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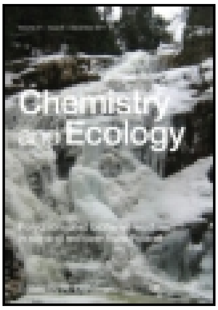

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Bioaccumulation and adverse effects of trace metals and polycyclic aromatic hydrocarbons in the common onion *Allium cepa* as a model in ecotoxicological bioassays

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The common onion *Allium cepa* can be easily used in ecotoxicological bioassays to evaluate the bioavailability and adverse effects of pollutants and complex mixtures like waste, industrial effluent or coal-mining drainage that contain elevated levels of trace metals and polycyclic aromatic hydrocarbons (PAHs). In this study, onions were exposed for 7 days to individual metals ($1\ \mu g \cdot mL^{-1}$), i.e., aluminium, copper, iron and manganese, or PAHs ($1.5\ ng \cdot mL^{-1}$), i.e., benzo[*a*]antracene and benzo[*a*]pirene. Biological effects, measured as growth inhibition of roots and leaves, were integrated with analysis of bioaccumulation in roots, bulbs and leaves. Copper, iron and benzo[*a*]pirene caused a significant inhibition in root development of newly formed tissues, whereas only slight variations were caused by other chemicals; the number of new root filaments and the length of the leaves did not show significant variations, thus not representing sensitive parameters to evaluate adverse effects of pollutants in *A. cepa*. Chemicals bioaccumulation was always significant in roots, whereas levels in bulbs and leaves exhibited increased levels only for manganese, and a decrease for aluminium. The overall results confirmed the sensitivity of the bioassays with *A. cepa*, suggesting their utility for future applications to evaluate the adverse effects of complex mixtures containing metals or PAHs.

Keywords: *Allium cepa*; trace metals; polycyclic aromatic hydrocarbons; bioaccumulation; toxicity; bioassay

1. Introduction

Heavy metals and polycyclic aromatic hydrocarbons (PAHs) are among the most important classes of pollutants, considering their natural origin and wide dispersion in the environment due to numerous anthropogenic activities [1]. These chemicals are generally involved in complex biogeochemical cycles in the earth's crust, atmosphere and water basins, and, being accumulated in the tissues of living organisms [2–6], can induce adverse effects at both molecular and cellular levels [7,8].

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Human exposure to metals and PAHs is responsible for several disturbances in the respiratory, digestive, immunitary, circulatory, endocrine and nervous systems, and also for chronic effects like mutagenicity and cancerogenity [9,10]. In plants, these pollutants can cause several phytotoxic effects, such as structural, physiological and biochemical changes in membranes, with consequent disturbances in membrane composition, rigidity, fluidity, influx of water and nutrients, alteration of the cell cycle and division, reduction in chlorophyll content and photosynthesis, and reduction in tissue growth [11–14]. In this respect, the possibility of measuring the bioaccumulation of chemicals and biological responses in plant tissues may be a valuable tool to assess the impact and toxicological effects of environmental pollutants [15].

The common onion *Allium cepa* has already been proposed as bioindicator organism to evaluate environmental contamination by various class of pollutants, including trace metals and PAHs [16]. Bioaccumulation in specific tissues and the inhibition of root and leaf elongation can be easily evaluated and previous studies have highlighted the practical advantages of such bioassays, including good sensitivity, reproducibility and rapidity of execution, in addition to simplicity and limited cost [17–19].

In this respect, *A. cepa* may be considered as a suitable indicator species to evaluate the potential toxicity of complex environmental mixtures like wastewaters, industrial effluent or coal-mining drainage, which are generally characterised by the presence of elevated levels of trace metals and PAHs. The coal industry is a major part of the economy in southern Santa Catarina (Brazil) and such mining activities generate acid effluents characterised by high concentrations of trace metals and PAHs [20,21]. Relatively few studies have attempted to evaluate the toxicity of coal-mining drainage [22] and the aim of this study was to validate a bioassay using *A. cepa* as a possible test for such environmental wastes. In light of this, bioaccumulation and adverse effects on tissues development were evaluated in onions exposed under laboratory conditions to aluminium (Al), copper (Cu), iron (Fe), manganese (Mn), benzo[*a*]antracene (B[*a*]A) and benzo[*a*]pirene (B[*a*]P), which represent the more abundant chemicals in coal-mining drainage.

