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RESEARCH ARTICLE

Evaluation of phenol detoxification by *Brassica napus* hairy roots, using *Allium cepa* test

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Abstract
Introduction Meristematic mitotic cells of *Allium cepa* constitute an adequate material for cytotoxicity and genotoxicity evaluation of environmental pollutants, such as phenol, which is a contaminant frequently found in several industrial effluents.
Results and discussion In the present work, *Brassica napus* hairy roots (HR) were used for phenol removal assays. The toxicity of post-removal solutions (PRS) and phenol solutions was analyzed. These HR removed the contaminant with high efficiency (100–80% for phenol solutions containing 10–250 mg/L, respectively). Phenol solutions treated with *B. napus* HR showed a significant reduction of general toxicity compared to untreated phenol solutions, since the IC50 values were 318.39 and 229.02 mg/L, respectively. Moreover, PRS presented lower cytotoxicity and genotoxicity than that found in phenol solutions untreated. The mitotic index (MI) observed in meristematic cells treated with PRS (100 and 250 mg/L of phenol) showed an increase of 35% and 42%, whereas the chromosome aberrations showed a significant decrease. According to these results, *B. napus* HR cultures could be used for the treatment of solutions contaminated with phenol, since we observed not only high removal efficiency, but also an important reduction of the general toxicity, cytotoxicity, and genotoxicity.

Keywords Cytotoxicity · Genotoxicity · Phenolic compound · Phytoremediation · Removal

Abbreviations

HR	Hairy roots
PRS	Post-removal solutions
MI	Mitotic index
IC50	Inhibitory concentration 50 value

1 Introduction

In the last few years, the increasing discharge of hazardous chemicals into the environment, has affected the balance of natural ecosystems and has consequently called the attention of several researchers and governmental agencies to the health of living organisms. Among the damages caused by toxic chemical agents to exposed organisms, genotoxic and mutagenic effects have shown to be of great concern due to

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P. S. González · G. A. Maglione · M. Giordana · C. E. Paisio · M. A. Talano · E. Agostini
Departamento de Biología Molecular, FCEFQN, Universidad Nacional de Río Cuarto, 5800, Río Cuarto, Córdoba, Argentina

G. A. Maglione
e-mail: gmaglione@gmail.com

M. Giordana
e-mail: micaelagiordana@hotmail.com

C. E. Paisio
e-mail: cpaisio@exa.unrc.edu.ar

M. A. Talano
e-mail: mtalano@exa.unrc.edu.ar

E. Agostini
e-mail: eagostini@exa.unrc.edu.ar

P. S. González (✉)
Departamento de Biología Molecular, FCEFQN, UNRC, Ruta 36 Km 601, CP 5800, Río Cuarto, Córdoba, Argentina
e-mail: psolangeg@hotmail.com

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their capacity to induce genetic damage (Leme and Marin-Morales 2009). As is well-known, phenols are commonly used in several agricultural applications, and they are also released from coal and petroleum refining activities. They constitute a risk to human health, due to their toxic, carcinogenic, mutagenic, and teratogenic effects (Entezari and Pétrier 2004; Paisio et al. 2009). Thus, they are considered as priority pollutants in the US EPA list and their discharge in the aquatic environment is beginning to be restricted. Therefore, the elimination of phenolic compounds from the environment is of great importance.

Conventional technologies play a key role for the removal of phenolics from water and soils. These methods, however, have serious limitations, so researchers have become increasingly interested in looking for biological alternatives, such as phytoremediation. Phytoremediation technology employs plants and/or *in vitro* cultures derived from plants, to remove or reduce the concentration of toxic organic and inorganic pollutants in air, soil, ground water, wastewater, and biowastes (Schnoor 1997). This technology has been recognized as a cheap and eco-friendly alternative, and has generated great interest in the last few years (Newman and Reynolds 2004). Most experiments for phytoremediation studies were performed with normal soil-grown or hydroponically grown plants (Dec and Bollag 1994; Flocco et al. 2002). However, some interesting results were obtained with the help of *in vitro* cell and tissue cultures (Suresh et al. 2005; Singh et al. 2006). In this context, hairy roots (HR) were used to assess the potential of several plant species to remove contaminants from the environment. For example, HR cultures of black nightshade (*Solanum nigrum*) metabolize and remove polychlorinated biphenyls (PCBs) from solutions spiked with PCB congeners (Macková et al. 1997; Rezek et al. 2007). Similarly, HR of indian mustard (*Brassica juncea)* and chicory *(Cichorium intybusand*) were used for removal of pesticides like dichlorodiphenyltrichloroethane (DDT) (Suresh et al. 2005). In addition, HR of several species have been used to compare the tolerance and removal of high levels of phenols (Santos de Araujo et al. 2006). In HR of many plant species, the role of peroxidase enzymes in phenolic metabolism was studied. They have been considered to be the key factor in the removal of phenol and chlorophenols from the culture medium (González et al. 2006; Santos de Araujo et al. 2006; Singh et al. 2006; Coniglio et al. 2008).

