Determination of vanadium accumulation in onion root cells (*Allium cep*a L.) and its correlation with toxicity

Letty Marcano, Ingrid Carruyo, Yusmary Fernández, Xiomara Montiel, and Zaida Torrealba.

Universidad del Zulia. Facultad Experimental de Ciencias. Departamento de Biología. Maracaibo. Estado Zulia. Venezuela.

ABSTRACT: The vanadium is a metal that presents great interest from the toxicological point of view, because of the numerous alterations that can take place in different biological systems. This work evaluated the capacity of vanadium accumulation and its correlation with genotoxic effects in root cells of *Allium cepa* L. The bulbs were cultivated in renovated filtered water each 24 h, at a temperature of $25 \pm 0.5°C$, in darkness and constant aeration. Treatments were carried out under the same experimental conditions, using water solutions of vanadium of 25, 50, 75 and 100μg/g for 0, 12, 24, 48 and 72 h. A control was carried out where metal solution was substituted by distilled water. After the treatment, the meristems were fixed with alcohol - acetic acid (3:1) and stained according to the technique of Feulgen. The capacity of accumulation was determined by GFAAS. The analysis of the results revealed an accumulation of the metal for all times and concentrations. No correlation was presented among vanadium accumulation, growth and mitotic index; however, positive correlation was given with the induction of chromosomic aberrations. In conclusion, vanadium is able to induce cytotoxic effect in the exposed roots, but only genotoxic effect was correlated with metal accumulation.

Introduction

Vanadium and vanadium compounds can be found in the earth's crust and in rocks, some iron ores, and crude petroleum deposits; it is used in making steel, rubber, plastics, ceramics, and other chemicals (WHO, 2001; Wong and Li 2002). Vanadium mainly enters the environment from natural sources and from the burning of fuel oils; it stays in the air, water, and soil for a long time and combines with other elements and particles (Miramand and Fowler, 1998).

Address correspondence to: Dra. Letty Marcano. Urbanización Monte Bello. Av 12 con calle Q Nº 12-21, Maracaibo, Estado Zulia, VENEZUELA.
Fax: (+58-261) 7483012. E-mail: letty_marcano@hotmail.com; lettymarcano@cantv.net

Determination of vanadium content of selected foods showed that beverages, fats, oils, and fresh fruit and vegetables contained the least vanadium ranging from <0.001 to 0.005 mg/kg. Grains, seafood, meats, and dairy products were generally within the range of 0.005 to 0.03 mg/kg, prepared food within 0.011 to 0.093 mg/kg, while dill seed and black pepper contained 0.431 - 0.987 mg vanadium/kg, respectively. The aerial portions of most plants are not correlated to soil vanadium levels (WHO, 2001). Principal exposures to vanadium are eating higher levels of it in certain foods and breathing air near an industry that burns fuel oil or coal (IPCS, 1999).

Vanadium is an essential trace element in some organisms (e.g., nitrogen-fixing bacteria); however, its essentiality in other organisms (e.g., humans and other mammals) remains an open question. Vanadium deficiency accounts for several physiological malfunctionings including thyroid, glucose, and lipid metabolism (Mukherjee *et al*., 1999).

Although the International Chemical Safety Card (IPCS, 1999) and the Environmental Protection Agency (U.S.EPA., 2004), have not classified vanadium as its human carcinogenicity, there are reports that conclude that exposure to vanadium pentoxide particles caused lung neoplasms in male rats and possibly in female rats, and in male and female mice (NTP, 2002). Animal studies suggest that intake exceeding 10 mg elemental vanadium leads to toxicological effects; these studies show that toxicity has induced hematological, biochemical, reproductive and developmental changes, excess vanadium built up in bone, kidney and liver; to cause pro-oxidative effects on glutathione, ascorbic acid, lipids and NADPH (Okeson *et al*., 2004; Younes and Strubelt, 1991; Domingo, 2002).

Considering to the vegetables as one of the primary links of the alimentary chain that are in contact with the contamination through the polluted soils, our goal was to determine the capacity of accumulation of the vanadium in onion root cells, using atomic absorption spectrometry technique and correlating the vanadium concentration with the genotoxic effect.

