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Preliminary study using *Eisenia fetida* as uranium (U) biomonitor in industrial landfill residue, cultivated *in vitro*

M.C. Tessari-Zampieri[1], J.E.S. Sarkis[2], M.L. Dias[3], M.A. Hortellani[4], C.B. Barbieri[5], M.A. Scapin[6]

[1]*dra.tessarizampieri.usp@gmail.com*,
[2]*jesarkis@ipen.br*,
[3]*marldias@yahoo.com.br*,
[4]*mahortel@ipen.br*,
[1,2,3,4]*Nuclear Energy and Research Institute of São Paulo University (IPEN/CNEN). Center for Laser Applications. Chemical and Isotopic Characterization Laboratory. Av. Prof. Lineu Prestes, 2242, Butantã, CEP 05508-000, São Paulo-SP, Brazil.*
[5]*cristinabbarbieri@gmail.com*,
[5]*Rio Grande do Sul State Forensic Institute-Rua Voluntários da Pátria, 1358, CEP 90230-010, Porto Alegre-RS, Brazil.*
[6]*professorscapin@gmail.com*,
[6]*Nuclear Energy and Research Institute of São Paulo University (IPEN/CNEN). Center for Chemistry and Environment. X-Ray Diffraction Laboratory. Av. Prof. Lineu Prestes, 2242, Butantã, CEP 05508-000, São Paulo-SP, Brazil.*

1. Introduction

Industrial landfills are designed to receive non-reactive or flammable solid residue that is containing low volumes of solvents, oils, or water. After its useful life end, which may last up to 32 years [1, 2], and proper closure procedures the landfill area can be reused/reclaimed. It can be used for social activities for surrounding community [3, 4], for this reason, the continuous monitoring of potentially toxic elements is necessary. A strategy that can be developed during its useful life or after the closure is environmental biomonitoring.

Environmental biomonitoring is a process that integrates environment quality data using a living species that indicates (Bioindicator) or accumulates (Biomonitor) potentially toxic elements. The *E. fetida* (Oligochaeta), popularly known as California red earthworm has a great capacity for assimilating soil components, including metals, by ingestion or dermal absorption, at contaminated soils [5].

Earthworms accumulate relatively high concentrations of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and other elements from anthropic sources [6]. The biological effects of depleted uranium accumulation in earthworms were evaluated by Giovanetti et al., [6]. This metal accumulation capacity is a fundamental characteristic in biomonitoring [7]. Consequently, the earthworm can be an excellent organism for this purpose. Therefore, the aim of this study was to evaluate *E. fetida* as uranium (U) biomonitor in industrial landfills residue.

2. Methodology

Residue samples were collected with a geotechnical sampler in an industrial landfill that received predominantly fertilizer industries residues, and identified as samples 1, 2, 3, and 4. Characterization was performed after the samples were oven-dried (FANEM-720C) at 70 ± 1°C and sieved to a grain size less than 0.065 mm. Analysis was carried out using a Wavelength Dispersive X-Ray Fluorescence (WDXRF) spectrometer Rigaku Co., model RIX 3000 (Rigaku Co, Japan) with X-ray tube with Rh anode, a 75 mm Be window, and a 60 kV maximum acceleration voltage, scintillation detector NaI (Tl) and flow-proportional counter.

Adult species of *E. fetida* earthworm were obtained through local suppliers and conditioned during 24 h on filter paper moistened with Milli-Q water to void their guts contents. Fifteen groups containing ten individuals each were conditioned in vessels containing 300 g of landfill residue. One vessel was filled with soil free of fertilizers (Pb analysis was carried out to verify) and used as a blank to control earthworm development. Reproduction was used to provide information on the tolerance and well-being *E. fetida* in residue samples.

E. fetida were initially sacrificed in liquid nitrogen and digested in acid mixture H_2O_2: HNO_3: H_2O (3:2:1), in microwave oven (MARS6-CEM). Dry residue samples (0.1 g) were homogenized to powder and digested according to Ivanova et al. [9]. Final solution was separated and diluted 10 folds in *E. fetida* muscle samples

and 500 folds in residue samples. A In (1 µg g^{-1}) solution was used as internal standard. U_{total} (^{238}U, ^{235}U, ^{234}U) analyses of the solutions were performed using a NexION®300 PerkinElmer ICP-MS.
Methodology was validated using standard reference material (NIST) SRM-2709-San Joaquin Soil, with recovery of 98.7% (U).
Earthworm muscle, and residue results were obtained with three replicates ± standard deviations.
Bioaccumulation factor (BAF) is a parameter representing U_{total} transfer from residues to earthworms indicating the earthworm's capacity to tolerate and bioaccumulate U. In this work U_{total} was calculated in a simplified manner according to the sum $^{234}U + ^{235}U + ^{238}U$. When this value is higher than one (BAF > 1), it indicates bioaccumulation of U_{total} in earthworms. BAF [8] was calculated according to Eq. (1).

$$BAF = \frac{U_{Total}\,(\text{Muscle})}{U_{Total}\,(\text{Residue})} \tag{1}$$

where, U_{Total} (Muscle) represents U_{total} concentration in muscles (µg g^{-1}), and U_{Total} (Residue) represents U_{total} concentration in Residue (µg g^{-1}).

