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Efficiency of co-composting process to remove genotoxicity from sewage sludge contaminated with hexavalent chromium

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ABSTRACT

This study focuses mainly on the genotoxicity of sewage sludge contaminated with hexavalent chromium (Cr(VI)). The effect was assessed by the micronucleus (MN) induction in *Vicia faba* roots, after exposure to solid co-composting matrix and aqueous extracts. Two different concentrations of co-composting of sewage sludge/palm waste were tested: mixture A:1/3 sludge + 2/3 palm waste and mixture B:1/2 sludge + 1/2 palm waste.

No effect was detected at different concentrations of solid matrices. However in the co-compost aqueous extracts, the MN frequency was significantly higher than in the direct contact. The evaluation of Cr(VI) toxicity showed a positive relationship ($R^2 = 0.77$) with MN frequency. After six months of co-composting, the MN rate decreased significantly by 70.4 and 77.2% with decreasing of Cr(VI) concentration with 58 and 58.6% for A and B respectively. These results indicate the efficiency of co-composting process to decrease the sludge genotoxicity by Cr(VI) stabilization.

1. Introduction

Chromium (Cr) is a transition metal element and, as such, has several forms depending on its electronic configuration. Chromium III is considered as being an essential micronutrient element for human, plant and animal metabolism (Anderson, 1981), and is relatively non-toxic (Jiang et al., 2013). Hexavalent chromium (Cr (VI)) is a known genotoxic carcinogen (Dixit et al., 2002) and is generally present in effluents of leather tanning industry, one of the major industrial activities of Marrakech city. Cr(VI) is found under different forms such as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) (Ucun et al., 2002), depending on both pH and total Cr (VI) concentration. High concentrations of Cr(VI) are considered as being most hazardous to animals and plants due to its high solubility, mobility, toxicity as well as having carcinogenic and mutagenic properties (Dixit et al., 2002). Wani et al. (2007) showed adverse effects on the composition and metabolic activities of microbes, and antagonistic effects on physiological processes of plants at high concentrations of Cr(VI) (Oves et al., 2013), especially on micronuclei induction in *Vicia faba* cell roots (De Marco et al., 1988).

Generally sewage sludge resulting from wastewater treatment plants (WWTP) are well known to be concentrated in fertilizing elements, in particular nitrogen and phosphorus, inspiring their use in agriculture. However, the presence of mutagenic toxic compounds in sewage sludge could be a drawback as an option for enhancing value of these agro-resources. Sewage sludge from WWTP of Marrakech city has been characterized and known to contain heavy metals such as chromium (Cr) (El Fels et al., 2014a). It is therefore, essential to remove chromium, especially in its toxic hexavalent from sewage sludge before use as fertilizer. Conventional procedures for removing chromium Cr(VI) from wastes, include several processes such as electrochemical treatment, ion exchange, chemical reduction which may be extremely expensive and non-environment-friendly.

For several years, production of compost has been one of the biological pathways for the disposal of non-hazardous solid wastes (Barje et al., 2012). Composting decreases the amount of solid waste and is considered as being one of the best methods to recycle sewage sludge waste and to reduce negative effects (El Fels el al., 2014a). However chemical analysis of organic matter is limited to

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offers the real information of about their toxicity. Several tests have been recommended to assess the quality and maturity of organic waste compost (Shukla et al., 2009; Shukla et al., 2009). Biotests are established alternatives that detect toxicity and can be useful in evaluating complex environmental toxicity. This biological approach provides an integrative parameter for the presence of compounds affecting the applied test system (Ansari and Malik, 2009).

Plant biotests have been recommended, and the *Vicia faba* micronucleus (MN) test is recognized as a sensitive assay to evaluate genotoxicity effects and chromosomal aberrations. It is widely used to evaluate the genotoxicity from both organic and inorganic compounds in soils (Cotelle et al., 1999; Marcato-Romain et al., 2009), wastewater and industrial effluents (Shukla et al., 2007), sediment (Chen and White, 2004), organic material such as sewage sludge or composts (De Simone et al., 2000; Kapanen et al., 2013) and water (Monarca et al., 2003). No study, however, has been conducted to test potentially polluted sludge with hexavalent chromium. In the present work, the *Vicia faba* root-micronucleus test was chosen to assess genotoxicity induced by raw sewage sludge contaminated with chromium and composts of sewage sludge mixed with palm waste, in order to evaluate the co-composting effect to remove the toxicity induced by Cr(VI).

2. Materials and methods

2.1. Co-composting trials

Co-composting trials were conducted for six months on a composting platform located in the plant nursery of Marrakesh. Two trials with different sewage sludge/palm waste ratios were followed:

-Mixture A (w/w): 1/3 sludge + date palm tree waste 2/3, total volume: (4m × 1m × 1m)

-Mixture B (w/w): 1/2 sludge + date palm tree waste 1/2, total volume: (4m × 1m × 1m).

