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Evaluation of allowed parameters for nickel in freshwater bodies using the *Allium cepa* test

Avaliação dos parâmetros permitidos de níquel para corpos de água doce utilizando o teste de *Allium cepa*

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Abstract

Nickel compounds are disposed in large amounts in the environment, making it an ecotoxicological relevant contaminant, since these can bioaccumulate along the food chain and/or can cause DNA damage to the exposed organisms. In this sense, Brazilian law establishes 0.025 mg/l as the utmost boundary for the total nickel concentration allowed in freshwater bodies that consists in a quarter of the established by United States Environmental Protection Agency (EPA). This study evaluated three nickel concentrations, in nickel chloride ($NiCl_2$) shape, having as reference the limit established by CONAMA (National Environmental Council), using seeds of *Allium cepa*, in order to measure the cytotoxic, genotoxic and mutagenic potentials of this element. At such concentrations, it was not observed toxicity or cytotoxicity, whereas for all three concentrations employed, there was induction of genotoxic and potentially mutagenic effects, especially cells bearing micronuclei, buds and chromosome stickiness. Thus, it is inferred that nickel chloride has aneugenic and/or clastogenic action. This study is also an alert for the water contamination by metals, since both concentration used, the lowest one and the one permitted by Brazilian law induced damage to the tested organism. The study suggests the necessity of review in the established parameters for such metal in freshwater bodies.
Keywords: Environmental mutagenesis. Heavy metals. Genotoxicity. Mutagenicity.

Resumo

Compostos de níquel são descartados em grandes quantidades no ambiente, fazendo dele um contaminante de relevância ecotoxicológica, uma vez que podem se bioacumular ao longo da cadeia trófica e/ou induzir danos genéticos aos organismos expostos. Nesse sentido, a legislação brasileira estabelece o limite máximo da concentração total de níquel permitida em corpos de água doce em 0,025 mg/l, que consiste em um quarto do estabelecido pela Agência de Proteção Ambiental dos Estados Unidos (EPA). O presente trabalho avaliou três concentrações de níquel, na forma de cloreto de níquel ($NiCl_2$), tendo como referência àquela estabelecida como limite pela resolução 357/2005 do CONAMA (National Environmental Council), utilizando sementes de *Allium cepa*, com o intuito de mensurar o potencial tóxico, citotóxico, genotóxico e mutagênico deste elemento. Em tais concentrações, não foi verificada toxicidade ou citotoxicidade, ao passo que, para as três concentrações empregadas, observou-se a indução de efeitos genotóxicos e potencialmente mutagênicos, principalmente de células portadoras de micronúcleos, brotos e aderências cromossômicas. Dessa forma, infere-se que o cloreto de níquel possui ação aneugênica e/ou clastogênica. Esse estudo também serve de alerta para a contaminação aquática por metais, visto que a menor concentração utilizada e a permitida pela legislação brasileira induziram danos no organismo testado. Sugere-se a necessidade de revisar os parâmetros estabelecidos para este metal em corpos de água doce.
Palavras-chave: Mutagênese ambiental. Metais pesados. Genotoxicidade. Mutagenicidade.

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Introduction

Metals are natural components of the ecosystem, being naturally incorporated into aquatic system by geochemistry processes. The elements cluster, designated heavy metals, occurs in natural systems at little concentrations and present density equal or higher than 5 g/cm3 (FADIGAS et al., 2002). These elements are featured as persistent, toxic, and bio cumulative. In aquatic environments they exist in solution, in hydrated-ion free form or complexed by organic and inorganic binders. They could still be present in the solid shape, due to several associations with sediments and floating particulate material (STUMM; MORGAN, 1996; IARC, 2012).

The environmental contamination by heavy metals has been increasing significantly in the last decades, mainly as a result of the modern industry development. Among the heavy metals, the nickel is highlighted, which performs as the 24th abundant metal in the world crust. The most important nickel sources are the nickel sulphide shape ores. The nickel compounds are industrially employed in electroplating and electroforming, in the nickel-cadmium battery production and electronic equipment. Moreover, nickel alloys, like stainless steel, are used in many products manufacture. The main sources of environmental contamination by nickel are nickel and by-products production and processing, as well as the products recycling and elimination of wastes containing this metal (IARC, 2012). According to Denkhaus and Salnokow (2002), the fossil fuel burning process is responsible for the main portion of nickel compounds present in the atmosphere.

