liu, D. (2003). "Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (*Helianthus annuus* L.)," *Bioresource Technol.* 86, 151-155.

- Martin, V. J. J., Burnison, B. K., Lee, H., and Hewitt, L. M. (1995). "Chlorophenolics from high molecular weight chlorinated organics isolated from bleached kraft mill effluents," *Holzforschung* 49 (5), 453-461.
- Miller, W. E., Peterson, S. A., Greene, J. C., and Callahan, C. A. (1985). "Comparative toxicology of laboratory organisms for assessing hazardous waste sites," *J. Environ. Qual.* 14, 569-574.
- OECD (1984). *Terrestrial Plants, Growth Test*, Guideline for Testing of Chemicals 208.
- Pettersen, R. (1984). *Chemical Composition of Wood*, The Chemistry of Solid Woods, R. M. Rowell (ed.), Advances in Chemistry Series 207, American Chemical Society, Washington D.C.
- Popa, V. I., and Teacă, C. A. (1998). "Influence of chlorolignin products on wheat plants (*Triticum aestivum* L.) growth and development," *Proceedings of the 7th International Conference on Biotechnology in the Pulp and Paper Industry*, June 16-19, 1998, Vancouver, Canada, Poster Presentations C231-C233 (Part 3).
- Popa, V. I., Popa, M., Bodârlău, R., and Teacă, C. A. (2000). "Biosynthesis of vegetal compounds in the presence of pollutants," *Rev. Roum. Chimie* 45(7-8), 725-729.
- Rao, S. S., Burnison, B. K., Rokosh, D. A., and Taylor, C. M. (1994). "Mutagenicity and toxicity assessment of pulp mill effluent," *Chemosphere* 28(10), 1859-1870.
- Ratsch, H. C., Johndro, D. J., and McFarlane, J. C. (1986). "Growth inhibition and morphological effects of several chemicals in *Arabidopsis thaliana* (L.) Heynh.," *Environ. Toxicol. Chem.* 5(1), 55-60.
- Schultz, E., Vaajasaari, K., Joutti, A., and Ahtiainen, J. (2002). "Toxicity of industrial wastes and waste leaching test eluates containing organic compounds," *Ecotoxicology and Environmental Safety- Environmental Research, Section B* 52, 248-255.
- Singh, S. N., Kulshreshtha, K., and Ahmad, K. J. (1997). "Impact of fly ash soil amendment on seed germination, seedling growth and metal composition of *Vicia faba* L.," *Ecological Eng.* 9, 203-208.
- Sodergren, A. (1993). *Bleached Pulp Mill Effluents: Composition, Fate and Effects in the Baltic Sea*, Swedish Environmental Protection Agency Report No: 4047., 150 pp.
- Suntio, L. R., Shiu, W. Y., and Mackay, D. (1988). "A review of the nature and properties of chemicals present in pulp mill effluents," *Chemosphere* 17 (7), 1249-1290.
- Tam, N. F. Y., and Tiquia, S. M. (1994). "Assessing toxicity of spent sawdust pig-litter using seed germination technique," *Resour. Conserv. Recycl.* 11, 261-274.
- Tana, J., Rosemarin, A., Lehtinen, K.-J., Hardig, J., Grahn, O., and Landner, L. (1994). "Assessing impacts on Baltic coastal ecosystems with mesocosm and fish biomarker tests: A comparison of new and old wood pulp bleaching technologies," *Science of the Total Environment* 145, 213-234.
- TAPPI (US Technical Association of Pulp and Paper Industry): "Acid insoluble lignin in wood and pulp," Method T 222 om-88, 1988.
- Teacă, C. A., and Popa, V. I. (2000). "Studies on the behaviour of some individual biological systems under chlorolignin products action," *Bulletin of Iasi Polytechnic Institute*, Fasc. 3-4, Tom XLVI(L), 129-136.

- Tiquia, S. M., Tam, N. F. Y., and Hodgkiss, I. J. (1996). "Effects of composting on phytotoxicity of spent pig-manure sawdust litter," *Environmental Pollution* 3, 249-256.
- Tripathy, A., and Sahu, R. K. (1997). "Effect of coal fly ash on growth and yield of wheat," *J. Environ. Biol.* 18(2), 131-135.
- Trollenier, G., and Hect-Bucholz, C. (1984). "Effect of aeration status of nutrient solution on microorganisms, mucilage and ultrastructure of wheat roots," *Plant and Soil* 80, 381-390.
- Tsiridis, V., Samaras, P., Kungolos, A., and Sakellaropoulos, G. P. (2006). "Application of leaching tests for toxicity evaluation of coal fly ash," *Environmental Toxicology* 21(4), 409-416.
- Utah State University (1996). *Current Nutrient Solution Wheat*, Crop Physiology Laboratory, (www.usu.edu/~cpl/nutrwht.html).
- Wang, W. (1991). "Literature review on higher plants for toxicity testing," *Water, Air & Soil Pollution* 59, 381-400.
- Wang, W., Gorsuch, J. W., and Lower, W. R. (1990). *Plants for Toxicity Assessment*, ASTM STP 1091, ASTM, Philadelphia.
- Wong, M. H., and Wong, J. W. C. (1989). "Germination and seedling growth of vegetable crops in fly ash amended soils," *Agric. Ecosyst. Env.* 26, 23-25.
- Wuncheng, W. (1985). "The use of plant seeds in toxicity tests of phenolic compounds," *Environment International* 11, 49-55.
- Zucconi, F., Pera, A., Forte, M., and Bertoldi, M. (1981). "Evaluating toxicity of immature compost," *Biocycle* 22, 54-57.

Article submitted: Dec. 7, 2007; Peer review completed: Jan. 31, 2008; Revised version received: Sept. 12, 2008; Article accepted: Sept. 16, 2008; Published: Sept. 23, 2008.