Research Article

Biosolid Soil Application: Toxicity Tests under Laboratory Conditions

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A large volume of generated sewage sludge makes its disposal a problem. The usage of sludge in agriculture is highlighted by a number of advantages. However, heavy metals and other toxic compounds may exercise harmful effects to soil organisms. This study evaluated the possible toxic effects of a biosolid sample, under laboratory conditions, for 30 days, using diplopods *Rhinocricus padbergi* and plants *Allium cepa* (onion) as test organisms. The data obtained demonstrated that the biosolid raw sample had genotoxic potential for *Allium cepa* root tip cells. In the diplopods exposed to biosolid sample, epithelium disorganization in the midgut and a reduction of the volume of the hepatic cells were observed after 7 days of exposure. After 30 days, the animals still showed a reduction of the volume of the hepatic cells, but in minor intensity. *Allium cepa* analysis showed genotoxicity, but this effect was reduced after 30 days of bioprocessing by diplopods. This study was important to know the effects as well as to determine how this waste could be applied concerning the soil living organisms and plants.

1. Introduction

In sewage treatment plants (STP), after the sewage had been treated, a sludge rich in organic matter and nutrients is generated as a waste, known as sewage sludge. The composition of this sludge is very variable since it depends on the source of the sewage treatment process and the seasonality [1]. Generally, the sewage sludge presents around 40% to 60% of organic matter, 4% nitrogen, 2% phosphorus, and other macro- and micronutrients, besides potentially toxic elements [2].

The generated sewage sludge still can go through processes in order to increase the solids and reduce the number of pathogenic organisms, generating a residue called biosolid, which is considered most innocuous than the sewage sludge itself [3]. Good quality fertilizers can be generated with the sludge stabilization, reducing its volume through the use of "sludge thickeners drying beds," filter presses, band presser, vacuum filters, and centrifugation [4]. According to Lambais and Do Carmo [5], chemical composition of the sludge depends on the origin of the wastewater. This way, the material is variable, but generally it is a compound rich in organic matter and essential nutrients for plants and microorganisms.

Currently, sewage treatment plants in different Brazilian cities are facing the problem of sludge disposal. The alternatives to the sewage sludge usual fate are landfill disposal, reuse in industry (light-weight aggregate production, bricks and ceramics manufacturing, and cement production), incineration, conversion into fuel oil, ocean disposal, recovery degraded soils, and agricultural use [2, 6].

In Brazil, there is a preference for the use of sludge in agriculture, since there is a considerable land availability and the costs would be relatively low. However, this practice is still incipient, so that the application is made without an adequate management [4]. According to Melo et al. [7], when incorporated into the soil, sewage sludge provides changes in physical properties such as density, aggregates size, and water retention capacity; on chemical properties such as pH, electrical conductivity, CEC, and increased levels of P and N and biological properties, usually by increasing soil microbial activity.

However, the main limitation observed during the evaluation of possible utilization of sewage sludge in agriculture

refers to the presence of metals and other persistent pollutants [8], which may be toxic to plants [9], microorganisms [10], and soil invertebrates [11]. Although other work related the phytoavailability of these metals to a variety of cultures, few studies relate the sewage sludge to genotoxic and mutagenic potential. The application of metal-rich biosolids in clay and sandy soils, compared with biosolids with low-metal concentration, causes a transient soil microbial community increase in mass and activity, with reduced carbon immobilization [5]. Many field studies, based on biosolids agronomic doses, reported soil biota stimulation, probably due to the addition of organic matter, which causes an increase in fertility. The application of this kind of compound, however, has shown inhibitory effect on soil invertebrates [12].

Thus, the aim of this study was to investigate the effects of a biosolid sample according to Brazilian standards for its application on soils, under laboratory conditions, using *Allium cepa* (plant) and *Rhinocricus padbergi* (terrestrial invertebrate) as test organisms.