In Brazil, for controlling and taxing the contaminants limits in water bodies, it is used the resolution no 357 of 25 March 2005 of the National Environmental Council (CONAMA). According to this resolution, the maximum total concentration limit of nickel allowed in bodies of fresh water is 0.025 mg/l Ni. On the other hand, according to the EPA (United States Environmental Protection Agency), the nickel limit on freshwater bodies is 0.1 mg/l, that is the quadruple of the established by CONAMA (EPA, 1995). In addition, the World Health Organization (WHO) (2008) mentions the value 0.07 mg/l as a guideline for drinking water, however this value is considered as provisional since there is hazard evidence in consequence of nickel exposure, but available information is considered limited by such organization.

Bioassays with vegetables have been shown efficient for monitoring the genotoxicity and mutagenicity of environmental pollutants (FERNANDES; MAZZEO; MARIN-MORALES, 2007; FONTANETTI; SOUZA; CHRISTOFOLETTI, 2012). Besides being more sensitive and simpler than many methods used to detect genotoxic effects, bioassays with plants have advantages concerning the low cost of equipment and materials needed, as well as the adaptation of plants for *in situ* studies. Moreover, it is possible to establish a good correlation between the plant test system and mammals test system (GRANT, 1982).

Considering that nickel is a heavy metal of growing importance in aquatic and terrestrial ecosystems pollution and has toxic and carcinogenic effects on living organisms (DENKHAUS; SALNOKOW, 2002; IARC, 2012), this study aimed to evaluate the genotoxic and mutagenic effects of this metal in three concentrations, using *A. cepa* bioassays. Thereby, this study aims to provide groundwork for the regulation and improvement of concentration parameters of this component in freshwater bodies.

Material and Methods

Nickel as a contaminant

To assembly the bioassays it was used nickel chloride hexahydrate PA, Synth brand, CAS number C.1055.01.AF and molecular weight 237.70 mol.

Three-nickel concentrations were used in the onion tests. The first one (N1) of 0.0125 mg/l, in

other words, it is half of the maximum concentration CONAMA defined. The second concentration used (N2), 0.025 mg/l is the actual limit concentration established by the resolution. The third concentration (N3) was 0.050 mg/l which consists the double of the maximum total concentration of nickel allowed in freshwater bodies, although it is the health advisory limit (HAL) established by EPA for this compound (EPA, 1995).

Biological material

The *A. cepa* seeds of the same batch, Baia Periforme variety, were submitted to germination under controlled temperature 22°C in Petri dishes lined with filter paper moistened with the tested solutions.

Bioassays with A. cepa

The positive control test was done by subjecting the seeds to two agents, whose concentrations are potentially cytotoxic and mutagenic: the herbicide trifluralin, with aneugenic action at a concentration of 0.019 ppm (FERNANDES; MAZZEO; MARIN-MORALES, 2007) and MMS (methyl methanesulfonate), with clastogenic action at concentrations of 4 x 10-4 mol/l (RANK; NIELSEN, 1997). The negative control was done with seeds subjected to germination in ultrapure water.

After the germination and root growth of about 2 cm, part of these were collected and fixed in Carnoy I (3 parts ethanol to 1 part of acetic acid). The other part was transferred to plates with ultrapure water for a recovery period of 48 hours. Subsequently, these rootlets were also collected and fixed in Carnoy I. Slides were made by the crushing common technique, with root meristems and root F1 region tissues, subjected to the Feulgen reaction (MELLO; VIDAL, 1978). In order to carry out counter-staining and facilitate the spreading of the cells, it was added to the material a drop of carmine acid (2%). Potentially toxic, cytotoxic, mutagenic and genotoxic changes to the cells of the meristematic region were analysed, amounting to 5,000 for each investigated sample. The same number of cells was analysed for the negative and positive controls. To confirm the results, there was a repetition bioassay, in which all the methodology described above was reapplied.

Assessment of the toxic, cytotoxic, genotoxic and mutagenic effects

The toxicity was evaluated by the seed germination rate. The germination rate is obtained by the ratio between the germinated seeds number and all the seeds exposed to germination. For this variable, the statistical analysis was performed by bilateral ANOVA, with a significance level of 0.05.

The cytotoxicity was checked by analysis of cellular morphological changes indicative of cell death and the mitotic index frequency (MI) according to the equation: MI = (number of dividing cells / total number of observed cells) x 100.