Materials and Methods

Condition of cultivation and treatments

Healthy and equal-sized bulbs (n = 12 onions), chosen from a population of the common onion *Allium cepa* L. (2n = 16), were used as study material. These are considered one of the best biological models for the study of environmental pollutants (Fiskesjo, 1985).

Before starting the experiment, the scales of the bulbs and the brownish button plate were removed, and the ring of the root primordial was left intact. The bulbs were allowed to germinate roots by placing them in beakers filled with filtered water, changed every 24 h, at a constant temperature of 25°C $\pm$ 0.5°C, and with a continuous airflow of 10 - 20 ml of air *per* minute, and protected against direct sun light. Once the roots had reached a length of 2 to 3 cm, they were placed in an aqueous solution of vanadium pentoxide (V_2O_5) at 25, 50, 75 and 100 µg/g for 0, 12, 24, 48 and 72 h. All tests were performed in triplicate, and each combination had its respective control, in which the solution of vanadium was substituted with distilled water.

Determination of vanadium accumulation

The capacity of accumulation was determined by graphite furnace atomic absorption spectrometry (GFAAS). For each time and concentration the roots were removed and dehydrated in a lyophilizer (Labconco model Lyph-lock 6) to - 46°C with a pressure 3400 x 10^{-3} mBAR, and their dry weight was determined. Vanadium was evaluated after digestion of the sample (0.1g) with concentrated nitric acid in closed vessels of Teflon into bombs of stainless steel (model 4745 Parr Instrument Company. USA) at 105-110ºC with conventional heating (Precision furnace) for 4h. Metal measurements were calibrated with standards solutions at 0, 10, 25, 50, 75 and 100 µg/g of V_2O_5 diluted with deionized H_2O. Lastly, the samples were analyzed by an atomic absorption spectrometry (Perkin Elmer model 3100), equipped with continuum source hollow cathode lamp 303-6078 for vanadium operated at 40 mA (wavelength of 318.4 nm, spectral banpass 0.7 nm) with an HGA-600 graphite furnace and an AS-60 autosampler. Pyrulytic coated graphite tube were used. The injection

TABLE 1.

**Capacity of accumulation expressed in dry weight (mg/g)
of the vanadium in tips roots of *Alium cepa* L.**

	Concentration (mg/g)			
Time (h)	25	50	75	100
0	0.814	0.814	0.814	0.814
12	5.068	6.802	11.597	12.431
24	5.917	7.167	12.964	17.045
48	9.054	9.442	13.405	27.256
72	13.654	14.321	15.448	40.900

volume was 20 μl. All glassware and plastic materials were rinsed with deionized water for at least 24 h in 10% v/v HNO_3 and then thoroughly rinsed with deionized water before use. The rehearsals were carried out in triplicate and the same ones were expressed in μg/g of dry weight (Bodenseewerk, 1984).

Determination of the effect of the vanadium on the longitude

Allium cepa bulbs were prepared as described by Fiskesjo (1985) using 3 bulbs for all experimental combination treatment and control. When the roots were 2 ± 0.5 cm long, the bulbs were placed directly in the test liquid which were changed every 24 h. During the incubation with vanadium, the length of the roots (4 of each bulb) was measured using a millimeter ruler.

Determination of the effect of vanadium pentoxide on the Mitotic Index (MI)

For each time of exposure and concentration, four (4) roots were cut to carry out the cytologist study. Following treatment, each one were placed in a solution of ethanol (99%) and glacial acetic acid (3:1) for 24 h, washed with distilled water three times, and then dyed in acetic-hydrochloric orcein. The "squash" technique was applied for the study of MI. An analysis of an average of 3,000 cells for each root tip was performed.

Effect of vanadium on the induction of Chromosomal Aberrations (ChA)

The induction of ChA was determined with the same previous procedure, evaluating simultaneously the morphological changes of the chromosome for each exposed meristem to the metal and comparing it with its respective control.

Statistical Analysis

A multivariate analysis of variance (MANOVA) was carried out to determine which of the two factors (time and concentration) had the greater effect. A student test analysis was carried out to determine the dependence of the concentration and the time of exposure on the percentage of ChA. Likewise a simple correlation analysis (Pearson) was carried out to determine the association among the vanadium accumulation with the different evaluated parameters: longitude, MI, ChA.