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2. Materials and methods

2.1. *Laboratory exposure and determination of physiological parameters*

Specimens of the common white onion *A. cepa* were treated following a previously reported toxicological protocol [18]. Fresh specimens of common onions, obtained from a local market and of similar size (30.0 ± 2.0 g) were carefully washed before removing the roots and leaves when present. The resulting bulbs were placed on the top of tests tubes filled with 50 mL of individual solutions of metals (Al, Cu, Fe and Mn at 1 $\mu g \cdot mL^{-1}$) or PAHs (B[*a*]A and B[*a*]P at 1.5 $ng \cdot mL^{-1}$) in 1 mM K-phosphate buffer at pH 6.5 ($n = 10$ for each treatment); control onions ($n = 10$) were placed on tubes filled with K-phosphate buffer. The selected chemical concentrations can be considered to be environmentally realistic, within the ranges commonly measured in wastewaters, industrial effluent and acid mine drainage [20,22] and, based on previous observations, these are expected to mediate sublethal effects in tested *A. cepa* [18]. Solutions were changed daily and exposures were carried out for 7 days at 25°C in the dark. After this, the development of newly formed tissues was evaluated in terms of length, weight and quantity of roots and length of leaves.

2.2. *Chemical analyses*

Analytical determinations of both trace metals and PAHs were carried out following previous validated methods [23]. Briefly, to analyse trace metals, tissues (roots, bulbs and leaves; $n = 10$)

were dried at 60°C to constant weight. Approximately 0.2 g of dried samples was digested under pressure with 5 mL nitric acid in a microwave mineralisation system (CEM Mars 5, CEM Corp., Matthews, NC, USA), with the following programme: stage 1, 600 W, 110°C for 15 min; stage 2, 600 W, 160°C for 20 min; stage 3, cooling at < 50°C. Al and Cu were analysed using atomic absorption spectrophotometry (AAS) with electrothermal atomisation and Zeeman effect (SpectrAA240Z , Varian, Mulgrave, VIC, Australia), whereas Fe and Mn were determined by AAS with an air–acetylene flame atomisation (SpecrAA 220FS, Varian); concentrations were expressed as $\mu g \cdot g^{-1}$ dry weight (dw). Quality assurance and quality control were tested by processing, with the same procedures, blank samples and Standard Reference Material (SRM-NIST 2977, National Institute of Standards and Technology, Gaithersburg, MD, USA). The concentration obtained for the SRM were always within the 95% confidence interval of certified values.

To determine PAHs (B[*a*]A and B[*a*]P), ~ 1 g of wet tissue ($n = 10$) was extracted in 5 mL 0.5 M potassium hydroxide in methanol using a microwave system (150 W, 55°C for 15 min) (Mars CEM, CEM Corp.). After centrifugation at 1000 g for 5 min, the methanolic solutions were concentrated using an evaporating centrifuge SpeedVac system (RC1009; Jouan, Nantes, France) and purified with solid phase extraction (Octadecyl C_{18}, 500 mg × 6 mL, Bakerbond; Mallinckrodt Baker, Phillipsburg, NJ, USA). A final volume of 1 mL was recovered with acetonitrile, and HPLC analyses were carried out with a water–acetonitrile gradient and fluorimetric detection. PAHs were identified by the retention time of appropriate pure standard solutions (EPA 610 Polynuclear Aromatic Hydrocarbons Mix). Quality assurance and quality control were performed by processing blank and reference samples (SRM-NIST 2977), and concentrations obtained for the SRM were always within the 95% confidence interval of certified values. The water content in tissues was determined and concentrations of PAHs expressed as $ng \cdot g^{-1}$ dw.

2.3. *Statistical analyses*

Biological and chemical parameters in control and exposed onions were compared by Student's *t*-test or one-way analysis of variance (ANOVA, type I error fixed at 0.05). The homogeneity of variance was checked by Levene's test and when necessary *post-hoc* comparison (Student Newman–Keuls) were applied to discriminate between means of values.