The genotoxic potential was evaluated by the chromosomal abnormalities index (CAI), for which it was computed the cells bearing nuclear buds, bridges and chromosome losses, c-metaphases, chromosome adhesions and polyploidy cells. The CAI frequency was obtained according to CAI= (number of cells bearing chromosome aberrations / total number of observed cells) x 100.

The mutagenic potential was evaluated by mutagenicity index (MtI), which takes into account the number of cells bearing micronuclei and chromosome breakage. The index was calculated as: MtI = (total number of cells with MN and breaks / total number of observed cells) x 100.

To verify the attachment or reparation of damage in the meristematic region cells, induction of micronuclei in the F1 region cells were also counted. A total of 5,000 cells were examined in this region for each sample and treatment. The values obtained in all tests were compared with those obtained in the negative control, using Mann-Whitney statistical test, with significance set at 0.05. The Bioestat 5.0 statistical program was used.

Results

The potentially cytotoxic, genotoxic and mutagenic chromosomal and nuclear aberrations were analysed in the cells of the meristematic region of A. cepa roots. The values obtained for the germination index at different Ni concentrations were not statistically significant compared to the negative control. Therefore, the Ni was not toxic at the tested concentrations.

Similarly, cellular morphological changes indicative of cell death (Figure 1A) and the mitotic index scores were not statistically significant, showing that there was no cytotoxicity for any of the Ni concentrations used. These results were observed in both bioassays (Table 1).

Table 1 - Mitotic index and observed cellular aberrations after the *A. cepa* meristematic cells exposure at different Ni concentration, in the continuous treatment and after its recovery, in both bioassays.

Treatment	MI		CAI		MtI		CD	
	CT	Rec	CT	Rec	CT	Rec	CT	Rec
NC	12.74 ± 2.81	13.88±5.13	1.17±0.16	0.85±0.24	0.58±0.14	0.35±0.15	0.0±0.0	0.0±0.0
MMS	14.94 ± 3.20	17.19±3.34	9.22±2.28*a,b	8.10±3.26*a,b	7.34±2.49*a,b	6.04±3.28*a,b	0.0±0.0	0.0±0.0
TRIF	11.34±2.52	11.82±1.62	4.24±1.27*a,b	5.18±0.88*a,b	2.07±1.02*a,b	3.11±0.67*a,b	0.0±0.0	0.0±0.0
N1	14.88±3.11	12.70±3.42	3.716±1.32*a,b	1.88±0.53*a,b	1.16±0.47*a	0.76±0.27*a,b	2.2±4.92	1.4±3.13
N2	18.82±7.13	12.58±1.38	4.016±0.48*a,b	3.44±0.57*a,b	2.33±0.82*a,b	1.32±0.41*a,b	1.2±2.68	5.4±6.50
N3	11.79±1.08	14.20±1.94	4.02±0.58*a,b	2.96±0.67*a,b	2.60±0.90*a,b	1.45±0.23*a,b	0.4±0.84	3.2±4.44
NCr	13.92±2.60	12.10±2.20	1.63±0.74	1.72±0.34	0.84±0.47	0.86±0.39	0.0±0.0	0.2±0.45
MMSr	13.35 ± 2.87	14.35±2.38	5.69±1.36*a,b	6.76±1.61*a,b	4.36±1.01*a,b	4.46±1.29*a,b	2.4±2.61	0.2±0.45
TRIFr	15.11±2.51	11.94±2.77	5.23±0.76*a,b	5.45±1.02*a,b	2.54±0.52*a,b	3.43±1.42*a,b	0.0±0.0	0.0±0.0
N1r	15.45±1.11	15.41±3.95	4.48±1.74*a,b	5.73±1.78*a,b	2.41±1.22*a,b	1.34±1.60*a,b	0.6±0.89	1.2±1.79
N2r	14.01±1.40	13.43±2.91	4.94±0.83*a,b	6.19±0.81*a,b	3.46±0.92*a,b	3.12±0.78*a,b	0.0±0.0	3.8±7.43
N3r	15.22±1.39	12.42±2.39	6.13±1.20*a,b	7.12±0.84*a,b	3.99±0.45*a,b	4.94±0.48*a,b	0.6±1.34	0.2±0.45

*a statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.05$

*b statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.01$.