In previous works, we established that *Solanum lycopersicum, Brassica napus*, and *Nicotiana tabacum* HR were able to remove phenol with high efficiency (Coniglio et al. 2008; González et al. 2008; Talano et al. 2010). It is not clear, however, whether the use of HR, reduces the toxicity of the treated phenol solutions. To evaluate the toxic/genotoxic risks of polluted solutions, toxicity, and genotoxicity tests employing microorganisms, plant cells, and mammalian cells have been used alone or in combination with chemical analysis (Žegura et al. 2009). Comparisons between plant and non-plant genetic assay systems indicate that higher plant genetic assays have a high sensitivity (i.e., few false negatives), and they are inexpensive and easy to handle. Among the higher plants species used to evaluate environmental contamination, the more frequently used are *Allium cepa, Vicia faba, Zea mays*, and *Nicotiana tabacum* (Grant 1994). In particular, *A. cepa* has been regarded as favorable to assess chromosome damages and disturbances in the mitotic cycle, because *A. cepa* cells have large chromosomes in a reduced number (Levan 1938). Thus, this test has been used to study the genotoxicity of a great variety of environmental pollutants, such as heavy metals, pesticides, aromatic hydrocarbons, and complex mixtures of pollutants (Leme and Marin-Morales 2009).

Due to phenol toxicity, the toxicity of remediated solutions needs to be taken into account for future application in phytoremediation. Then, the aim of the present study was to evaluate the toxicity, cytotoxicity, and genotoxicity of phenol solutions before and after the application of *B. napus* HR, using *A. cepa* test.

2 Materials and methods

2.1 Plant material

HR cultures of rapeseed (*B. napus*) were obtained by inoculation of sterile leaf explants with *Agrobacterium rhizogenes* strain LBA 9402, as previously described (Agostini et al. 1997). They were sub-cultured every 30 days in Murashige–Skoog liquid medium (Murashige and Skoog 1962), enriched with vitamins and kept in an orbital shaker at 100 rpm, at 25°C±2°C in the darkness. After this period of time, roots were harvested and used for the experiments described below.

2.2 Phenol removal assays

Removal assays were carried out using 10, 50, 100, 150, 200, and 250 mg/L of phenol. These solutions were used because concentrations of the same order of magnitude can exist in heavily contaminated sites and in industrial effluents (Paisio et al. 2009).

Phenol solutions were treated with 4 g of HR/100 mL phenol solution and 5 mM H_2O_2 in a total volume of 750 mL. The reaction mixture was incubated during 1 h at 25°C±2°C in an orbital shaker at 100 rpm (González et al. 2006). After incubation, residual phenol and H_2O_2 in the reaction mixtures, denominated post-removal solutions (PRS), were measured through spectrophotometric assays.

2.3 Phenol determination

Phenol determinations were carried out following the method of Wright and Nicell (1999). Aliquots of 100 μL of each sample were mixed with 100 μL of 4-aminoantipyrine (20.8 mM), 100 μL potassium ferricyanide (83.4 mM), and 700 μL of sodium bicarbonate (0.25 M pH 8.4). After 5 min, the absorbance of the colored compound formed was determined at 510 nm, which was proportional to the concentration of phenol in the range of $0–10^{-4}$ M. The results were expressed as removal efficiency, calculated according to Agostini et al. (2003).

2.4 Hydrogen peroxide determination

Residual H_2O_2 in PRS was evaluated using the procedure described by Sergiev et al. (1997). Samples of 500 μL were mixed with 500 μL of 10 mM potassium phosphate buffer, pH 7. Then, 1 mL of 1M KI was added and the mixture was homogenized. The absorbance was measured at 390 nm, in a Beckman spectrophotometer, and the data were transformed to H_2O_2 concentration using a calibration curve, which was carried out with known concentrations of H_2O_2 (Merck). The concentration of H_2O_2 in standard solutions was determined spectrophotometrically using ε_{240nm}=43.6 M^{-1} cm^{-1}.