3. Results

Variations in the physiological parameters of roots and leaves were estimated after 7 days of treatment and the results are shown in Figure 1. A significant inhibition of root development was mediated by Cu and Fe ($p < 0.05$), while only slight, not statistically significant variations were caused by Al, B[*a*]A and B[*a*]P. Particularly marked effects were observed for onions exposed to Cu ($p < 0.05$), determining a reduction of the length and weight of tissues of ~ 90%, compared with controls; more moderate variations were determined by Fe inhibiting root development of ~ 35%. The number of roots and leaves length were almost constant in control and treated onions, thus appearing as less sensitive physiological parameters (Figure 1).

Concentrations of trace metals and PAHs in newly formed roots and leaves, and in the bulbs of treated onions are shown in Figure 2. A statistically significant bioaccumulation was always observed in roots for all the chemicals, with values increasing from ~ 35 to 100 $\mu g \cdot g^{-1}$ for Al, from 12 to up to 30,000 $\mu g \cdot g^{-1}$ for Cu, from 40 to 120 $\mu g \cdot g^{-1}$ for Fe, from 35 to 600 $\mu g \cdot g^{-1}$ for Mn, from 22 to 160 $ng \cdot g^{-1}$ for B[*a*]A and from ~ 8 to 130 $ng \cdot g^{-1}$ for B[*a*]P ($p < 0.05$)

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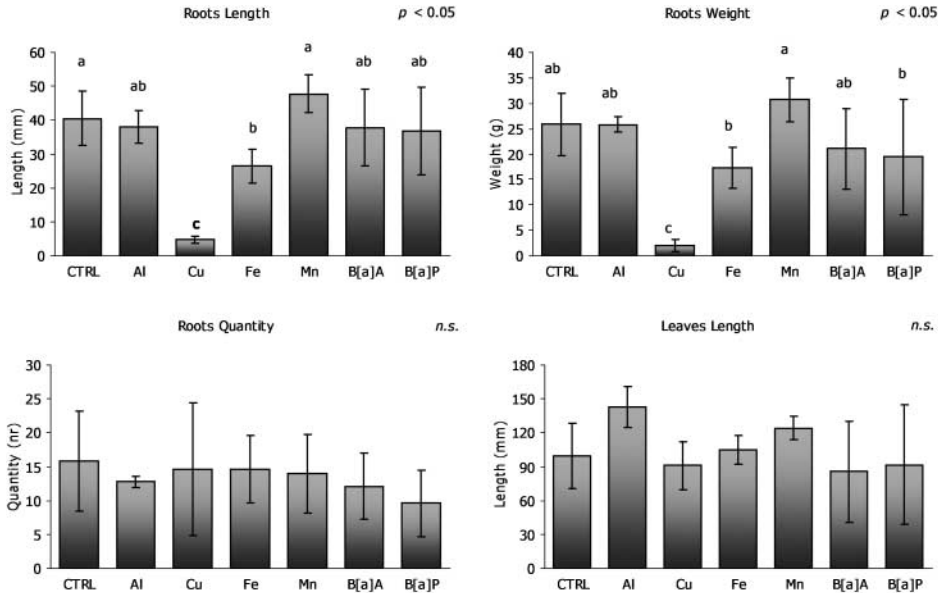

Figure 1. Changes in physiological parameters of *Allium cepa* exposed for 7 days to individual solutions of Al, Cu, Fe and Mn ($1\,\mu g \cdot mL^{-1}$) or to B[*a*]A and B[*a*]P ($1.5\,ng \cdot mL^{-1}$), compared with control onions. Evaluated parameters were related to newly developed roots (length, weight and quantity) and leaves (length). Results are expressed as mean ± SD ($n = 10$). Different letters indicate significant differences as determined by ANOVA and *post-hoc* test (n.s., not significant).

(Figure 2). No significant variations in chemicals levels were obtained in bulbs of treated *A. cepa* with the exception of Mn increasing from < 15 to $\sim 200\,\mu g \cdot g^{-1}$ ($p < 0.05$) and Al, decreasing by $\sim 50\%$, from about 100 to $50\,\mu g \cdot g^{-1}$ after 7 days of exposure ($p < 0.05$) (Figure 2). Also, leaves did not generally show variations in terms of bioaccumulation of chemicals and only Mn values significantly increased from ~ 20 to $27\,\mu g \cdot g^{-1}$ ($p < 0.05$) (Figure 2).