MI: Mitotic index; **CAI**: chromosomal abnormalities rate; **MtI**: mutagenicity index; **CD**: cell death; **CT**: Continuous treatment;Rec: Recovery treatment; **NC**: negative control; **MMS**: methyl methanesulfonate; **TRIF**: Trifluralin; **N1**:Solution containing 0.0125 mg/l of Ni; **N2**:Solution containing 0.025 mg/l of Ni; **N3**:Solution containing 0.050 mg/l of Ni; **NCr**: negative control in the repetition; **MMSr**: methyl methanesulfonate in the repetition; **TRIFr**: Trifluralin in the repetition; **N1r**:Solution containing 0.0125 mg/l of Ni in the repetition; **N2r**:Solution containing 0.025 mg/l of Ni in the repetition; **N3r**:Solution containing 0.050 mg/l of Ni in the repetition.

The observation of cells bearing chromosomal aberrations (Figure 1B - H), numerically expressed by CAI resulted, however, in statistically significant differences compared to negative control, for all the Ni concentrations evaluated. The significance was maintained after the recovery treatment and confirmed

by the experiment repetition (Table 1), demonstrating that Ni has a genotoxic potential. The potentially genotoxic changes sum showed a dose-dependent response in the repetition bioassay.

Among the genotoxic effects observed (Table 2), nuclear buds (Figure 1B) showed statistically significance for all three Ni concentrations evaluated in the first bioassay and, it only happened in the highest concentration (0.050 mg/l) in the repetition, even though the values obtained for the further concentrations are numerically similar to those observed in the first bioassay. Thus, nuclear buds occur in large numbers in both bioassays, although the significance has demonstrated distinct patterns between them, moreover, especially the first bioassay showed that the recovery treatment is not effective to mitigate this effect

Table 2 - Potentially genotoxic changes (chromosomal bridges and losses, C-metaphase, chromosomal adhesion and nuclear buds) found in *A. cepa* meristematic cells treated with different Ni concentrations, in the continuous treatment and after its recovery, in both bioassays.

	Genotoxic Effects									
Treatment	Nuclear buds		Chromosome bridges		Chromosome losses		C-metaphases		Chromosome adhesion	
	CT	Rec	CT	Rec	CT	Rec	CT	Rec	CT	Rec
NC	1.0±1.22	1.0±0.70	1±0.71	2.6±1.82	0.2±0.45	0.2±0.45	0.4±0.55	0.2±0.45	3.0±1.87	1.0±0.71
MMS	7.4±1.52^{*a,b}	6.4±2.97^{*a,b}	4.4±1.52^{*a,b}	8.2±4.14^{*a}	4.2±1.64^{*a,b}	2.4±2.30	1.2±1.79	0.0±0.0	3.8±1.79	6.0±1.41^{*a,b}
TRIF	12.8±7.12^{*a,b}	13.2±6.10^{*a,b}	3.0±2.91	4.2±2.28	1.6±1.95	0.8±0.45	0.8±1.79	0.0±0.0	2.2±1.64	4.2±1.92^{*a,b}
N1	8.2±3.56^{*a,b}	3.0±1.73^{*a}	4.8±2.77^{*a}	3.2±1.30	1.4±1.67	0.0±0.0	0.4±0.55	0.0±0.0	9.6±2.97^{*a,b}	4.2±1.64^{*a,b}
N2	6.4±3.36^{*a,b}	3.6±3.36	3.6±1.34^{*a,b}	4.8±1.92	1.6±1.14	1.4±0.55^{*a}	0.0±0.0	0.2±0.45	2.2±1.3	6.0±3.08^{*a,b}
N3	5.8±4.20^{*a,b}	10.6±19.80	2.6±2.30	3.4±1.95	0.8±0.45	1.0±0.71	0.2±0.45	0.2±0.45	4.0±2.0	5.2±2.49^{*a}
NCr	2.8±3.56	4.0±1.73	1.4±0.89	2.6±1.51	0.2±0.45	0.2±0.45	0±0	0±0	2.2±1.79	2.0±1.22
MMSr	2.8±1.64	10.4±3.91^{*a,b}	2.4±2.79	6.6±3.13	1.6±0.55^{*a,b}	0.6±0.55	1.0±1.22	0.6±1.34	3.5±3.51	3.8±2.28
TRIFr	12.6±6.80^{*a}	10±2.55^{*a,b}	6.8±1.78^{*a,b}	3.6±2.88	1.4±1.14	0.4±0.55	0.2±0.45	0.0±0.0	4.8±2.28	5.2±2.28^{*a,b}
N1r	7.4±3.58	6.8±4.44	4.4±2.30	8.8±1.92^{*a,b}	0.4±0.55	0.8±0.84	0.4±0.55	0.0±0.0	6.4±1.14^{*a,b}	7.0±0.71^{*a,b}
N2r	6.2±3.27	12.8±4.66^{*a,b}	4.6±3.91	6.8±3.70	0.6±0.55	0.0±0.0	0.4±0.55	0.6±0.89	3.4±0.89	7.4±4.16^{*a,b}
N3r	9.4±7.09^{*a}	11.8±3.49^{*a,b}	5.2±3.83	2.4±1.94	1.2±1.30	0.8±1.30	0.4±0.55	0.6±0.89	4.4±1.82	5.4±4.72