2.5 *Allium cepa* L. test

Commercial onion bulbs (*A. cepa*), weighing between 2 and 4 g, were used in the assay. The outer scales of the bulbs and the dry bottom plate were removed, without destroying the root primordia, before the beginning of the test. The basic protocol of Fiskesjö (1985, 1993) was followed. Briefly, onions were grown in mineral water for 48 h and the bulbs with satisfactory root lengths (10–15 mm) were used in the study, while those with exceptionally long or short roots were discarded. Then, groups of five bulbs were exposed during 5 days to the following solutions: (a) Phenol concentrations of 10, 50, 100, 150, 200, and 250 mg/L, to evaluate phenol toxicity, (b) H_2O_2 (0.5 mM), to evaluate the toxicity of this substrate, since it was used in phenol treatment and concentrations below 0.5 mM were detected in PRS, (c) PRS originally containing 100 and 250 mg/L of phenol, (d) mineral water as negative control, (e) methyl methanesulfonate 10 mg/L as positive control. These solutions were changed each day.

For the evaluation of cyto and genotoxicity, three root tips from each onion were removed after two division cycles (48 h) and fixed in 95% ethanol–glacial acetic acid (3:1) for 24 h. They were then transferred to 70% ethyl alcohol and stored at 4°C. For microscopic assay, the root caps were removed and the root tips were hydrolyzed for 10 min with HCl 1N. The caps were washed with distillate water and were placed on a slide. Microscope slides were prepared by squashing, and stained with 2% Orcein–45% acetic acid.

All the assays were done by quintuplicate (five bulbs of each concentration and negative control). Of each bulb, three root tips were used for the microscopic assays.

Mitotic index (MI) was determined by scoring approximately 15,000 cells (900–1,000 cells per slide). MI was calculated as the percentage ratio of dividing cells and total number of observed cells and the results were expressed as percentage of the control.

The number of chromosomal aberrations was analyzed by counting 7,500 cells per treatment, being 500 cells per slide, comprising a total of 15 slides and they were classified as aneugenic and clastogenic aberrations.

To assess toxicity, after 5 days of exposition to the tested samples, the root length and other macroscopic parameters (morphological alterations, color change, root tip shape, presence of swelling (c-tumors), hooks or twists in the roots, were recorded as indicative of general toxicity (Fiskesjö 1985, 1993). The inhibitory concentration 50 value (IC50), which indicates the concentration of phenol or PRS that produces 50% of growth inhibition, was determined.

2.6 Statistical analysis

Statistical analysis was performed using STATISTICA 7.1 software package. All the data were analyzed using ANOVA. In all cases, $p \leq 0.05$ was statistically significant. Dunnett test was used for comparing several treatment groups with a control. IC50 value was calculated using the EPA Probit Analysis Program (US EPA 1998).

3 Results

3.1 Phenol removal assays using *B. napus* HR

In previous works, we found that *B. napus* HR constitute an appropriate biotechnological tool for phenol removal, since this system was able to oxidize high phenol concentrations with high efficiency in a short time (Coniglio et al. 2008). In the present study, phenol removal efficiencies obtained with *B. napus* HR were 100% to 97% for phenol solutions from 10 to 50 mg/L, and between 87% and 80% for solutions containing from 100 to 250 mg/L of phenol, respectively (Fig. 1).

3.2 Toxicity analysis of phenol, H_2O_2, and PRS

In order to compare the toxicity of PRS, it was necessary to evaluate the toxicity of the different components of the reaction mixture. Thus, the toxicities of phenol solutions (from 10 to 250 mg/L) and H_2O_2 0.5 mM were analyzed

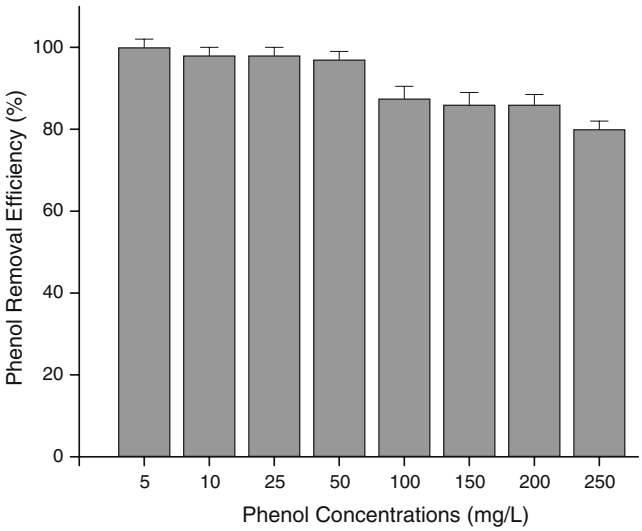

Fig. 1 Phenol removal efficiencies of different phenol solutions using *B. napus* hairy roots. The results were expressed as removal efficiency ±standard error

using *A. cepa* bulbs. In addition, two PRS with different initial phenol concentration (100 and 250 mg/L) were also evaluated.

The degree of toxicity of the analyzed samples was assessed through the mean value of root length (Table 1). Onion roots exposed to mineral water (negative control) had an average length of 29.6 mm and showed normal morphology.