4. Discussion

A large number of industrial activities throughout the world produce increasing volumes of waste effluents, as well as sewage or mining drainages containing various classes of hazardous chemicals, like trace metals and PAHs. These polluted effluents often represent a relevant environmental issue with severe implications for human health and ecosystems, especially in developing countries where monitoring of industrial wastes and mining drainage may be limited for technical and economical reasons. In this respect, the possibility to test the adverse effects of waste effluents using simple and relatively low-cost bioassays might be of crucial importance [18,19].

Various plants have already been tested in laboratory bioassays due to their versatility. Among these, the common onion *A. cepa* presents some practical advantages, including good sensitivity and the possibility of testing several environmental conditions and complex matrices like wastewaters [18,19,24–26].

In a previous study, a noticeable sensitivity was observed for physiological responses of *A. cepa*, with significant effects even at the lower Cu exposure levels of $0.1\,\mu g \cdot mL^{-1}$; these characteristics, combined with the possibility of performing easy and low-cost experiments confirmed *A. cepa* as an eligible model for ecotoxicological bioassays [18,19].

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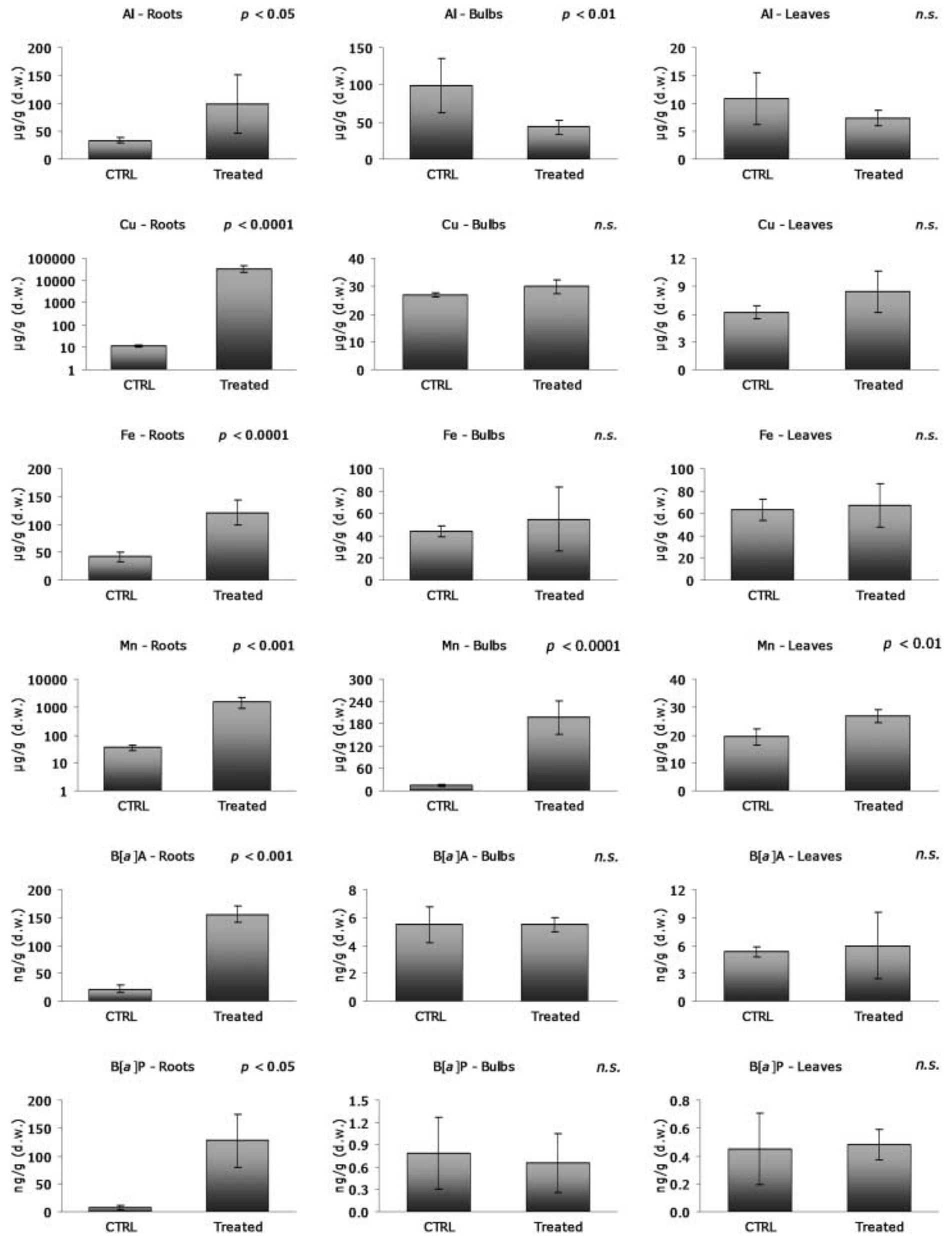