*a statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.05$
*b statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.01$.
CT: Continuous treatment; Rec: Recovery treatment; **NC**: negative control; **MMS**: methyl methanesulfonate; **TRIF**: Trifluralin; **N1**: Solution containing 0.0125 mg/l of Ni; **N2**:Solution containing 0.025 mg/l of Ni; **N3**:Solution containing 0.050 mg/l of Ni; **NCr**: negative control in the repetition; **MMSr**: methyl methanesulfonate in the repetition; **TRIFr**: Trifluralin in the repetition; **N1r**:Solution containing .,0125 mg/l of Ni in the repetition; **N2r**:Solution containing 0.025 mg/l of Ni in the repetition; **N3r**:Solution containing 0.050 mg/l of Ni in the repetition

Polyploidy cells (Figure 1C) were also observed, although not in significant number. The chromosomal adhesion number (Figure 1D) presented statistical significance only for Ni lower concentrations and after the recovery treatment, tendency asserted in both assays. Chromosome losses (Figure 1E) and C-metaphases (Figure 1F) did not express significant values for the tested concentrations. Likewise, chromosome bridges (Figure 1G - H) were more significant at lower Ni tested concentrations in the first bioassay and, only for the lowest concentration tested in the repetition one.

Figure 1

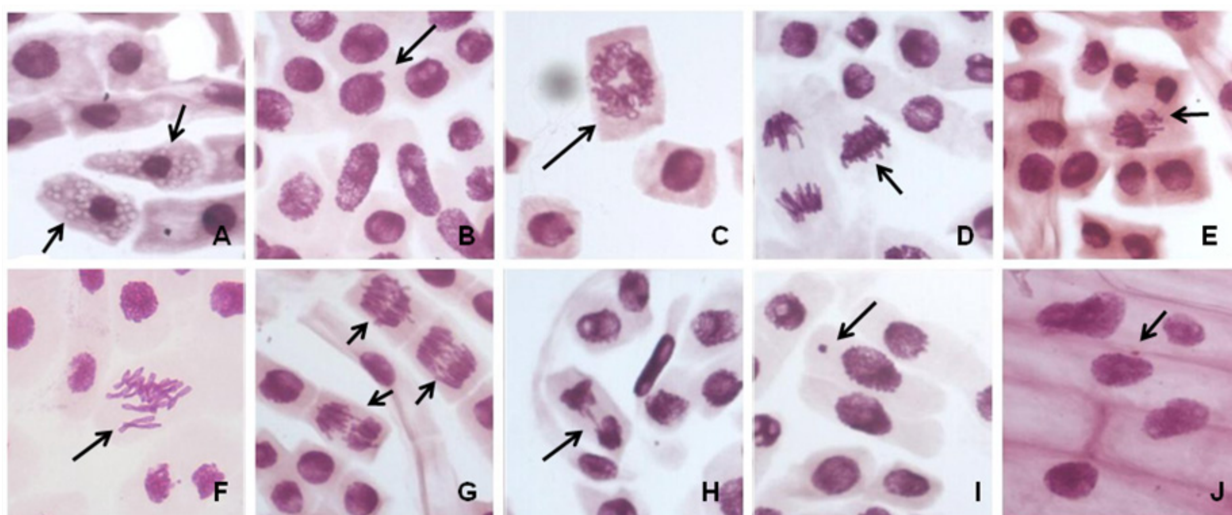

Figure 1 - Nuclear abnormalities and chromosomal aberrations in *Allium cepa* exposed to different Ni concentrations. **A**. Cytoplasm vacuolization - cell in cells death process (arrows); **B**. Cell bearing nuclear bud (arrow); **C**. Polyploid metaphase (arrow); **D**. Metaphase with chromosomal adherence (arrow); **E**. Metaphase with chromosome loss (arrow) **F**. C-metaphase (arrow); **G**. Anaphases with chromosome bridges (arrows); **H**. Telophase with chromosome bridge (arrow); **I**. Cell with micronucleus (arrow); **J**. Micronucleus in F1 region cell (arrow).