The results were analyzed by means of the statistical program STATISTIX V.Y. for Windows.

Results

Capacity of accumulation

The results regarding the accumulation in tip roots of *Allium cepa* exposed at different concentration and time with the metal are presented in Table 1. The sample controls presented an accumulation of 0.814 (μg/g) of vanadium. In the exposed meristems, the metal content in the roots ranged from 5.068 to 40.90 μg/g dry weight, showing a direct relation with the concentration and time of exposure. The results of the variance analysis showed that both effects are significant, but the effect of the concentration was 1.84 times higher than the effect of the time of contact. However, the interaction of both effects together was not significant (Table 2).

TABLE 2.

Variance analysis for the effect of time and concentration on vanadium accumulation expressed in dry weight (μg/gr)

Variation source	DF	SS	MS	F	p
Time (A)	3	345.651	115.217	4.96	P < 0.01
Concentration (B)	3	639.109	213.036	9.17	P < 0.05
(AxB)	9	209.038	23.2264	-	-

Effect on the longitude

It was observed a decrease in the longitude in the exposed roots (Fig. 1). This effect was dependent on the time of treatment and the concentration. Both effects are significant, but the effect of the times of treatment was 1.13 higher than the effect of the concentration (Table 3).

Effect on the Mitotic Index (MI)

Analysis of the results from the effect of the concentration and the time of exposure on the MI show a decrease in MI of exposed roots dependent on the concentration and time of treatment (Fig. 2). Both effects are significant, but the effect of the times of treatment was 4.56 higher than the effect of the concentration (Table 4).

Induction of Chromosome Aberrations (ChA)

Table 5 shows the induction of ChA for all concentrations used, which becomes significant ($p < 0.01$) beginning with 12 h of exposure. In figure 3, changes were observed in the organization and morphology of the chromosomes in the root tips exposed to metal. It was observed for all the concentrations and times longer than 12 h, the induction of stickiness (Fig. 3a), formation of anaphasic bridges (Fig. 3b) and c-mitotic effect (Fig. 3c).
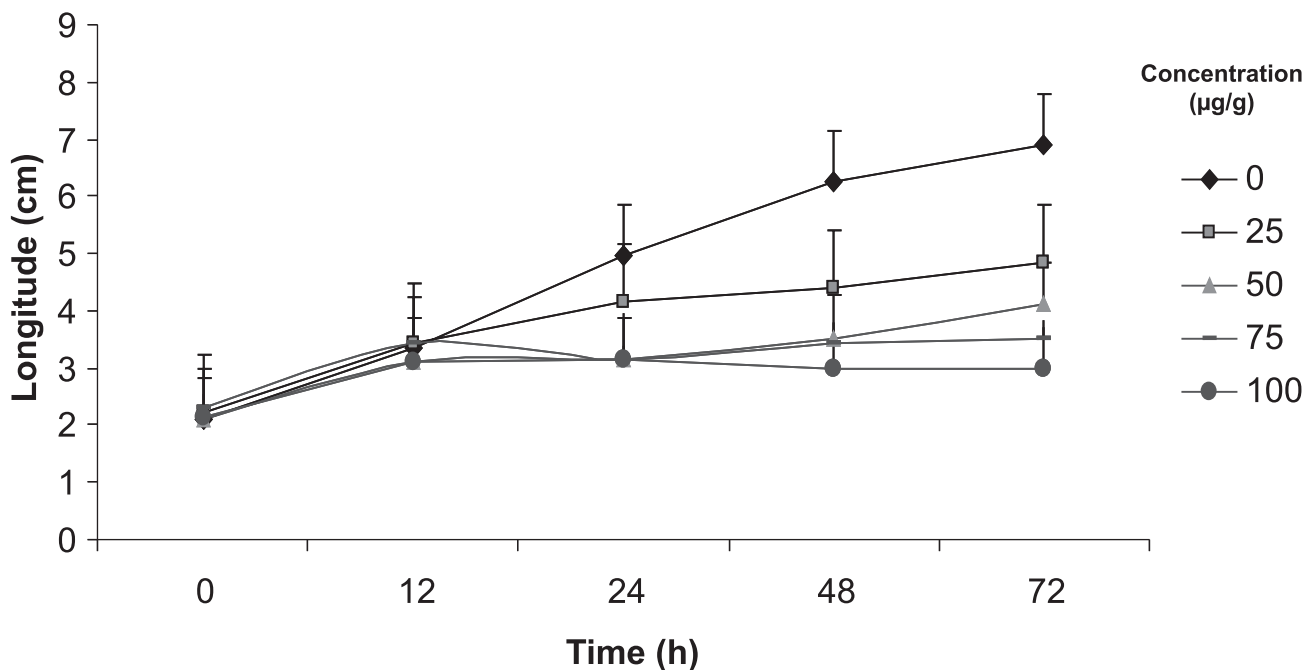
FIGURE 1. Effect of Vanadium at different concentrations and times of exposure on the longitude in tips roots of onion (*Allium cepa* l.) SD is marked

