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Review

# Genotoxic and mutagenic effects of sewage sludge on higher plants

Maria Nilza Corrêa Martins [a], Victor Ventura de Souza [b], Tatiana da Silva Souza [a,b,*]

[a] Programa de Pós-Graduação em Genética e Melhoramento, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, CEP 29500-000 Alegre, ES, Brazil

[b] Departamento de Biologia, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, CEP 29500-000 Alegre, ES, Brazil

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ABSTRACT

Sewage treatment yields sludge, which is often used as a soil amendment in agriculture and crop production. Although the sludge contains elevated concentrations of macro and micronutrients, high levels of inorganic and organic compounds with genotoxic and mutagenic properties are present in sludge. Application of sludge in agriculture is a pathway for direct contact of crops to toxic chemicals. The objective of this study was to compile information related to the genotoxic and mutagenic effects of sewage sludge in different plant species. In addition, data are presented on toxicological effects in animals fed with plants grown in soils supplemented with sewage sludge. Despite the benefits of using sewage sludge as organic fertilizer, the data showcased in this review suggest that this residue can induce genetic damage in plants. This review alerts potential risks to health outcomes after the intake of food cultivated in sewage sludge-amended soils.



**Contents**



## 1. Introduction

The increasing use of sewage sludge for growing different crops, such as pineapple, banana, coffee, sugar cane, guava, papaya, and corn (Costa and Costa, 2011) is due to its considerable percentage of organic material and of macro and micronutrients essential for plants; substituting—even if only partially—mineral fertilizers (Nascimento et al., 2004).

Despite facilitating increased productivity and having many economic advantages (due to the high price of chemical fertilizers), the application of sewage sludge to agricultural soils, in the long run, can lead to the introduction of organic and inorganic compounds, with genotoxic and mutagenic potential (Rank and Nielsen, 1998). When this occurs, these compounds can be translocated to the plants and then transferred to other organisms via the food chain (Grotto et al., 2013). Therefore, rigorous regulation of these soil additives is needed, as well as studies that determine

* Correspondence to: Departamento de Biologia, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, UFES, Rua Alto Universitário, s/número Guararema, 29500-000 Alegre, ES, Brazil.

*E-mail address:* tatianas.souza@hotmail.com (T.d.S. Souza).

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short and long-term environmental risks.

Genetic toxicity bioassays with higher plants are particularly suitable to monitoring soils supplemented with sewage sludge, because these organisms are direct targets of the possible contaminants. In addition to detecting deleterious substances, even at low concentrations, several genetic biomarkers, from point mutations to chromosomal aberrations, can be evaluated from different organs like leaves, endosperm, pollen grains, and roots (Grant, 1994). Despite this, few genotoxicity studies on sludge have been performed with higher plants.

This work aims to gather information on the possible deleterious effects of sewage sludge on the genetic material of higher plants. Furthermore, data are presented on toxicological effects in animals fed with plants grown in soils supplemented with sewage sludge.

## 2. Sewage sludge: composition and genotoxicity mechanisms

The composition of sewage sludge varies as a function of its origin; in other words, if it comes from a predominantly residential and/or industrial area, the time of year it was processed, and the treatment technology used to process it in the different Wastewater Treatment Plants (WTPs) (Lima et al., 2011).

Generally, sewage sludge has agronomic appeal because it has high moisture content, abundance of organic material (proteins, carbohydrates, lipids), and macro and micronutrients essential to the growth and development of plants (Singh and Agrawal, 2008). Ramulu (2002) related an increase in the quantity of pathogens in soils fertilized with sewage sludge. However, to be used in agriculture, sewage sludge must go through processes that aim to decrease the number of pathogenic organisms in it (viable helminth eggs, fecal coliform, *Salmonella*, and enteric viruses); creating a residue termed a biosolid (Haynes et al., 2009).

The problem of sludge application to an agricultural soil arises due to high concentrations of potentially toxic elements which may accumulate in soil due to long term uses (Singh and Agrawal, 2008), constitute phytotoxic problems (Udom et al., 2004). The genotoxicity of sewage sludge has been primarily attributed to heavy metals such as arsenic (As), barium, (Ba), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se) and Zn (Zn) (Srivastava et al., 2005a, 2005b; Amin et al., 2009a, 2009b; Amin, 2011). Table 1 shows standards for maximum allowed concentrations of heavy metals in sewage sludge in accordance the legislation of different countries.