Samples containing 10 and 25 mg/L of phenol did not affect root length of *A. cepa*, whereas solutions containing 50 to 150 mg/L of phenol produced root growth inhibition but it was not statistically significant. On the contrary, a significant inhibition of root length (50% and 58%) was observed in bulbs exposed to 200 and 250 mg/L phenol solutions, respectively ($p<0.05$). Root length of onion bulbs exposed to PRS containing originally 100 mg/L of phenol, was similar to those obtained with 100 mg/L phenol solution without treatment. However, onion bulbs treated with PRS originally containing 250 mg/L of the pollutant, showed an increase of 34% in the root length, compared with phenol solution of the same concentration.

The differences observed in the roots number from bulbs exposed to all phenol solutions tested were not statistically different compared to the control ($p>0.05$) (Table 1). The bulbs exposed to PRS presented higher root number than those treated with phenol solutions containing 100 and 250 mg/L of the pollutant, but the results were not statistically significant ($p>0.05$).

The solution containing 0.5 mM H_2O_2 did not affect significantly neither root length nor root number.

To evaluate macroscopic morphological abnormalities, the presence of hooks, swelling (c-tumors), or twists were analyzed as toxicity indicators (Fiskesjö 1985, 1993; Aganovic-Musinovic et al. 2004) (Table 1). Swelling (c-tumors) and twists were not observed. However, all the studied solutions induced hook formation, but only 200 and 250 mg/L phenol solutions exerted significant changes ($p<0.05$). Moreover, these roots presented a brown color and the highest number of hooks. Similarly, *A. cepa* roots treated with PRS presented brown color, but a reduction not significant statistically of hook number was observed, compared with untreated phenol solutions of the same concentration.

Based on the results shown in Table 1, we determined IC50 values using Probit analysis. For phenol solutions, the IC50 value was 229 mg/L, whereas for PRS was 318 mg/L, which represents an important decrease in acute toxicity of PRS.

Table 1 Mean values of root length, root number, and hook number in *A. cepa* cells exposed to H_2O_2, different phenol concentrations and PRS

Treatments	Root length (X±SE) (mm)	Root number per bulb (X±SE)	Hook number per bulb (X±SE)
Negative control	29.6±8.2	53.5±19.6	5.1±2.7
Positive control	20.2±3.2	55.5±14.3	9.2±2.7
H_2O_2 0.5 mM	29.0±7.1	56.5±12.3	4.8±2.2
Phenol (10 mg/L)	29.1±2.2	54.8±12.6	6.0±2.5
Phenol (25 mg/L)	29.5±5.6	57.0±13.8	6.8±4.0
Phenol (50 mg/L)	25.1±5.5	62.6±13.2	7.4±4.7
Phenol (100 mg/L)	22.9±4.0	54.6±15.6	8.2±3.4
Phenol (150 mg/L)	22.9±4.7	49.6±17.5	7.2±3.9
Phenol (200 mg/L)	14.6±5.0[a]	47.6±28.4	10.0±3.6[a]
Phenol (250 mg/L)	12.4±3.6[a]	43.8±27.9	11.0±3.1[a]
PRS (100 mg/L)	23.5±3.3	57.4±15.4	7.2±2.1
PRS (250 mg/L)	16.6±2.0[a]	70.6±26.5	8.8±2.4

X Mean, *SE* standard error

[a] Values significantly different from negative control value ($p<0.05$) or the corresponding phenol solutions without treatment

3.3 Cytotoxicity and genotoxicity analysis

Cytotoxic and genotoxic effects of phenol, H_2O_2, and PRS were estimated on the basis of MI and chromosome aberrations. The results of positive and negative controls were included in the tables.

3.3.1 Mitotic index

MI is summarized in Table 2 and Fig. 2a. The MI was used as a parameter to assess the cytotoxicity of different solutions. The MI of bulbs grown in control solution was 8.3, whereas the MI obtained for the bulbs treated with H_2O_2 0.5 mM was similar to the control. When bulbs were treated with phenol solutions, the reduction of MI observed was concentration dependent. The lowest MI found was 3.3 for 250 mg/L phenol solution, with a significant decrease of 60% compared with the control ($p<0.05$).

On the other hand, the bulbs exposed to PRS (100 and 250 mg/L), showed a significant increase in the MI (from 6.3 to 8.5 and from 3.3 to 4.7) compared to the MI of bulbs exposed to phenol solutions of the same concentration but without treatment.

3.3.2 Chromosome aberrations

In this study, the percentage of total aberration produced by phenol and PRS was evaluated (Table 1 and Fig. 2b), but H_2O_2 (0.5 mM) was not analyzed since toxic effects (such as inhibition in root length, decrease in MI and increment in hook number) were not observed at this concentration. However, the presence of chromosome aberrations should not be totally discharged.