Figure 2. Bioaccumulation of Al, Cu, Fe, Mn, B[*a*]A and B[*a*]P in both the newly formed roots, bulbs and newly formed leaves of exposed *Allium cepa*, compared with basal levels in control onions. Results are expressed as mean ± SD ($n = 10$). Level of significance (p) indicates statistical differences between treated and control onions, as determined by Student's t-test (n.s., not significant).

The aim of this study was to extend our knowledge on the sensitivity of bioassays with *A. cepa*, for a wide range of trace metals ($1\,\mu g \cdot mL^{-1}$Al, Cu, Fe and Mn) and selected PAHs ($1.5\,ng \cdot mL^{-1}$B[*a*]A and B[*a*]P). The obtained results indicated that exposure to Cu, Fe and B[*a*]P can induce significant inhibition in the length and weight of onion roots, confirming that these tissues may be considered more appropriate for detecting the toxicicological effects of pollutants in wastewaters [18]. However, both the number of newly formed roots and the length of the leaves did not exhibit any significant variation between control onions and those exposed to chemicals, thus not representing valid parameters.

The suitability of the root as an optimal end point tissue in bioassays with *A. cepa* was further supported by their ability to concentrate pollutants from the exposure medium; in fact, bioaccumulation of both the trace metals and PAHs was always significant, whereas only Mn exhibited increased concentrations in bulbs and leaves of treated onions; because Mn may act as cofactor in some enzymatic processes, a possible function in onion metabolism cannot be excluded.

Interestingly, the concentrations of Al decreased significantly in bulbs of treated *A. cepa*, suggesting the involvement of a possible detoxification mechanism as a function of the intensity of exposure. However, additional data should confirm this hypothesis, also considering that there are no data on the capability of *A. cepa* to regulate the concentration of toxic metals; the potential transfer of chemicals between roots, bulbs and leaves also remains to be elucidated [18]. However, in a previous study, complete inhibition of tissue development and a significant decrease in copper concentrations in onion bulbs was observed after acute exposure ($10\,\mu g \cdot mL^{-1}$) to the metal, suggesting that plants were suffering a toxic state with general inhibition of their metabolic status. This may be of particular importance when *A. cepa* is tested under field conditions or with complex environmental matrices, because the presence of elevated levels of pollutants might drastically compromise the general status of the organisms, thus leading to an underestimation of chemical bioaccumulation [18].

In general, our results are in good agreement with previous studies revealing a significant reduction in root elongation as a consequence of exposure to various elements, such as Al, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn [19,24–27]. In some cases, inhibition of root development was associated to the onset of various toxic effects, such as a significant decrease in the mitotic index and the formation of chromosome aberrations during anaphase–telophase [19,24–27].

Bioassays with *A. cepa* also provided useful results for evaluating the effects of organic xenobiotics including petroleum hydrocarbons, PAHs, pesticides and other chemical agents in complex mixtures [19]. Formation of chromosomal aberration or micronuclei was detected in root cells of onion exposed to various aromatic compounds, including benzene and B[*a*]P [19,28,29]. The effects of this class of pollutants on tissue development have been poorly investigated. Exposure to fluoranthene at concentrations ranging from 2 to $10\,\mu g \cdot mL^{-1}$ caused a clear reduction in root length (up to $\sim$40%) and weight ($\sim$50%), suggesting that PAHs might promote disorder in the production of plant hormones controlling growth and development via the division, elongation and differentiation of cells [30].