TABLE 3.

Variance analysis for the effect of time and concentration of vanadium on roots longitude (cm).

Variation source	DF	SS	MS	F	P
Time (A)	6	93.2650	15.5442	118.81	P < 0.01
Concentration (B)	4	55.1639	13.7910	105.41	P < 0.01
(AxB)	24	49.0321	2.04301	15.62	P < 0.01

*Correlation between accumulation and deleterious effect*

The correlation analysis of the accumulation of the metal with the different deleterious effects revealed no association among the metal accumulated with the longitude ($r = 0.0037$; $p > 0.05$), nor with the mitotic index ($r = -0.0216$; $p > 0.05$); for the case of the chromosomal anomalies, a highly significant positive correlation is presented with the accumulation ($r = 0.5423$; $p < 0.01$), with a coefficient of determination ($R^2 = 0.2940$) that

indicates that 29.4% of the aberrations takes place for effects of the accumulated metal.

Discussion

Capacity of accumulation

There are few studies related to the bioaccumulation of vanadium. In general it appears than the organisms do not concentrate vanadium from the environment and
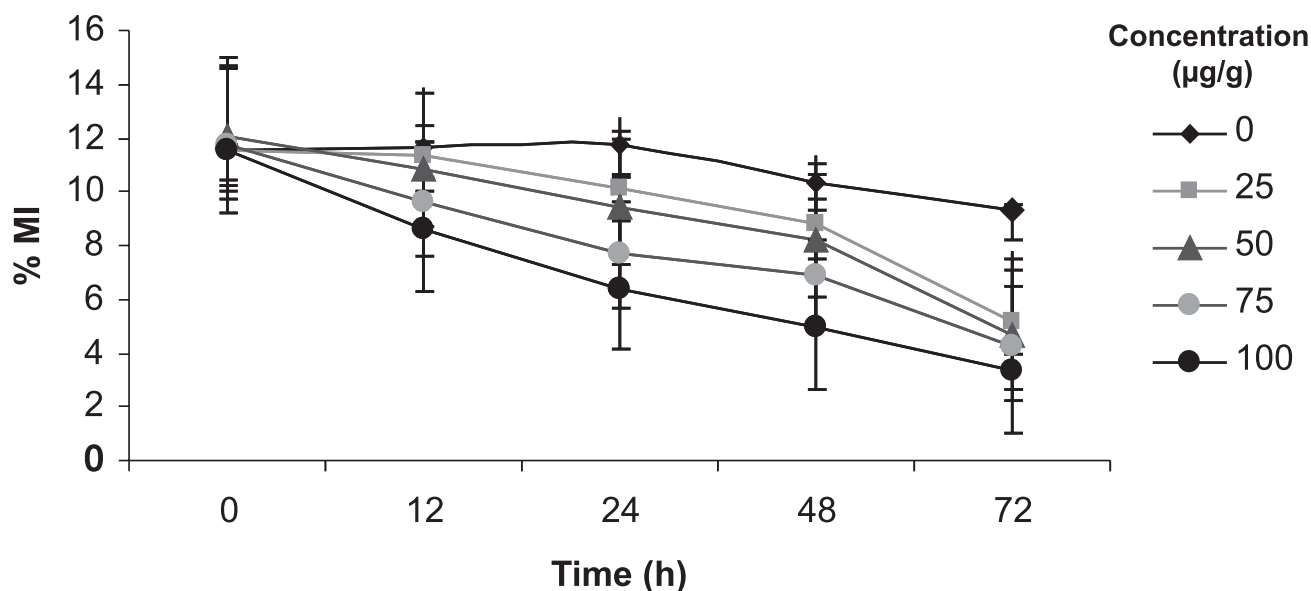
FIGURE 2. Effect of Vanadium at different concentrations and times of exposure on the mitotic index (MI) in tips roots of onion (*Allium cepa* L). SD is marked