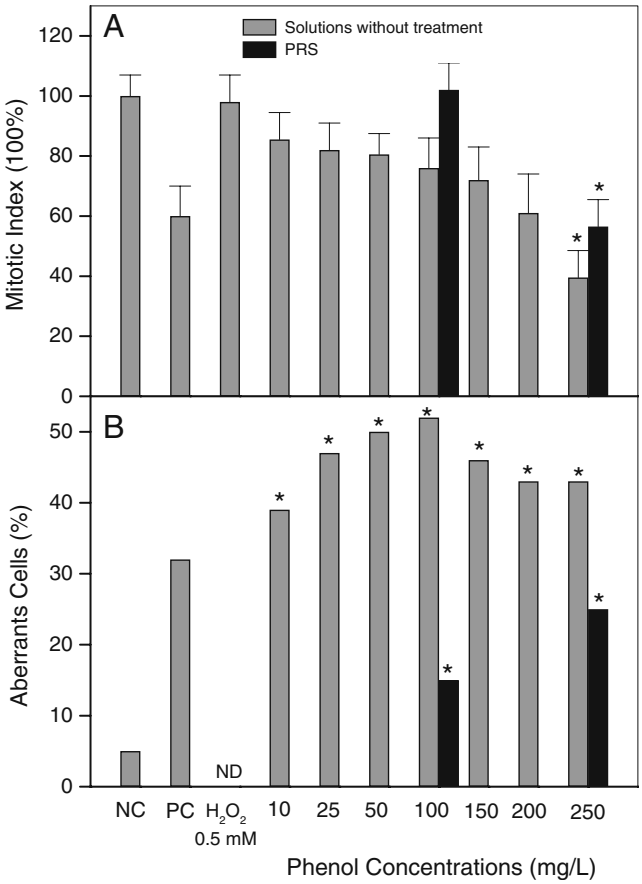

Fig. 2 Mitotic index (in percent) (**A**) and aberrant cells (in percent) (**B**) of *Allium cepa* root tips induced by different treatments: H_2O_2 0.5 mM, different phenol concentrations and PRS. *NC* Negative control, *PC* positive control, *ND* non-determinate. Standard errors were presented by *error bars*. Asterisks indicate significantly differences from negative control value ($p<0.05$)

Table 2 MI and number of different chromosomal abnormalities in *A. cepa* meristematic cells exposed to H_2O_2, different phenol concentrations, and PRS

			Chromosomic Aberrations			
	MI±SE (%)	No. of cell examined	Clastogenic	Aneugenic	Total aberrant cells	Aberrant cells (%)
Negative control	8.3±1.0	500	0	25	25	5
Positive control	5.1±1.1	500	nd	nd	160	32
H_2O_2 0.5 mM	8.2±0.9	nd	nd	nd	nd	nd
Phenol (10 mg/L)	7.1±0.9	500	10	185	195[a]	39[a]
Phenol (25 mg/L)	6.8±0.8	500	35	200	235[a]	47[a]
Phenol (50 mg/L)	6.7±0.7	500	35	215	250[a]	50[a]
Phenol (100 mg/L)	6.3±1.0	490	39	221	260[a]	52[a]
Phenol (150 mg/L)	6.0±1.1	485	34	189	223[a]	46[a]
Phenol (200 mg/L)	5.1±1.5	500	35	180	215[a]	43[a]
Phenol (250 mg/L)	3.3±0.9[a]	470	38	165	203[a]	43[a]
PRS (100 mg/L)	8.5±0.9[a]	500	0	75	75[a]	15[a]
PRS (250 mg/L)	4.7±0.9[a]	480	0	120	120[a]	25[a]

SE Standard error, *nd* non-determinate

[a] Values are significantly different from negative control value ($p<0.05$) or the corresponding phenol solutions without treatment

The highest number of aberrations was observed in metaphase and anaphase, in all the analyzed treatments. Chromosomal abnormalities were classified into two different classes: clastogenic aberrations, as a consequence of chromosomic rupture, and aneugenic aberrations, as a result of spindle inhibition during which anaphase chromosomes lay on the metaphase plate instead of moving towards their respective poles.

In the meristematic cells of *A. cepa* exposed to different phenol concentrations, the percentage of aberrant cells were found to be statistically higher than that found in control treatment. These cells showed both aberrations; however, a greater number of aneugenic abnormalities were observed.

Meristematic cells exposed to PRS showed a significant decreasing of percentage of aberrant cells from 52% to 15% and from 43% to 25% compared to their corresponding phenol solutions of 100 and 250 mg/L ($p<0.05$), and it is noteworthy that PRS did not produce clastogenic effects.