In our study, we did not measure a marked inhibition of root development in onions exposed to PAHs, with significant effects measured for root weight after exposure to B[*a*]P; however, it is important to note that the previously cited fluoranthene concentrations [30] were at least three orders of magnitude higher than those reported here for B[*a*]A and B[*a*]P. However, in both these studies, environmentally relevant PAHs concentrations were tested, and the different biological responsiveness at increasing pollution levels confirms the suitability of bioassays using *A. cepa*, with good sensitivity even at low chemical concentrations.

The coal extraction and processing industry is a major economic resource for the southern region of Santa Catarina, Brazil. This activity generates effluents characterised by low pH levels (< 3.0) and elevated concentrations of trace metals and PAHs, representing a critical environmental issue. The common onion, *A. cepa*, might thus represent an important additional tool for assaying potentially harmful acid mine drainages, also considering that the biological effects of waste effluents on the living organisms are still not well studied. In this respect, our results confirm the suitability of tests based on *A. cepa* for future applications on monitoring and managing these contaminants.

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References

[1] L. Camus, S.R. Birkely, M.B. Jones, J.F. Børseth, B.E. Grøsvik, B. Gulliksen, O.J. Lønne, F. Regoli, and M.H. Depledge, *Biomarker responses and PAH uptake in* Mya truncata *following exposure to oil-contaminated sediment in an Arctic fjord (Svalbard)*, Sci. Total Environ. 308 (2003), pp. 221–234.

[2] J. Li, X. Shang, Z. Zhao, R.L. Tanguay, Q. Dong, and C. Huang, *Polycyclic aromatic hydrocarbons in water, sediment, soil, and plants of the Aojiang River waterway in Wenzhou, China*, J. Hazard. Mater. 173 (2010), pp. 75–81.

[3] G. Bonanno and R. Lo Giudice, *Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators*, Ecol. Indic. 10 (2010), pp. 639–645.

[4] A. Sánchez-Chardi, C. Peñarroja-Matutano, M. Borrás, and J. Nadal, *Bioaccumulation of metals and effects of a landfill in small mammals. Part III: Structural alterations*, Environ. Res. 109 (2009), pp. 960–967.

[5] J.M. Reichmuth, P. Weis, and J.S. Weis, *Bioaccumulation and depuration of metals in blue crabs (Callinectes sapidus Rathbun) from a contaminated and clean estuary*, Environ. Pollut. 158 (2010), pp. 361–368.

[6] A.H. Arias, C.V. Spetter, R.H. Freije, and J.E. Marcovecchio, *Polycyclic aromatic hydrocarbons in water, mussels (*Brachidontes *sp.,* Tagelus *sp.) and fish (*Odontesthes *sp.) from Bahía Blanca Estuary, Argentina*, Estuar. Coast Shelf Sci. 85 (2009), pp. 67–81.

[7] P. Kakkar and F.N. Jaffery, *Biological markers for metal toxicity*, Environ. Toxicol. Pharmacol. 19 (2005), pp. 335–349.

[8] D.M. Lowe, M.N. Moore, and J.W. Readman, *Pathological reactions and recovery of hepatopancreatic digestive cells from the marine snail* Littorina littorea *following exposure to a polycyclic aromatic hydrocarbon*, Mar. Environ. Res. 61 (2006), pp. 457–470.

[9] C. Szu-Chich and L. Chung-Min, *Health risk assessment on human exposed to environmental polycyclic aromatic hydrocarbons pollution sources*, Sci. Total Environ. 366 (2006), pp. 112–123.

[10] J. Yan, L. Wang, P.P. Fu, and H. Yu, *Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list*, Mutat. Res. 557 (2004), pp. 99–108.

[11] A. Heikens, W.J.G.M. Peijnenburg, and A.J. Hendriks, *Bioaccumulation of heavy metals in terrestrial invertebrates*, Environ. Pollut. 113 (2001), pp. 385–393.

[12] S. Khan, L. Aijun, S. Zhang, Q. Hu, and Y.G. Zhu, *Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation*, J. Hazard. Mater. 152 (2008), pp. 506–515.

[13] K.S. Arun, C. Carlos, L. Herminia, and S. Avudainayagam, *Chromium toxicity in plants*, Environ. Int. 31 (2005), pp. 739–753.

[14] L. Tamas, J. Huttova, I. Mistrik, M. Simonovicova, and B Siroka, *Aluminum-induced drought and oxidative stress in barley roots*, J. Plant Physiol. 163 (2006), pp. 781–784.