2. Materials and Methods

2.1. Rhinocricus padbergi. Adult specimens of *R. padbergi* with a mean size of 5.0 cm were collected at the campus of the São Paulo State University (UNESP), Rio Claro, in order to avoid intraspecific differences related to either diplopod size or age. After collection, the specimens were maintained in the laboratory for 3 weeks for acclimation, in a terrarium containing soil, tubercles, and decomposing pieces of tree trunks from the capture area. The experimental temperature was $21 \pm 2°C$ and the photoperiod was a 12 : 12-h light/dark cycle.

2.2. Allium cepa. All assays were carried out with only one kind of seeds of *A. cepa* (variety Baia Piriforme) to avoid different responses in the several stages of the process.

2.3. Control Soil. The control soil was obtained from the site where diplopods were collected at a depth of 0–20 cm, in the UNESP Campus of Rio Claro, SP. Soil samples were homogenized, dried at room temperature, and sieved with 4 mm mesh.

2.4. Biosolid Sample. The wastewater treatment plant where the biosolid was collected occupies an area of 20 hectares. The facility serves approximately 80% of the 318.785 inhabitants [13] of a city in São Paulo state where sewage is treated by conventional activated sludge process. In October 1999, the plant received the license of Producer of Agricultural Amendments, by the Ministry of Agriculture. The product produced in the facility is a biosolid classified as soil conditioner. The brand name is Sabesfértil (SP-09599 00001-0). Biosolid samples were collected and stored in plastic boxes wrapped with dark plastic bags and maintained in a cold room (4°C), until use.

2.5. Chemical Analysis of Samples. The concentration of trace elements (As, Ba, Cd, Cu, Cr, Hg, Mo, Ni, Pb, Se, and Zn) and the 16 priority organic compounds (PAHs) in the biosolid and control soil was determined followed the Standard Methods for the Examination of Water and Wastewater 21th Edition 2005 (SM21) and USEPA. The characterization of samples was measured by TASQA Laboratory (Paulínia, São Paulo, Brazil). Analyses of trace elements were performed by inductively coupled plasma emission spectrometry (ICP-AES). The PAHs analyses were performed by atomic absorption spectrometry. Chemical and physicochemical analyses, as well as a characterization of control soil sample based on macro- and micronutrients (N, Ca, Mg, P, K, S, Fe, Mn, Cu, and Zn), C/N ratio, organic matter, cation exchange capacity (CEC), and base-saturation percentage, were carried out by the Instituto Campineiro de Análise de Solo e Adubo (ICASA), Campinas, São Paulo, Brazil.

2.6. Calculating Biosolid Quantities for Application

2.6.1. Application of Sewage Sludge. According to the law 375/2006 of the Environmental National Council (Conselho Nacional do Meio Ambiente, CONAMA) [14], the maximum annual application of sewage sludge and derivatives in tons per hectare shall not exceed the quotient between the quantity of nitrogen recommended for the crop (in kg/ha), following the official recommendation for São Paulo State and the nitrogen content available in the sewage sludge or derivatives (in kg/t), calculated as N recommended (kg/ha)/N available (kg/t).

To determine the nitrogen available in the sewage sludge and/or biosolid, mineralization fractions were calculated. According to CONAMA [14], this fraction represents 40% of undigested and 20% of digested sewage sludge.

2.6.2. Preparation of Soil and Residue Sample for the Bioassays with R. padbergi. Two glass terraria with capacity for 22.5 L were filled with 5 Kg of control soil each. After physicochemical analysis of soil samples and biosolid, the following bioassays were set up with control soil (CS) and soil + biosolid (SB):

(1) CS: 5 Kg of control soil;

(2) SB: 5 Kg of control soil + 234.4 g of biosolid.

Twenty specimens of *R. padbergi* were then placed in each terrarium, where they remained for 30 days to assess the toxicity of contaminants present in biosolid. The animals were monitored for 90 days. Six animals per bioassay were dissected for histological analyses, three animals on the seventh day, and three diplopods in 30th day of exposure.