Figures 3 and 4 show normal division phases of meristematic cells of *A. cepa* and some chromosomal abnormalities that were found in meristematic cells exposed to phenol and PRS. The presence of chromosome bridges and breaks (chromosome rupture) are examples of clastogenic effects. On the other hand, chromosome losses, delays, and C-metaphase result from aneugenic effects.

4 Discussion

In a previous work, we found that rapeseed HR constitutes an appropriate biotechnological tool for phenol removal (Coniglio et al. 2008), as was also demonstrated in the present study. However, to verify the detoxification after applying a phytoremediation process, it was necessary to analyze the toxicity of PRS, as well as the toxicity of the different components of the reaction mixture, such as H_2O_2 and phenol, which can remain in the medium after remediation. The *A. cepa* test has often been used for the determination of cytotoxic and/or genotoxic effects of various substances, and it has shown high sensitivity to detect hazardous environmental chemicals (Fiskesjö 1985). Moreover, this test has been used to demonstrate the mutagenic activity of effluents and surface waters (Kungolos et al. 2006; Žegura et al. 2009). In the present study, toxic effects of phenol and PRS were evaluated based on the values of root length as well as root morphology of *A. cepa*.

Cytotoxicity and genotoxicity were estimated through several cytological parameters, such as MI and number and types of chromosome aberrations. It was shown that the concentration of H_2O_2 analyzed (0.5 mM) produced neither toxic nor cytotoxic effects on *A. cepa* bulbs, since the root length and MI did not change compared to the negative control.

It must be noted that data obtained from literature demonstrated that H_2O_2 could be toxic over a wide range of concentrations (0.1 to 0.88 mM) for several organisms, such as marine algae, *Daphnia magna*, fishes, and bacteria (Twiner et al. 2001; Meinertz et al. 2008). In addition, Paisio et al. (2009) showed that survival of *Rhinella arenarum* larvaes was not significantly affected by H_2O_2 concentrations ranging from 0.1 to 0.6 mM. However, in that study, a solution containing 1 mM H_2O_2 was toxic and produced a significant mortality. On the other hand, Radić et al. (2010) used a high H_2O_2 concentration (300 mM) as a positive control mutagen and they detected a decrease of 60% in root length and significant inhibition of MI (52%) compared to the negative control. This concentration induced also a great number of aberrations, and the main effect observed was stickiness followed by laggards and c-

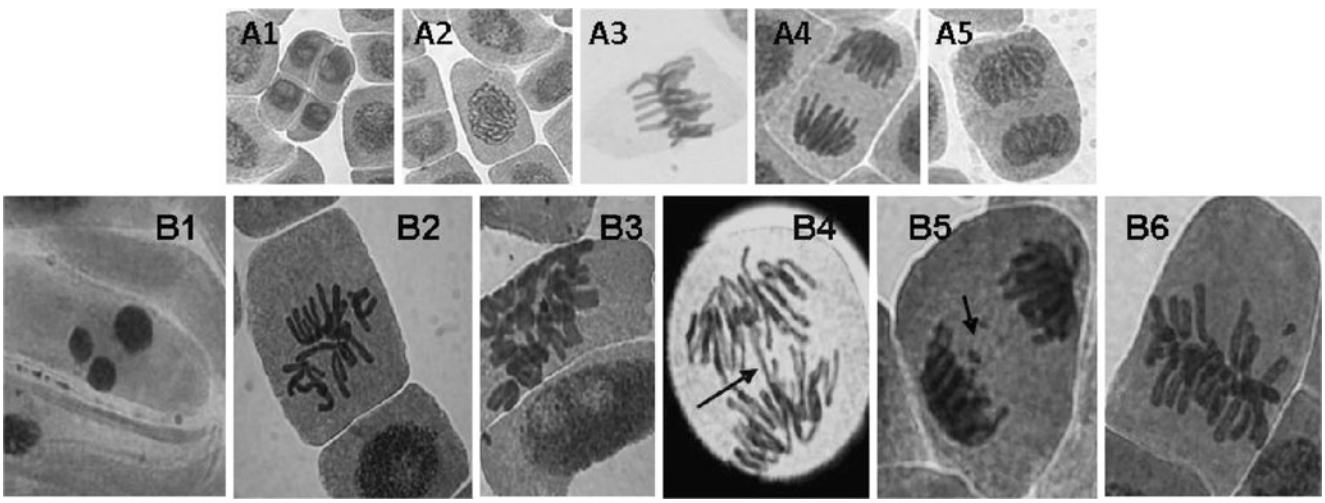

Fig. 3 Normal division phases of meristematic cells of *A. cepa*: interphase (*A1*), prophase (*A2*), metaphase (*A3*), anaphase (*A4*), and telophase (*A5*). Chromosome aberrations in cells exposed to phenol: multinucleated cell (*B1*), chromosome loss (*B2*), C-metaphase (*B3*), bridges (*B4*), chromosome breaks (*B5*), chromosome loss (*B6*)