TABLE 4.

Variance analysis for the effect of time and concentration of vanadium on Mitotic Index (%).

| Variation source	DF	SS	MS	F	p
Time (A)	4	851.834	212.958	2913.14	P < 0.01
Concentration (B)	4	186.447	46.6117	637.62	P < 0.01
(AxB)	16	85.5552	5.34720	73.15	P < 0.01

there is not indication of biomagnification in the food chain (WHO, 2001; Martin and Kaplan, 1998), reported that the reception of vanadium in the roots and the leaves of the bean plants is under the detection limits and Tudares and Villalobos (1998), established that vanadium levels in plants are low and with many variations among the species, and each depends directly on the content of the metal in the soil and factors like the acidity and humidity.

The accumulation presented in the sample controls, should be taken of fresh cultivation, because the bulbs were acquired in the market, being the same ones were used for the consumption of the populations, this allows to possibly infer the grade of contamination environmental product of the use of the compound agrochemical, which increases the absorption in crops (Bodenseewerk, 1984).

For the exposed meristems, the analysis revealed an accumulation of the metal for all times and concentrations. It is accentuating proportionally, reflect-

ing a bioaccumulation of metal in the studied biological model that is in accordance with that reported by Tudares and Villalobos (1998), who established that the bioaccumulation of the metal depends on the physiochemical characteristics of the soil, as well as the studied species. So, the cells meristematic of *Allium cepa* can be considered like a species with a half capacity of bioaccumulation, of toxicological importance if it is considered that it is part of the population basic diet. Recently reports of other plants have been given as the tobacco, certain mosses (*Hypnum cupressiforme*) and some mushrooms (*Amanita muscaria),* can accumulate around 100 times more vanadium than other metals (WHO, 2001).

The mechanism of cellular incorporation of vanadium is not very well known; however, reports exist that relate it with certain oligoelements required for the cellular functions, some of which are cations such as zinc, for which the vanadium could act as antagonistic (Okeson *et al*., 2004). It has also been related with ex-
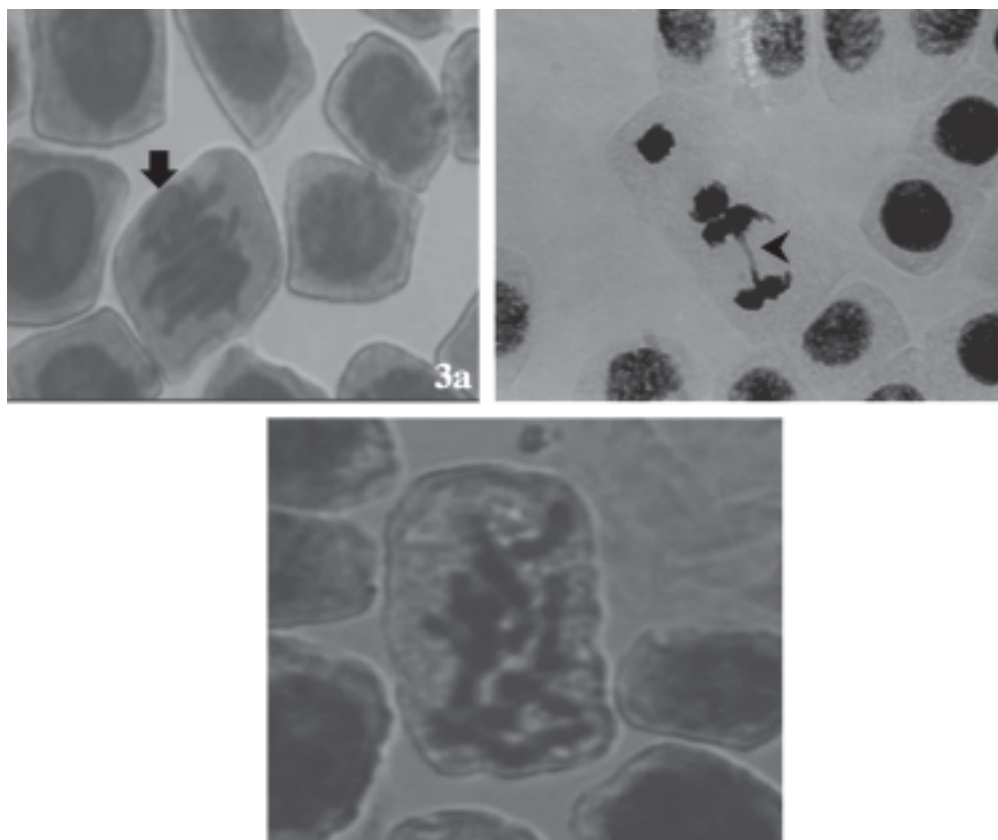
FIGURE 3. Chromosomal Anomalies (ChA) induced by vanadium in tips roots onion (*Allium cepa* L).
Fig. 3a. Stickiness (75 μg/g) at 12 h. 1,000 X
Fig. 3b. Telophasic bridges (75 μg/g) at 12 h. 1,000 X
Fig. 3c. C-mitotic effect. (100 μg/g) at 48 h. 1,000 X