2.6.3. Histology of the Midgut. The diplopods were anesthetized with chloroform, placed in Petri dishes containing isotonic salt solution, and dissected under the dissecting scope. The midgut was removed and fixed in paraformaldehyde 4%. Following that, the organ was dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, and 95%), embedded in resin (Leica historesin), and kept in the refrigerator for 24 h. Later, the material was transferred to plastic moulds containing inclusion resin. After polymerization, 6 μm slices were obtained with the help of a Leica

RM2245 microtome. For histological analyses, sections were stained with hematoxylin and eosin.

2.7. Germination of A. cepa Seeds in Residue Samples at Time 0 (t_0) and after Exposure to Diplopods after 30 Days (t_{30}).

Approximately 100 seeds of *A. cepa* were allowed to germinate at 22°C in Petri dishes containing raw biosolid sample (B) and soil from each terrarium: CS and SB samples were collected and placed in Petri dishes for the germination of *A. cepa* seeds, at time 0 (t_0). Positive controls were made with the aneugenic herbicide trifluralin (TRIF) at the concentration of 0.019 ppm [15] and methyl methanesulfonate (MMS), a clastogenic agent at the concentration of 10 mg/mL [16]. Negative control (NC) consisted of seeds allowed to germinate in ultrapure water and the environmental control (CS) consisted of seeds allowed to germinate in the control soil.

After 30 days of exposure by diplopods, soil samples from each terrarium were collected for the tests with onion seeds.

2.7.1. Preparation of Slides of A. cepa.

After germinating and reaching 2 cm in length, root tips were collected and fixed with Carnoy (3 : 1 ethanol/acetic acid). Samples were then stained with the Feulgen reaction [17], with acid hydrolysis for 11 minutes. Root tips were sectioned to remove the meristem and region F_1. To intensify the staining and spread cells, one drop of 2% acetic carmine was added. All samples were lightly pressed between slide and coverslip. Coverslips were removed with liquid nitrogen and slides were mounted with Enthelan. The material was analyzed under light microscope, at magnification of 400x.

2.7.2. Evaluation of the Cytotoxic, Genotoxic, and Mutagenic Effects on Meristematic Cells of A. cepa.

A total of 5000 cells were examined for each treatment at t_0 and t_{30} and for the negative and positive controls. Cytotoxicity was assessed based on morphological alterations indicating cell death, and the mitotic index (MI) calculated as MI = (number of dividing cells/total number of observed cells) × 100. The cells in death process present a vacuolated cytoplasm, which is outcome of the cytoplasmatic organelles digestion by lysosomal enzymes [18]. They can still present enhanced cytoplasmatic volume, which can lead to a rupture of the plasmatic membrane exposing the cell content to the outer media [19].

Genotoxicity was evaluated based on the number of cells with chromosome aberration (CA). For the CA analyses, several aberrations within different cell divisions (metaphase, anaphase, and telophase) were considered such as C-metaphase, chromosomal adherence, multipolar anaphase and telophase, and chromosome bridge and loss [20]. The frequency of CA was calculated as CAI = (number of cells with chromosome aberrations/total number of observed cells) × 100. Mutagenicity index (IMt) was determined based on the occurrence of cells with micronuclei (MN) and chromosome breaks, calculated as IMt = (total number of cells with MN and breaks/total number of observed cells) × 100. The results obtained in all treatments at the different periods were compared with the negative control and soil control at

their corresponding times with the Mann-Whitney test, with significance set at 0.05.

2.7.3. Evaluation of Micronuclei in Cells of the F_1 Region of A. cepa.

The damage to meristematic cells was assessed based on the number of micronuclei in cells of the F_1 region, which is composed by differentiated cells and is located about 1 mm above the meristematic region [21]. A total of 5000 cells were examined per treatment. The results obtained for all samples were compared with the negative control and soil control at their corresponding times with the Mann-Whitney test, with significance set at 0.05.