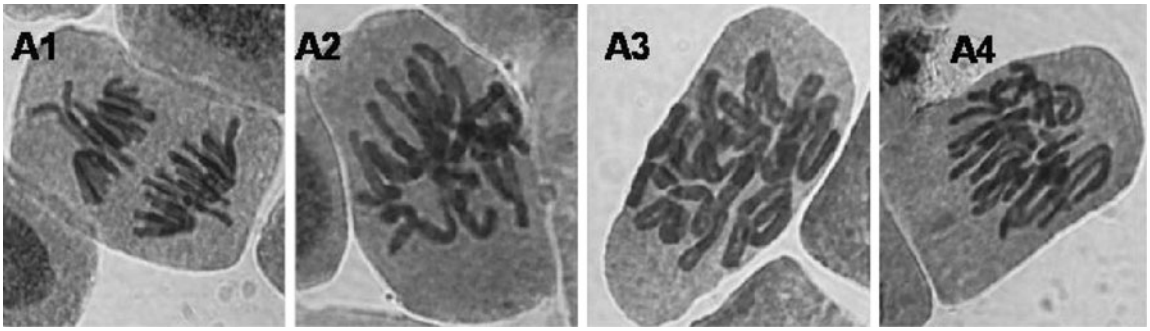

Fig. 4 Chromosome aberrations observed in meristematic cells of *A. cepa* exposed to PRS. Anaphase with advanced chromosomes (*A1*), abnormal metaphase (*A2*), C-metaphase (*A3*), and poliploid cell (*A4*)

methaphase (c-mitosis). However, it should be noted that the H_2O_2 concentration used by these authors was 600 times higher than those tested in the present work. As it was expected, the analyzed concentration did not produce toxic or cytotoxic effects. Thus, chromosomic aberrations were not analyzed in this study, although the presence of chromosome aberrations should not be totally discharged.

Considering that H_2O_2 toxicity is variable and depends on the concentration used as well as the organism tested, it is very important and necessary to determine H_2O_2 residual concentrations after remediation treatments, previous to the release of the remediated solution in the environment.

When phenol toxicity was evaluated, solutions containing 200 and 250 mg/L of the pollutant were phytotoxic, since the root number was lower and even they were shorter than the control; moreover, they presented morphological abnormalities (hooks) and brown coloration. Similarly, Ateeq et al. (2002), showed a dose-dependent root growth inhibition on *A. cepa* bulbs treated with herbicides. They found that pentachlorophenol was more toxic than butachlor, since 50% effective concentration (EC50) was 0.73 and 5.13 mg/L, respectively. In a similar study, with another herbicide, 2,4-dichlorophenoxyacetic acid, at concentrations from 25 to 100 mg/L, a high toxicity was observed. This compound induced striking changes both on the general growth and shape of the cells (Ateeq et al. 2002). These reports, as well as our results, indicate that phenolic compounds are toxic and genotoxic for meristematic root cells of *A. cepa*. These data are relevant to find an adequate remediation treatment for effluents containing phenolic compounds.

On the other hand, in bulbs exposed to PRS (250 mg/L), a toxicity reduction was observed, as we found an increase in root length of 34%, compared with their respective control solution. A parameter frequently used as indicator of acute toxicity is the IC50 value. The IC50 value obtained in the present study was 229.02 mg/L, whereas Arambasic et al. (1995), using *A. cepa* test, obtained a higher IC50 value for phenol (284.36 mg/L) after 48 h of treatment. The minor value of IC50 obtained by us may be associated with a higher exposition time (120 h) of the bulbs to the polluted solutions. In this sense, the increase in the exposition time seems to be responsible of the decrease in the IC 50 values. For instance, low IC50 values from 120 to 220 mg/L were obtained for other plants species, such as *Lactuca sativa, Raphanus sativus, Abutilon theophiasti, Milliacecum panicum*, and *Cucumis sativus* after 96–120 h of exposure (Wang 1985, 1987).

It is important to note that the increase in root number and length observed in the bulbs treated with PRS, as well as the higher IC50 value and the decrease of hook, compared with not treated phenol solutions, could indicate an important reduction in the toxicity of the treated solutions. These results are consistent with those described by Paisio et al. (2010) which also showed that the toxicity of 100 and 250 mg/L phenol solutions treated with *B. napus* HR, decreased significantly compared to phenol solutions without treatment, using the AMPHITOX bioassay.