The mutagenic potential (Tables 1), on the other hand, was detected at all Ni tested concentrations and shown to be statistically significant compared to the negative control, except for the lower concentration (0.0125 mg/l) where there was less statistical significance in the first bioassay. These values remained significant after the recovery, in both bioassays.

Among the two mutagenic potentially changes computed in the meristematic region, only micronuclei (Figure 1I) were significant, and it was maintained at all concentration levels, even after recovery, as shown in the table 3. It can also be seen that the increase of the Ni concentration is followed by an increase in the micronuclei number both in the continuous treatment and the recovery, which occurred in both bioassays. This trend was also observed in the micronuclei evaluation in the root F^1 region (Fig. 1J) according to table 4. It was also observed a reduction in the F^1 region micronuclei amount in relation to the meristematic region in all treatments, although the values remained significant, showing the micronuclei fixing itself in the F_1 region, which were induced in the meristematic region.

Table 3 - Potentially mutagenic changes (micronuclei and chromosome breakages) found in *A. cepa* meristematic cells treated with different Ni concentrations, in the continuous treatment and after its recovery, in both bioassays.

Treatment	**Mutagenic Effects**			
	Micronuclei		Chromosome breakages	
	CT	Rec	CT	Rec
NC	2.9±1.64	3.6±2.07	0.0±0.0	0.0±0.0
MMS	69.8±23.99*a,b	59.8±29.24*a,b	0.2±0.45	0.8±1.13
TRIF	22.8±8.58*a,b	31.0±6.81*a,b	0.2±0.45	0.2±0.45
N1	10.8±3.70*a	7.8±2.95*a	0.2±0.45	0.0±0.0
N2	24.0±5.30*a,b	13.0±5.00*a,b	0.4±0.55	0.0±0.0
N3	26.0±8.45*a,b	14.4±2.79*a,b	0.4±0.55	0.0±0.0
NCr	9.0±5.52	8.6±3.91	0.6±1.34	0.2±0.44
MMSr	43.8±10.03*a,b	43.6±11.93*a,b	0.0±0.0	1.6±1.14
TRIFr	24.0±5.10*a,b	33.0±13.00*a,b	1.6±1.52	1.8±2.16
N1r	23.4±11.59*a	32.8±16.42*a,b	0.8±0.83	1.0±1.0
N2r	35.2±8.23*a,b	30.4±7.16*a,b	0.6±0.54	2.2±1.09*a,b
N3r	39.0±3.81*a,b	48.8±4.89*a,b	1.0±1.73	1.0±1.41

*a statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.05$

*b statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.01$.

CT: Continuous treatment; Rec: Recovery treatment; **NC**: negative control; **MMS:** methyl methanesulfonate; **TRIF**: Trifluralin; N1:Solution containing 0.0125 mg/l of Ni; **N2**:Solution containing 0.025 mg/l of Ni; **N3**:Solution containing 0.050 mg/l of Ni; **NCr**: negative control in the repetition; **MMSr**: methyl methanesulfonate in the repetition; **TRIFr**: Trifluralin in the repetition; **N1r**:Solution containing 0.0125 mg/l of Ni in the repetition; **N2r**:Solution containing 0.025 mg/l of Ni in the repetition; **N3r**:Solution containing 0.050 mg/l of Ni in the repetition.

Table 4 - Comparison on the micronuclei amount observed in *A. cepa* meristematic and F1 region cells, treated with ultrapure water, MMS, trifuralin a Ni solutions in different concentrations, in the continuous treatment and in the recovery treatment, in the both bioassays.