3. Results

3.1. Chemical Characterization of Samples.

To follow a Brazilian standard for application of biosolids on soil, data regarding the agronomic potential and fertility of control soil became necessary. The values obtained are presented in Table 1. The results obtained by physical-chemical and trace elements analysis of control soil and biosolid sample are presented in Table 2. The concentrations of arsenic and copper found in the control soil were above the limits determined by CETESB-195/2005-E [22], but below the limits for intervention in agricultural areas. Levels of barium, lead, copper, chromium, molybdenum, nickel, and zinc found in the biosolid were high, but below the maximum level allowed by CONAMA (Table 2). None of the 16 PAHs, priority by EPA, were detected by analyses.

3.2. Histology of the Midgut of Diplopods.

Animals from control soil presented the midgut (Figure 1(a)) as the histological pattern described for the species [23], being, therefore constituted by a pseudostratified epithelium with brush border (arrow head in Figure 1(a)). The epithelium showed principal cells with nuclei of round to oval morphology, located in middle apical region and regenerative cells in the basal portion; the epithelium is followed by a muscular layer and hepatic cells layer covered by an external membrane. The hepatic cells had an irregular morphology, spherical nucleus, and cytoplasm with cytoplasmic granules of varied content. Among the hepatic cells, some hemocytes were observed, generally isolated.

The group exposed for one week to SB showed epithelium disorganization (arrow in Figure 1(b)) with disruption in several places, indicating an epithelium renewal. The hepatic cells layer showed disorganization with volume reduction in some cells (Figure 1(c)).

After 30th day of exposure, animals from SB sample showed the minor disorganization of the hepatic cells layer when compared to the midgut of the animals exposed for 7 days (Figure 1(d)).

3.3. Cytotoxic, Genotoxic, and Mutagenic Effects on A. cepa.

The mitotic index of cells examined for B, SB, TRIF, MMS, CS, and NC at 0 and after 30 days of exposure to diplopods is presented in Table 3. Seeds exposed to B and SB samples presented the highest mitotic index in both periods of exposure. However, no sample was statistically significant

TABLE 1: Fertility parameters of the control soil.

Sample	pH CaCl$_2$	g/dm^3 OM	mg/dm^3 P res	K	Ca	Mg	H + Al	SB	CEC	% V	Ca/Mg	Mg/K
						mmol/dm^3 TFSA					Ratio	
Soil	6.20	18	3.0	0.8	2	1	88	3.9	91.9	4.2	2.0	1.25

OM: organic matter; CEC: cation-exchange capacity; V: base saturation.

TABLE 2: Physicochemical and metal analysis of the control soil and biosolid sample.

Parameter	CS (mg/kg)	B (mg/kg)	Method	G (mg/kg)	MCA (mg/kg)
	Samples				
Arsenic	16.8	<LQ	SM21 3120B	3.5	41
Barium	5.91	158	SM21 3120B	75	1300
Cadmium	<LQ	<LQ	SM21 3120B	<0.5	39
Total calcium	25.4	3939	SM21 3120B	—	—
Organic carbon (g/kg)	12.6	279	SSSA Cap40	—	—
Lead	49.3	174	SM21 3120B	17	300
Copper	37.2	276	SM21 3120B	35	1500
Electric conductivity (μs/cm)	115	5389	SM21 3120B	—	—
Chromium	31.2	224	SM21 3120B	40	1000
Total sulfur	151	11864	SM21 3120B	—	—
Total phosphorus	182	17027	SM21 3120B	—	—
Total magnesium	<LQ	358	SM21 3120B	—	—
Mercury	<LQ	1.08	EPA 7470A	0.05	17
Molybdenum	3.64	9.55	SM21 3120B	<4	50
Nickel	13.0	82.3	SM21 3120B	13	420
Nitrate (mg/Kg)	4.40	6.79	SM21 4500-NO^{-3} E	—	—
Nitrite (mg/kg)	0.06	1.39	SM21 4500-NO^{-2} B	—	—
Ammoniacal nitrogen (mg/kg)	31.8	167	SM21 4500-NH$_3$ E	—	—
Kjeldahl nitrogen (mg/Kg)	476	21620	SM21 4500-Norg B	—	—
pH	6.20	8.01	EPA 4095 C	—	—
Total potassium	406	2152	SM21 3120B	—	—
Selenium	<LQ	<LQ	SM21 3120B	0.25	100
Total sodium	<LQ	<LQ	SM21 3120B	—	—
Solid content	0.86	0.24	SM21 2540B	—	—
Moisture (g/g)	0.14	0.76	SM21 2540B	—	—
Zinc	23.2	825	SM21 3120B	60	2800