Each mixture was carefully homogenized, moisture was adjusted by watering to 60% (optimal value for composting), and then the mixtures were windrowed. The windrows as pile of $4m^3$ were turned over manually with a weekly frequency to enable mixture ventilation. Homogenous samples were taken after aerating the mixture at A0 and B0 first day of co-composting, respectively for mixture A and B, and at A6 and B6 the final stage (six months) of composting, respectively for mixture A and B. Homogeneous samples (1 kg) were obtained by careful mixing of several sub samples taken at different points (length and height) of the windrow and quarted. Samples were stored at −20 °C before analysis.

2.2. Physico-chemical analysis

The physico–chemical parameters were analyzed as shown by El Fels et al. (2014a). The temperature was measured every day at different levels (length and height) of the windrow with sensors equipped with data memory (PH 0700115 model 1.20, Ector-Traçability software, ECTOR France). The samples were dried out at 105 °C. The pH was measured in an aqueous extract of the compost at room temperature (1 g/10 ml of distilled water). Total organic carbon and ash contents were calculated measured after calcination in a muffle furnace at 600 °C for 6 h. Total Kjeldahl nitrogen (TKN) was assayed in 0.5 g samples by using classical Kjeldahl procedure, by steam distillation according to (AFNOR, 2004) T90-1110 standard.

2.3. Determination of total Chromium (Cr)

After humid mineralization with DigiPrep Jr (SCP Sciences) the concentration of total Cr, in the co-composted substrates was determined by ICP OES (ICP OES Thermo IRIS Intrepid II XDL Duo). The freshly determined weight of each sample was ground in liquid nitrogen, then placed in 70% HNO_3 overnight at room temperature, and for 1 h at 80 °C in DigiPrep the; H_2O_2 (30%) was added for 25mn at 55 °C. The digested samples were removed from the DigiPrep (Hot plate) and allowed to cool. Nitric acid concentration of the resulting extracts was adjusted to a maximum final concentration of 10%. The digested samples were centrifuged and further analysed to quantify total amount of Cr during co-composting.

2.4. Hexavalent chromium (VI) assay

Cr(VI) was analysed by the colorimetric method (MA, 2015 200-CrHex 1.0; Ahluwalia and Goyal 2013) in aqueous extracts prepared from sewage sludge(100 g/L). The centrifuged aqueous extract of the sewage sludge raw material, and the different co-composted stages of mixture A and B, were analysed after complexing Cr(VI) with 1,5-diphenylcarbazide agent in acidic solution (H_2SO_4, 2N). The color complex formed with Cr(VI) absorbing light at 540 nm, was measured with an ultra-violet visible (UV–vis) spectrophotometer (Secomam S750). A calibration curve was also prepared using potassium dichromate ($K_2Cr_2O_7$) in the range of 0.1–1 mg/L Cr(VI).

2.5. Vicia faba *micronucleus test*

Vicia faba seedlings were prepared according to Ma et al. (1995) and Marcato-Romain et al. (2009). Dry *Vicia faba* seeds were soaked for 4 h in water. Seed coats were removed and seeds were left to germinate between two layers of moist cotton. After 4 days, primary roots, having reached 2 to 3 cm in length, were selected for the MN assay. Their tips were cut off to promote the growth of secondary roots. For each experiment, five independent replicates were made. These germinated seedlings were used as starting material in the MN tests as described below.

2.5.1. Direct exposure of seedlings to solid matrices

Six months after the start of the co-composting process, mixtures A and B were mixed (w/w) with Lufa 2.2 standard soil to reach final ratios of 25%, 50%, 75% and 100% of co-composting substrates; and 100 g of each concentration were dispatched in 6 cm diameter and 6 cm high cylindrical pots. Secondary roots were collected after three days of exposure. As reported by Song et al. (2007), the positive control made use of Lufa 2.2 soil, mixed with 10^{-5} M solution of maleic hydrazide. Moisture content was maintained at all times to 2/3 of water holding capacity by introducing water in the under cups.

2.5.2. Root exposure to co-composting aqueous extracts and to Cr(VI) solutions

Aqueous co-composting time extracts were prepared by stirring 100 g of the solid matrices in 1000 ml distilled water for 24 h at room temperature. After centrifugation at 5000 rpm supernatants were recovered and used as such for exposure.

Primary roots of germinated seedlings were exposed to aqueous co-composted extracts at the onset of the experiment and 6 months after. In parallel, they were also exposed during 24 h, to five hexavalent chromium concentrations, ($34\ 10^{-3}$, $17\ 10^{-3}$, $34\ 10^{-4}$, $17\ 10^{-4}$, $34\ 10^{-6}$ mM), prepared as soluble salts of $K_2Cr_2O_7$, followed by a 24 h recovery period in water to allow cells to undergo one complete cell cycle. Maleic hydrazide (MH) (10^{-5}M) and the water