	Micronuclei			
	Continuous Treatment		Recovery	
	Meristematic	F_1	Meristematic	F_1
NC	2.9±1.64	2.8±0.84	3.6±2.07	3.4±1.14
MMS	69.8±23.99[*a,b]	36.8±6.68[*a,b]	59.8±29.24[*a,b]	19.4±8.41[*a,b]
TRIF	22.8±8.58[*a,b]	17.6±8.02[*a,b]	31.0±6.81[*a,b]	18.2±1.64[*a,b]
N1	10.8±3.70[*a]	9.6±1.14[*a,b]	7.8±2.95[*a]	9.2±4.55[*a]
N2	24.0±5.30[*a,b]	19.4±4.39[*a,b]	13.0±5.00[*a,b]	10.0±3.32[*a,b]
N3	26.0±8.45[*a,b]	21.0±5.89[*a,b]	14.4±2.79[*a,b]	10.4±3.64[*a,b]
NCr	9.0±5.52	4.8±1.92	8.6±3.91	3.0±1.73
MMSr	43.8±10.03[*a,b]	25.0±3.39[*a,b]	43.6±11.93[*a,b]	10.4±1.34[*a,b]
TRIFr	24.0±5.10[*a,b]	13.8±2.17[*a,b]	33.0±13.00[*a,b]	14.0±4.30[*a,b]
N1r	23.4±11.59[*a]	12.0±2.82[*a,b]	32.8±16.42[*a,b]	14.6±4.83[*a,b]
N2r	35.2±8.23[*a,b]	14.2±2.95[*a,b]	30.4±7.16[*a,b]	13.4±2.51[*a,b]
N3r	39.0±3.81[*a,b]	14.6±2.51[*a,b]	48.8±4.89[*a,b]	23.0±4.00[*a,b]

*a statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.05$

*b statistically significant values when compared to the negative control, by Mann-Whitney test, $p<0.01$.

CT: Continuous treatment; Rec: Recovery treatment; **NC**: negative control; **MMS**: methyl methanesulfonate; **TRIF**: Trifluralin; **N1**:Solution containing 0.0125 mg/l of Ni; **N2**:Solution containing 0.025 mg/l of Ni; **N3**:Solution containing 0.050 mg/l of Ni; **NCr**: negative control in the repetition; **MMSr:** methyl methanesulfonate in the repetition; **TRIFr:** Trifluralin in the repetition; **N1r:**Solution containing 0.0125 mg/l of Ni in the repetition; **N2r**:Solution containing 0.025 mg/l of Ni in the repetition; **N3r**:Solution containing 0.050 mg/l of Ni in the repetition.

Discussion

The availability of safe water both for the ecological balance maintenance and for human consumption is a very important issue not only locally but it is also a worry of nations and international organizations (WHO, 2008). Currently, there is a growing care to establish safe parameters for the presence of certain substances in freshwater bodies and also improve previously established parameters. Hereupon, bioassays with higher plants have been often used for monitoring environmental pollutants genotoxicity and mutagenicity. In addition, the advantages of using this kind of biological indicator, plants are usually more susceptible to heavy metals (FISKEJÖ, 1988).

Studies have shown that the nickel amount absorbed by the root system is proportional to the concentration of that presented in the medium

(CATALDO; GARLAND; WILDUNG, 1978; PAIVA et al., 2003; YUSUF; FARIDUDDIN; HAYAT, 2011). Akbaş, Dane and Meriç (2009) observed phytotoxic effect (EC 50) at a Ni concentration of 0.25 mmol/l, such value is equivalent to 14.67 mg/l of Ni or 239.5 times the highest concentration tested here. In experiments with pigeon pea (*Cajanus cajan*), Ni toxicity was certified. In this study, the germination decreases with increasing concentration of this element in the solution, since the lowest concentration used was 0.5 mmol/l (RAO; SRESTY, 2000). However, the toxicity may be avoided in plants by mechanisms that maintain the intracellular metallic ions in a non-toxic shape (BRIAT; LEBRUM, 1999), like organic amino acids, which represent possible chelating of these ions, avoiding toxicity and cytotoxicity for the plant (FOY; CHANEY; WHITE, 1978). Cytotoxic effect induction by Ni2+ is more severe than that induced by other metal ions such as copper and zinc (CHAKRAVARTY; SRIVASTAVA, 1992). However, in this study, even having twice the maximum Ni concentration permitted by the Brazilian law, it was not able to cause toxic or cytotoxic effects to *A. cepa*.

In contrast, the Ni2+ genotoxicity was found even in the lowest concentration evaluated, which represents the half of the maximum Ni concentration permitted by law. According to Briat and Lebrum (1999), transition metals such as Ni2+, have unpaired electrons which are good catalysts for the oxygen reduction reaction, which generates O2-, which in aqueous solution form H2O2 yielding OH- by Haber-Weiss reaction. The hydroxyl radicals produced in excess oxidize biological molecules, leading to greater cell damage and ultimately conducting to cell death (YUSUF; FARIDUDDIN; HAYAT, 2011). It is even known that metal bindings with the cell nucleus cause pro-mutagenic damage, including DNA base changes, DNA and proteins inter and intra-molecular crossing, rearrangement and depurination, and the chemical reactions involved in these injuries are characteristics of an oxidative attack (BRIAT; LEBRUM, 1999).