CS: control soil; B: biosolid; LQ: limits of quantification; IV: inconsistent value; G: guidelines of quality for soil (mg/kg) and groundwater in São Paulo State, according to CETESB (195/2005-E); MCA: maximum concentration allowed in sewage sludge or derivative product, according to CONAMA (375/2006).

TABLE 3: Mean and standard deviation of the mitotic (MI), the chromosome aberration (CA), and mutagenicity indexes (IMt) in meristematic cells of *Allium cepa* after exposure to control soil, negative and positive controls, and biosolid samples.

Samples		MI t_0	MI t_{30}	ICA t_0	ICA t_{30}	IMt t_0	IMt t_{30}
Controls	NC	13.66 ± 1.37	11.2 ± 1.78	1.2 ± 0.83	2 ± 1	0.2 ± 0.44	0.6 ± 0.54
	CS	14.21 ± 0.90	9.48 ± 1.07[2]	2 ± 0.70	1.6 ± 1.14	0.6 ± 0.54	0
	MMS	11.53 ± 0.88	5.73 ± 1.15	8.6 ± 3.04[*1]	14.2 ± 2.94[*1]	25.6 ± 8.5[*1]	24.8 ± 7.29[*1]
	TRIF	9.47 ± 0.54	9.31 ± 1.35	51.4 ± 8.79[*1]	23.6 ± 4.82[*1]	12.2 ± 3.42[*1]	3.6 ± 0.89[*1]
Raw	B	15.36 ± 0.99	NA	2.15 ± 1.12	NA	0.16 ± 0.18	NA
Combination	SB	15.07 ± 0.56	15.03 ± 0.79	21 ± 5.83[*1]	10.6 ± 3.33[*1,2]	1.2 ± 1.3	0.4 ± 0.54

NC: negative control; CS: control soil; MMS: positive control; TRIF: positive control; B: raw biosolid sample; SB: soil + biosolid.
t_0: time of mixing and t_{30}: after 30 days of exposure to the diplopods.
[*] Statistically significant values when compared to the negative control, by Mann-Whitney test, $P < 0.05$.
[1] Statistically significant values when compared to the control soil, by Mann-Whitney test, $P < 0.01$.
[2] Statistically significant values when compared to the same treatments at 0 and 30 days.
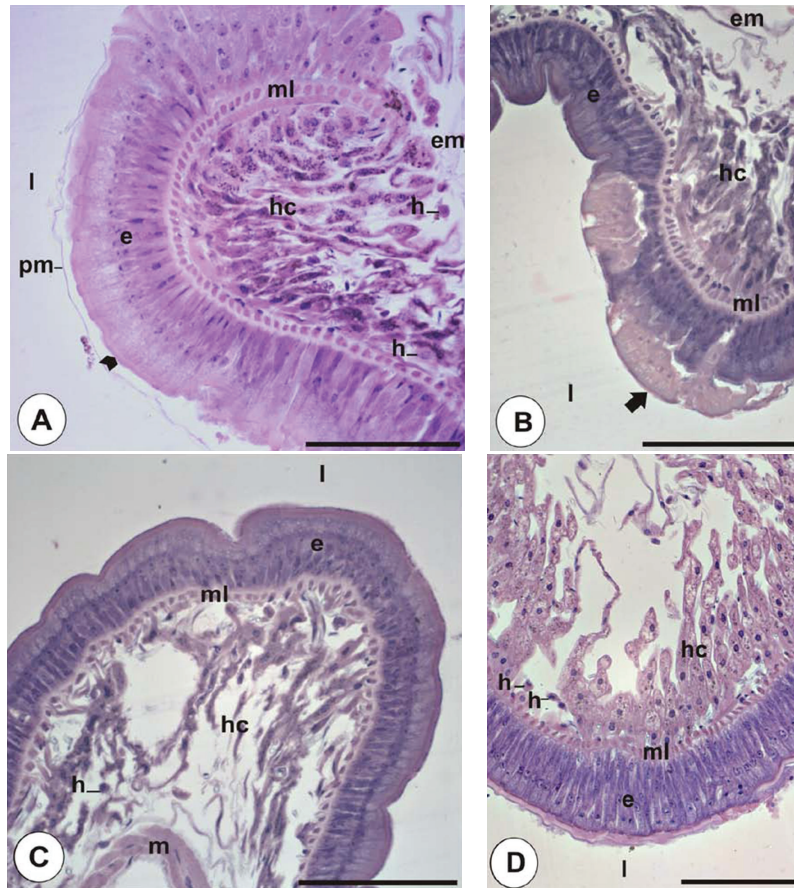NA: not available.