It has generally been assumed that heavy metals are immobile in managed agricultural soils (McBride, 1995). However, some factors such as soil texture, pH and metal organic-complexation can increase heavy metals mobility and result in plant uptake or leaching of these elements to groundwater (Udom et al., 2004). It is known that Cu, Mn and Zn are essential micronutrients which are readily absorbed by plants roots and translocated to shoots. Already Ni is required by plants in very small amounts (Udom et al., 2004, Emamverdian et al., 2015). Pb can be absorberd by plant roots, but is not translocated to the shoots at high levels (Udom et al., 2004). Although Cd is not essential elements for plants, they get easily absorbed and accumulated in different parts of plants (Udom et al., 2004, Emamverdian et al., 2015).

There are records of significant accumulation of heavy metals in plant grown in soils amendment with sewage sludge. Henning et al. (2001) reported that *Zea mays* plants exhibit high levels of Pb, Cu, and Zn in their tissues. Antolín et al. (2005) concluded that *H. vulgare* grains displayed a considerable increase in heavy metal levels. Rangel et al. (2006) documented an increase in Mn and Zn content in leaves and corn grains. Amin and Sherif (2001) applied aerobically digested sewage sludge to corn at different rates. It was noticed that M1 corn grains accumulated the four analyzed metals in the order as follows: Ni > Pb > Cd > Cr. According to authors, this accumulation pattern suggested that there was a selective uptake of these metals probably due to both their different solubility in the soil solution and different transfer coefficients, and thus made it immediately in available to the plants.

**Table 1**
Standards for maximum concentrations of heavy metals in sewage sludge (dry weight).

| Metals | Brazil (mg/kg) | Europe (mg/kg) | US (mg/kg) |
|---|---|---|---|
| Arsenic (As) | 41 | – | 75 |
| Barium (Ba) | 1300 | – | – |
| Cadmium (Cd) | 39 | 20–40 | 85 |
| Chromium (Cr) | 1000 | – | – |
| Copper (Cu) | 1500 | 1000–1750 | 4300 |
| Lead (Pb) | 300 | 750–1200 | 840 |
| Mercury (Hg) | 17 | 16–25 | 57 |
| Molybdenum (Mo) | 50 | – | 75 |
| Nickel (Ni) | 420 | 300–400 | 420 |
| Selenium (Se) | 100 | – | 100 |
| Zinc (Zn) | 2800 | 2500–4000 | 7500 |

Brazil: Maximum permissible concentration in the sewage sludge and by-products according to the National Council of the Environment CONAMA (375/2006) (Conama, 2006). Europe: Limiting concentration values for trace metals in sludge used for agriculture according to the Council of the European Communities (86/278/CEE). US: Maximum concentration of heavy metals allowed in the sewage sludge according to the Environmental Protection Agency of the United States (USEPA) (CFR Part 503) (Usepa, 1993).

Metals interact in different ways with the cellular machinery: by competition with other metals, by binding to DNA, to specific amino-acids or to specific sites (Mateuca et al., 2006). Cr, Cu, Mn and iron (Fe) can directly generate oxidative injury, which leads to production of oxygen free radicals species (ROS) in plants, resulting in cell homeostasis disruption, DNA strand breakage, defragmentation of proteins, or cell membrane and damage to photosynthetic pigments, which may trigger cell death. In contrast, Al, Cd, Ni, Hg, and Zn indirectly inflict oxidative stress via multiple mechanisms including glutathione depletion, binding to sulfhydryl groups of proteins, inhibiting antioxidative enzymes, or inducing ROS-producing enzymes like NADPH oxidases (Emamverdian et al., 2015).

The consequences of mutagen–target interactions may lead to different types of DNA damage such as gene mutations, chromosome mutations or numerical chromosome changes (Mateuca et al., 2006). $As^{3+}$, $Pb^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ caused a dose-dependent increase of micronuclei frequencies in *Allium cepa*, *Tradescantia* and *Vicia faba*. Cu gave negative responses. The ranking of genotoxic potencies was in the decreasing order: $As^{3+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+}$ (Steinkellner et al., 1998). In contrast, Cu caused a dose dependent increase in micronuclei frequencies in *V. faba* and *Pisum sativum* (Souguir et al., 2008). Seth et al. (2008) observed that Cd induced chromosome aberrations, mitotic abnormalities and micronucleus formation in *A. cepa*. The authors also reported significantly increase of DNA damage detected by comet assay. Mutagenic effects of Cd were evaluated on nine microsatellite loci. No microsatellity instability was observed in *Lactuca sativa* leaves, but a 2-bp deletion in one lettuce root was detected among the simple sequence repeats analyzed (Monteiro et al. 2009). Arya et al. (2013) reported Pb induced decrease in mitotic index and increase of chromosomal aberrations, DNA fragmentation and micronucleus frequency in *Allium cepa* and *V. faba*. Truta et al. (2013) reported that Zn significantly increased ana-telophase aberrations and metaphase disturbances in barley (*Hordeum vulgare*). Oladele et al. (2013) concluded that Pb is much more genotoxic than Zn to bambara groundnut (*Vigna*