isting reports that in deficiencies in iron, it could be increased the absorption of metals like the vanadium (NIOSH, 1997).

The results of the variance analysis, for the study of the effect of the concentration and the time of exhibition, in the accumulation the metal of the roots, indicate that subjecting the root tips to high concentrations for a short time leads to more accumulation than exposing them to low concentrations for longer times. This results are in agreement with those reported by Martin and Kaplan (1998) who points out that the reception of vanadium in the roots and the superior parts of bean plants in 18 months of experiment was perceptible during the initial period, suggesting the short term bioreadiness.

Effect on the longitude

Analysis of the results of this study on the effect of the concentration and the time of exposure to the vanadium on the roots longitude show a decrease in the longitude in the exposed roots. It was dependent on the concentration and the time of treatment. It was observed that both effects are significant, but the time of contact was higher than the effect of the concentration, which indicates that subjecting the root tips to low concentrations for longer times, is more deleterious than exposing them to high concentrations for a short time. The results coincide with those reported by Cortizo and Etcheverry (1995), and Wang and Liu (1999), who point out a reduction of the growth induced by vanadium in I*ris L* and soybean, respectively. On the other hand (Donghua *et al*., 1994; Marcano *et al*., 1999), settle down that the toxic effect of lead and cadmium respectively in the same biological model, reflected by a blockade in the growth of the treated roots that increases with the get higher of concentration and time of exposure.

Effect on the Mitotic Index (MI)

MI is considered a parameter that allows one to estimate the frequency of cellular division. All the experiments were carried out when the roots reached 2 to 3 cm in length, at which stage the root tips are in a dy-

TABLE 5.

Frequency of % chromosomal anomalies induced by vanadium in meristematics cells of *Allium cepa* L.

Chromosomal anomalies	Time (h)	0 (µg/g)	25 (µg/g)	50 (µg/g)	75 (µg/g)	100 (µg/g)	TOTAL ChA
	12	0	7.3	12	46.3	75.3	140.9
Stickiness	24	0	7.3	12	42	64	125.3
	48	0	10	33	37.3	53.3	133.6
	72	0	12.6	30.3	27.3	24	94.2
	12	0	0	0	0.83	2	2.83
Chromosomic	24	0	0	0	1.16	2.33	4.19
Bridges	48	0	0	0	2.5	2.16	4.66
	72	0	0	0	2	1.66	3.66
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
C-mitosis	48	0	0	0	0	13	13
	72	0	0	0	0	21	21

namic balance, when the number of cells in the division phase are equal to the number of cells in the differentiation phase.