FIGURE 1: Micrographs of the *R. padbergi* midgut stained with HE. (A) Animals exposed for 7 days to the control soil; (B, C) animals exposed for 7 days to soil + biosolid; (D) animals exposed for 30 days to soil + biosolid. e: epithelium; em: external membrane; h: hemocyte; hc: hepatic cells; l: lumen; m: muscle fibers; ml: muscular layer; pm: peritrophic membrane; arrow head in (A) brush border; arrow in (B) epithelium renewal. Scale bars = 100 μm.
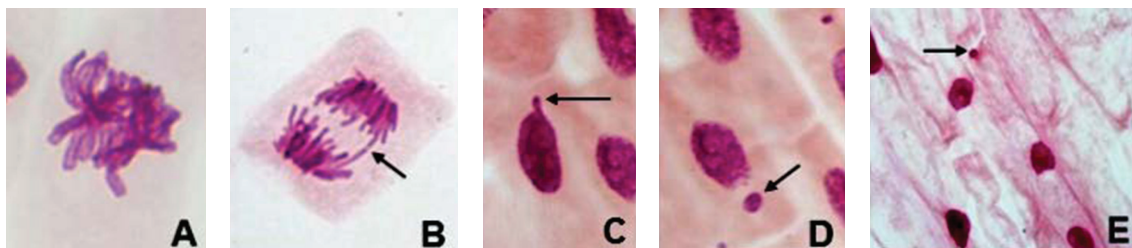

FIGURE 2: Chromosomal aberrations in *Allium cepa* exposed to raw biosolid (B) and control soil + biosolid (SB); (A) chromosomal adherence; (B) anaphase with polyploidy and chromosome bridge (arrow); (C) nuclear bud (arrow); (D) meristematic cell carrying micronuclei (arrow); (E) micronuclei in cells of the F_1 region (arrow).

when compared to the negative control. In comparison to the values obtained between the CS and B samples there was a significant difference. There were no cells in the cell death process for all samples.

B and SB samples induced genotoxic effects (Table 3), at t_0 and t_{30} ($P < 0.05$), characterized by chromosome aberrations, compared to the negative control and/or control soil. However, the genotoxic effect was significantly reduced after the bioprocessing by diplopods in the SB group. The most frequently observed alterations in the present study were cells in metaphase with chromosomal adherence (Figure 2(a)), polyploidy (Figure 2(b)), anaphase with chromosomal bridge (Figure 2(b)), and nuclear bud (Figure 2(c)).