Among the genotoxic effects observed, chromosome stickiness, which was observed more significantly after recovery treatment, is considered a result of aneugenic effect (LEME; ANGELIS; MARIN-MORALES, 2008). The most expressive changes, however, were nuclear buds. Nuclear buds could be due to poliploidization events, whose excess material is expelled from the cell (FERNANDES; MAZZEO; MARIN-MORALES, 2007), those derive from aneugenic effect, once that by the mitotic spindle inactivation, the centromere division is blocked, occurring absence of chromosomes distribution between daughter cells, then forming a cell with duplicate genetic material (FERNANDES; MAZZEO; MARIN-MORALES, 2007).

Fiskejö (1988) featured the Ni2+ as a mutagenic element, due to its ability to induce chromosomal breakage in the *A. cepa* genetic material. In the concentrations tested here, chromosomal breakages happened in low amounts and presented no significant values. Chromosomal breakages and bridges are considered because of clastogenic effect (LEME; ANGELIS; MARIN-MORALES, 2008). Similarly to the breakages, chromosomal bridges were few expressive, being observed in significant amount only in the lowest tested concentration for both bioassays, so that for this parameter, it was not characterized the clastogenic action.

However, the nickel mutagenic potential found here, has its significance in the micronuclei amount observed. Micronuclei can derive from both acentric fragments that came from clastogenic activity and from entire chromosomes, showing chemical compounds aneugenic activity (FENECH, 2000). According to Yamamoto and Kikuchi (1980) it is possible to analyse the local action of micronucleus inductor agents based on its relative size. Although it has not been taken affective measures about the micronuclei diameter, the present work counts on two positive controls with distinct and known action ways; the trifuralin with aneugenic effect

(FERNANDES; MAZZEO; MARIN-MORALES, 2007) and the MMS with clastogenic one.

Tiny dimension micronucleus like those obtained by the MMS where observed, indicating a supposed clastogenic effect resulting from chromosomal breakage derived from this ion action. In their experiments with *Helianthus annuus*, Chakravarty and Srivastava (1992) observed clastogenic effects, concentration and time dependent related to Ni2+. The DNA fragments rupture can also be related to oxidative damage induced by transition metals (BRIAT; LEBRUM, 1999). The reactive oxygen species attack the purine and pyrimidine bases and on the deoxyribose of the DNA can increase the chromosome fragmentation likelihood, leading to an increased micronuclei formation (YI et al., 2007). Some of the observed micronuclei, however mostly resemble those seen in the control performed with trifluralin, for being larger, probably derived from abnormalities caused by the mitotic spindle inactivation and/or dysfunction.

The chromosomal aberrations study becomes important due to its effectiveness on predicting the xenobiotics action mechanism, such as with heavy metals, on the living organisms genetic material, since they may have harmful action, even at nontoxic concentrations, like the ones tested here. Such study was of paramount importance, since by means of the chromosomal aberrations test it was possible to predict that the Ni2+ has aneugenic and clastogenic action for the used test-organism. The genotoxicity and mutagenicity of this element was proven even after the recovery period. These data allow us to infer, therefore, that the nickel concentration in water, currently permitted by Brazilian law, has genotoxic and mutagenic potential to the employed organism, which therefore suggests greater caution regarding this metal disposal in the environment. Moreover, the findings are worrisome because such concentrations tested here are lower than the maximum value allowed in the potability standards established by WHO (World Health Organization) and adopted by the Brazilian legislation, which is 0.07 mg/l (PADILHA et al., 2012). Owing to the good correlation between this plant test system and animal models, it can be inferred that the Ni2+ presents a hazard to ecosystems and population exposed to this contaminant.

Conclusion

According to the above, it is possible to conclude that nickel, although in trace concentrations is essential for living organisms, it can show harmful effects at higher concentrations, although they show no toxicity, as that the one allowed in freshwater bodies by the Brazilian law. This observation demonstrates both the subtle heavy metals action, like a xenobiotic, in the environment, drawing attention to their disposal, as well as shows the *A. cepa* test sensibility, which was capable of delivering responses even in a Ni2+ low concentration.

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