[15] R. Bocchetti, D. Fattorini, B. Pisanelli, S. Macchia, L. Oliviero, F. Pilato, D. Pellegrini, and F. Regoli, *Contaminant accumulation and biomarker responses in caged mussels,* Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas*, Aquat. Toxicol. 89 (2008), pp. 257–266.

[16] D.M. Leme and M.A. Marin-Morales, *Chromosome aberration and micronucleus frequencies in* Allium cepa *cells exposed to petroleum polluted water – a case study*, Mutat. Res. 650 (2008), pp. 80–86.

[17] F.R. Espinoza-Quiñones, N. Szymanski, S.M. Palácio, A.N. Módenes, M.A. Rizzutto, F.G.J. Silva, A.P. Oliveira, A.C. Oro, and N. Martin, *Inhibition effect on the* Allium cepa *L. root growth when using hexavalent chromium-doped river waters*, Bull. Environ. Contam. Toxicol. 82 (2009), pp. 767–771.

[18] R. Geremias, D. Fattorini, V.T.D. Fávere, and R.C. Pedrosa, *Bioaccumulation and toxic effects of copper in common onion* Allium cepa *L.*, Chem. Ecol. 26(1) (2010), pp. 19–26.

[19] D.M. Leme and M.A. Marin-Morales, Allium cepa *test in environmental monitoring: A review on its application*, Mutat. Res. 682 (2009), pp. 71–81.

[20] R. Laus, R. Geremias, H.L. Vasconcelos, M.C.M. Laranjeira, and V.T. Fávere, *Reduction of acidity and removal of metal ions from coal mining effluents using chitosan microspheres*, J. Hazard. Mater. 149 (2007), pp. 471–474.

[21] P.T. Pavei, C.T.B. Menezes, R.M. Lattuada, and G. Perin, *PAH toxicity effluents from acid mine drainage in southern Santa Catarina, Brazil,* SETAC Europe 17th Annual Meeting, Porto, Portugal, 2007.

[22] R. Geremias, R. Laus, J.M. Macan, R.C. Pedrosa, M.C.M. Laranjeira, J. Silvano, and V.T. Fávere, *Use of coal mining waste for the removal of acidity and metal ions Al (III), Fe (III) and Mn (II) in acid mine drainage*, Environ. Technol. 29 (2008), pp. 863–886.

[23] P. Kumar, R.K. Tewari, and P.N. Sharma, *Modulation of copper toxicity-induced oxidative damage by excess supply of iron in maize plants*, Plant Cell Rep. 27 (2008), pp. 399–409.

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[24] M. Yıldız, I.H. Ciğerci, K. Konuk, A.F. Fidan, and H. Terzi, *Determination of genotoxic effects of copper sulphate and cobalt chloride in* Allium cepa *root cells by chromosome aberration and comet assays*, Chemosphere 75 (2009), pp. 934–938.
[25] R. Srivastava, D. Kumar, and S.K. Gupta, *Bioremediation of municipal sludge by vermitechnology and toxicity assessment by* Allium cepa, Bioresour. Technol. 96 (2005), pp. 1867–1871.
[26] M.B. Arambasic, S. Bjeli, and G. Subakov, *Acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on* Allium cepa *L.,* Lepidium sativum *L. and* Daphnia magna*: Comparative investigations and the practical applications*, Water Res. 29 (1995), pp. 497–503.
[27] T. Bortolotto, J.B. Bertoldo, F.Z. Silveira, T.M. Defaveri, J. Silvano, and C.T. Pich, *Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays*, Environ. Toxicol. Pharmacol. 28 (2009), pp. 288–293.
[28] G. Fiskesjö and C. Lassen, *Benzo[*a*]pireno and nitrosoguanidine in the* Allium *test*, Mutat. Res. 97 (1982), p. 188.
[29] J. Rank and M.H. Nielsen, *Evaluation of the* Allium *anaphase–telophase test in relation to genotoxicity screening of industrial wastewater*, Mutat. Res. 312 (1994), pp. 17–24.
[30] M. Kummerova and T. Kmentovà, *Photo induced toxocity of fluoranthene on germination and early development*, Chemosphere 56 (2004), pp. 387–393.

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