Meristematic cells with micronuclei (Figure 2(d)) and chromosome breaks were examined to assess the mutagenicity. Statistical analysis revealed no mutagenic effect of the samples examined at both times of exposure compared to the negative control and/or control soil (Table 3). Although micronuclei has been observed in meristematic and F_1 region (Figure 2(e)), the values were not statistically significant

TABLE 4: Mean and standard deviation for micronuclei in cells of the meristematic region (M) and F_1 of *Allium cepa* after exposure to control soil, negative and positive controls, and biosolid samples.

Samples		t_0		t_{30}	
		M	F_1	M	F_1
Controls	NC	0.2 ± 0.44	0.2 ± 0.44	0.6 ± 0.54	0.2 ± 0.44
	CS	0.6 ± 0.54	0	0	0.2 ± 0.44
	MMS	$21.2 \pm 7.08^{*1}$	$4.6 \pm 2.07^{*1}$	$20.6 \pm 5.12^{*1}$	$4.6 \pm 0.89^{*1}$
	TRIF	$12.2 \pm 3.42^{*1}$	$2.6 \pm 1.14^{*}$	$3.6 \pm 0.89^{*1}$	$2.6 \pm 0.89^{*1}$
Raw	B	0.14 ± 0.14	0.05 ± 0.04	NA	NA
Combination	SB	1 ± 1	0.6 ± 0.54	0.4 ± 0.54	0

NC: negative control; CS: control soil; MMS: positive control; TRIF: positive control; B: raw biosolid sample; SB: soil + biosolid.
t_0: time of mixing and t_{30}: after 30 days of exposure to the diplopods.
[*] Statistically significant values when compared to the negative control, by Mann-Whitney test, $P < 0.05$.
[1] Statistically significant values when compared to the control soil, by Mann-Whitney test, $P < 0.01$.
[2] Statistically significant values when compared to the same treatments at 0 and 30 days.
NA: not available.

(Table 4) and a decrease for these values was observed after 30 days of exposure to diplopods.

4. Discussion

Researches about sewage sludge disposal in soil are focused on its effects on soil fertility, plant development, and contamination by heavy metals and organic compounds [24]. However, few studies have been conducted to evaluate the toxic, genotoxic, and mutagenic potentials of the sewage sludge disposal on exposed plants and animals.

Chemical analysis of biosolid sample showed the presence of trace elements. According to the literature, these elements tend to induce genotoxic and/or mutagenic effects on plants [25, 26] and tend to concentrate in the terrestrial invertebrates tissues because their rate of absorption frequently surpasses their rate of elimination [27].

Several authors have used higher plants to diagnose and monitor the action of chemicals and environmental pollution. Among these, *Allium cepa* (onion) has been used in determination of cytotoxic, genotoxic, and mutagenic effects of substances [15, 28] and complex environmental samples [29–36].

In this study, *A. cepa* assays were carried out to assess abnormalities in dividing cells and to estimate the potential of B and SB samples to induct chromosome aberrations. The mitotic index of B was statistically significant compared to control soil. In our study, the mitosis stimulation observed in B and SB treatments may be due to the phosphorus and nitrogen presence and abundant elements in domestic sewage [37, 38].

Chromosomal aberrations are recognized as important consequences of the genotoxic environmental chemicals actions [39], to which many organisms, including humans, are exposed. Epidemiological studies have linked high chromosomal aberrations frequencies at significant cancer developing risk [40]. For this reason, numerous biological tests for chromosomal aberrations evaluation have been developed, in order to ensure the environmental quality [15, 31].

The most common aberrations observed in this study were metaphase with chromosomal adherence, polyploidy anaphase, and anaphase with chromosomal bridge and nuclear bud. According to the literature, all aberrations may be caused by the action of some trace elements/metals. Some metals are potentially genotoxic/mutagenic and are strongly related to environmental pollution. Several studies with plants have shown that the genotoxic effects of metals can cause changes in chromosome structure, chromosome number, and also the disturbances in the mitotic apparatus [25, 26]; they have the ability to inhibit mitotic spindle formation, leading to an abnormal chromosomes distribution and polyploidy [25, 41–43].