Analysis of the results from the effect of the concentration and the time of exposure on the MI, showed a decrease in % MI of exposed roots. It was dependent on the concentration and time of treatment. Both effects are significant, but the effect of time of contact was higher than the effect of the concentration, which indicates that subjecting the root tips to high time for low concentrations is more deleterious than exposing them to high concentrations for short times (Domingo, 2002), reported a reduction statistically significant of the mitotic index induced by vanadium in human lymphocyte cultures. On the other hand, Donghua *et al.* (1994), Marcano *et al.* (1999) and Wierzbicka (1999), reported in the same biological model the blockade induced by other heavy metals in the exposed cells, coinciding with the reports that point out the toxicity of the metal in diverse biological systems (Migliore *et al.*, 1993; Owusu-Yaw *et al.*, 1990; NTP, 2002; Okeson *et al.*, 2004).

Induction of Chromosome Aberrations (ChA)

The results of these experiments show a clastogenic effect, evidenced by the induction of ChA for the concentrations used, which becomes significant (p< 0.01) beginning with 12 h of exposure. For all the concentrations and times longer than 12 h, the induction of stickiness was evidenced. This phenomenon has been reported as indicative of high toxicity (Marcano *et al.*, 1998). Other anomalies observed were formation of anaphasic bridges and c-mitotic effect, possibly for the effect of vanadium inhibiting the formation of the mitotic spindle followed by random scattering of the condensed chromosome (Owusu-Yaw *et al.*, 1990). These anomalies are considered inductor aneuploids and poliploids (Marcano *et al.*, 1998).

The Student test analysis for related samples determined a significant dependence on the concentration, the time of exposure and the percentage of ChA (p < 0.01); these increase proportionally to the increase of the concentration and the time of exposure, until they reach a point that, because of the blocking effect of MI, the % of ChA begins to decrease. Similar results have been reported in other biological systems (Zhong *et al.*, 1994; Okeson *et al.*, 2004). The clastogenicity induced by the metal can cause cellular death, due to its effect on the induction of DNA strand breaks (Migliore *et al.*, 1993; Chan and Kim, 1993).

Correlation between accumulation and deleterious effect

The correlation analysis of the accumulation of the metal with the different deleterious effects could support the hypothesis that vanadium does not affect these two parameters directly. Possibly the inhibitory effect on the same ones can be related with the route of absorption of nutritious necessary for the growth of the meristems. In this respect, there have been reported harmful effects of vanadium (10 - 20 mg/L) on the plants, altering the process of nitrogen fixation (WHO, 2001).

For the case of the chromosomal anomalies, the results indicate that the accumulated metal can act in the mechanisms involved in DNA repair. This is supported by reports that establish that the metal interferes with the enzymatic complexes of molybdenum and iron (Chan and Kim, 1993; Tudares and Villalobos, 1998), causing disorders at cellular level that can bear to damages to DNA. Nielsen and Uthus (1990), report the narrow similarity of vanadium with phosphorus and the impact that this exercises on phosphorylation reactions, indispensable processes in the regulation of the cellular cycle, for what can settle down the genotoxic effect taken place by the vanadium is directly related with the penetration power and accumulation of the metal, as well as their interaction with enzymes required for diverse metabolic processes (Yang *et al.*, 2004; Okeson *et al.*, 2004). These results agree with reports in other biological systems (Miramand and Fowler, 1998; Abdallah and Moustafa, 2002).

Conclusions

The capacity of accumulation of vanadium was demonstrated in the studied biological model, for what we can suggest as a good model for studies of contamination for heavy metals. Equally, an inhibitory effect of vanadium on the growth of the meristems and the mitotic index was showed, as well as the induction of chromosomal aberrations. However, the analysis correlation of the accumulation of the metal with the different deleterious effects revealed no association among the metal accumulated with the longitude, nor with the mitotic index. Nevertheless, a direct correlation among the accumulative metal and the induction of chromosomal anomalies was showed (genotoxic effect). It is recommended to carry out future studies relating the vanadium accumulation with their toxicity in other biologi-

cal models to try to elucidate the different biological roles of the element.

Acknowledgments

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