Cytotoxic and genotoxic effects were estimated on the basis of MI and chromosomal aberrations. The reduction of MI observed in meristematic cells treated with phenol solutions, was concentration dependent, showing a decrease of 60% at the highest concentration (250 mg/L). This change is related to the cytotoxic effect of phenol on the exposed cells. Chauhan et al. (1998), for instance, suggested that the decrease of MI can be attributed to the effect of environmental chemicals on DNA/protein synthesis of the biological system. These results are in agreement with the studies conducted on *A. cepa* and *V. faba* exposed to tannery waste leachates, which showed significant decrease of mitosis and root growth (Chandra and Gupta 2002; Chandra et al. 2004). However, PRS (100 and 250 mg/L) showed different results since these solutions presented an increase in MI of 35% and 42% compared with control phenol solutions.

The increase in MI observed can be attributed to the reduction of the toxicity and cytotoxicity. Hoshina (2002) established that MI values lower than negative control could indicate alterations in the growth and development of the exposed organism.

The genotoxic study of root tips exposed to different phenol concentrations, showed changes in the organization and morphology of the chromosomes. The chromosome aberrations were significantly increased in the meristematic cells of *A. cepa*, exposed to all phenol concentrations assayed, compared to the control. The main types of chromosome aberrations detected were bridges, vagrant chromosomes, stickiness, loss, and fragment. Moreover, clastogenic and aneugenic effects were produced by these phenol solutions.

Stickiness is considered to be a chromatid-type aberration (Badr 1986). However, stickiness has been shown to be a result of DNA condensation (Österberg et al. 1984) and entanglement of inter-chromosomal chromatin fibers which led to subchromatid connections between chromosomes (Chauhan et al. 1986). Liu et al. (1992) suggested that sticky chromosomes reflect a highly toxic effect, usually of an irreversible type, and probably lead to cell death.

On the other hand, the presence of bridges could be attributed to chromosome breaks, stickiness and breakage and re-union of the broken ends. Sticky bridges might be also the result of incomplete replication of chromosomes by defective or less active replication enzymes (Sinha 1979; Badr et al. 1992).

The spindle irregularities like vagrant chromosomes were also observed after phenol treatment but it was at a lesser extent. The induction of this aberration leads to the separation of unequal number of chromosomes in the daughter cells with unequal-sized or irregularly shaped nuclei at interphase (El-Gharmery et al. 2003). In this sense, Ateeq et al. (2002) indicated abnormalities in the cytokinesis, observed as c-mitosis in *A. cepa* cells treated with butachlor, due to this compound acted as a potent spindle inhibitor.

All the above mentioned aberrations were induced by different phenol concentrations, which demonstrate the high phenol toxicity. For this reason, it is very important to select an effective method for phenol removal, which would be able to reduce the phenol concentration and, also to reduce the toxicity of PRS after the application of a removal treatment.

In this work, we proposed the use of *B. napus* HR as a suitable system for phenol removal. A genotoxic study, using *A. cepa* bulbs, was performed after treatment. The bulbs treated with PRS showed significant decreases in the percentage of aberrant cells. These decreases were from 52% to 15% and from 43% to 25% compared with 100 and 250 mg/L untreated phenol solutions, respectively. Moreover, it is important to note that the PRS did not induce clastogenic effects. Multinucleated cells, micronuclei, and deformed nuclei were practically not detected for the treatment analyzed, which indicates a genotoxicity reduction of these solutions; although these results might be confirmed by the micronucleous test (Maron and Ames 1983).

The reduction of the aneugenic effects and the absence of clastogenic effects are of great importance, since the aneugenic effects can be reversible (Amat et al. 2002).

In this context, the high phenol removal efficiency and the marked reduction of general toxicity, cytotoxicity as well as the genotoxicity after treatment with *B. napus* HR, makes this phytoremediation system an efficient method to be applied for phenol detoxification.

5 Conclusion

B. napus HR constitutes an efficient biotechnological tool for phenol removal. However, the toxicity of PRS is an aspect poorly studied. Thus, the present study used *A. cepa* test as a reliable method for detection of general toxicity, cytotoxicity and genotoxicity of H_2O_2, phenol and phenol solutions remediated with *B. napus* HR. It is important to remark that H_2O_2 at the concentration used in this study (0.5 mM), produced neither toxic nor cytotoxic effect.

Phenol solutions from 10 to 250 mg/L showed genotoxic effects on meristematic cells, since an increase of chromosome aberrations were observed. Moreover, high concentration of phenol solutions (200 and 250 mg/L) produced toxicity and cytotoxicity effects.

PRS, showed an important reduction of general toxicity, as well as in cytotoxicity and genotoxicity after treatment with *B. napus* HR, compared to phenol solutions without treatment.

In conclusion, the use of *B. napus* HR cultures could be an important tool to remove phenol, as well as to reduce the toxicity, cytotoxicity, and genotoxicity of solutions contaminated with this compound.

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