In this study was observed the presence of some elements such as barium, lead, copper, chromium, mercury, molybdenum, nickel, and zinc. Although their concentrations are within the standards established by CONAMA [14], according to De Godoy and Fontanetti [44], these standards do not take into account aspects such as the possible interaction of toxic metals and plants, as well as the lack of information regarding the influence of sludge on the soil fauna, including animals that promote humification, aeration, and enrichment of the same.

Like earthworms and collembola [8, 12, 45, 46], the diplopods are considered excellent test organisms for studying the organic amendment effects in the soil ecosystem due to their direct exposure and their sensibility to pollutants; therefore, the diplopods have been used as bioindicators of soil pollution and ecotoxicological assessment [27, 44, 47, 48].

The stimulation of soil biota revealed in some field studies using agronomic dosage of biosolids [49] is probably linked to the soil fertility enhancement, especially due to the contribution of the organic matter. However, in some laboratory investigations, the biosolid and sewage sludge application has caused inhibitory effects [50] and tissue damage on soil invertebrates [44, 47, 48].

Morphological changes can be employed in investigations of chemicals toxicity and in monitoring of acute and chronic effects, in impacted environments [51]. Analyses

involving morphology and histology of tissues in invertebrates have been widely used to identify different damage types caused by harmful substances to animals [52–55].

Preliminary histological analysis of the midgut of diplopods, exposed for 7 and 30 days to the SB sample, according to sewage sludge disposal for Brazilian law may help clarify the mechanisms used by animals in an attempt to detoxify the contaminants present in the biosolid when applied to the soil. It is inferred that the high rate of epithelium renewal of the midgut of these animals may be to maintain the integrity of the organ and an attempt by the body to compensate the damage suffered after the ingestion of contaminated soil [27]. Similar results were obtained by other authors in response to acute diplopods exposure to industrial soil contaminated with polycyclic aromatic hydrocarbons [27] and sewage sludge samples from different sewage treatment plants [47, 48].

Tissue changes related to defense and detoxification may be reversible, being present only as a result of an altered metabolic status of the organism [56]. In invertebrates, the intracellular accumulation of potentially toxic compounds in insoluble forms and in physiologically inactive ones is an efficient mechanism for detoxification of these elements [27, 57]. In diplopods, the midgut and the hepatic cells work actively in this process [47, 57].

The biotransformation of toxic compounds requires the consumption of energy reserves [27]. In the present study, after 7 days of exposure, the reduction of the volume of hepatic cells of the animals exposed to SB sample could be due to the large energy demand for detoxification of the toxic compounds found in the soil. However, after 30 days of exposure, a decrease in the aggression intensity in hepatic cells of the diplopods' midgut was observed, probably due to SB sample stabilization.

At the same time, there was a reduction of SB genotoxicity in onion seeds exposed to this sample. It is inferred that the genotoxicity reduction was obtained again due to SB sample stabilization for bioprocessing of this by diplopods and subsequent immobilization of trace elements. However, under field conditions, the animals would be chronically exposed to this residue, given the frequent application of this. In this sense, it is indispensable to develop studies that evaluate the subchronic exposure effects in an attempt to measure whether responses are sufficient to maintain the organ integrity and/or if will elapse in more severe cellular/tissue damage.

5. Conclusion

There is evidence that biosolids use will lead to increased soil fertility and trace elements levels; therefore, the environmental significance of such increases needs to be examined.

The results of this study reinforce the need for more research to evaluate the biological effects of waste to be discharged into the environment in different ecosystems compartments, as well as different levels of biological organization, even when toxic agents are present at low concentrations, since the sample studied in accordance with Brazilian standards for sewage sludge disposal has shown toxic and genotoxic potential to onion seeds and terrestrial invertebrates.

In this sense, studies that evaluate the subchronic exposure effects in an attempt to measure how this provision can be harmful or not to the environment compartments and receptor organisms are necessary.

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