3. Results and Discussion

Validation demonstrated that the chosen analytical method acceptable since a high recovery (98.7%) was obtained for U determination [10]. Fertilizer free control soil (Table I) presents lower levels of the analyzed elements and was used to monitor species development. The WDXRF analysis results for residue samples are described in Table I. These major and minor constituents are associated with the ore processing steps for fertilizer production.
Major constituents Al and Fe are elements present in all residue samples, evidencing a similarity between the samples collected in landfill. Ca and Mg differ because they can be from rocks with different constituents such as kieserite and anhydrite, respectively, used in the potassium salt production process [11]. In addition, Ca is present in apatite, fluorapatite, hydroxyapatite, and chlorapatite series, to produce phosphorus salt [12].
Minor constituents Cl, Mn, Cu, and Zn are elements present in all residue samples. High Cl concentrations in landfill residue differ significantly from control soil, as it is part of the separating process of KCl from sylvinite ore with NaCl, where Cl is discarded [11]. After selective separation of the minerals and final product drying, granulation and particle size reduction, the residues are discarded in industrial landfill.
In control soil, Pb concentration was lower than in landfill residue indicating that the soil was indeed fertilizer free. Pb is found naturally in phosphatic rocks in small proportions, after processing, high concentrations remain in fertilizer and other part is discarded in landfills [13].

Table I. WDXRF analyses results in residue samples and control

Elements	Control (n=5)	Sample 1 (n=5)	Sample 2 (n=5)	Sample 3 (n=5)	Sample 4 (n=5)
Mg (%)	0.10±0.01	3.6±0.5	0.45±0.06	0.66±0.09	1.0±0.1
Al (%)	4.0±0.1	11.6±0.2	12.0±0.2	12.2±0.2	10.3±0.2
Ca (%)	0.33±0.01	12.4±0.2	0.35±0.01	0.101±0.002	0.35±0.01
Fe (%)	1.06±0.01	3.66±0.05	3.94±0.06	3.04±0.04	4.54±0.06
Cl (µg g^{-1})	0.011±0.001	70±7	24±2	11±1	28±3
Mn (µg g^{-1})	7±1	420±43	76±8	77±8	80±8
Cu (µg g^{-1})	15±1	6.1±0.3	5.2±0.3	2.9±0.3	4.9±03
Zn (µg g^{-1})	6.0±0.5	34±3	10±1	5.4±0.5	8.7±0.8
Pb (µg g^{-1})	0.19±0.02	5.2±0.5	10±1	3.5±0.4	3.0±0.3

During the experiments, the specimens used did not present any health or growth problems, and *E. fetida* adapted well in landfill residues samples. They have reached maximum size after 60 days and started their reproduction. The presence of a large amount of immature and mature cocoons in vessels during experiments is an evidence of *E. fetida* fertilization and reproduction. Table II show U_{total} concentration in control soil and landfill residue, values ranged from 0.804 to 5.81 µg g^{-1} and are in accordance with the defined Radioactive Materials Natural Occurrence (NORM) [14] value for U that is 80 µg g^{-1}. Control soil U_{total} was 3.1±0.1 µg g^{-1}, U occurs as a soil component, originating from rocks in earth's mantle [1, 15]. Background U concentration in soil is about 2 µg g^{-1} [1]. No records of U_{total} determination in fertilizer production residues were found in the literature but is anthropogenic sources from ore processing and from disposal of solid residue from mining, milling, and production operations. Phosphatic rocks usually employed as source phosphorus in phosphatic fertilizer production [16] are rich in U. U values may vary regionally, i.e. in phosphatic region states Pernambuco and Paraiba (Brazil), range from 10 to 530 µg g^{-1} [15].

Earthworms are important carriers due to their food habits, absorption, and adsorption. Earthworm transfer elements present in the soil to terrestrial biota, Table 2 show the results of U_{total} analyses in muscles. Our results were performed in microhabitat with landfill residue, this indicates that the values obtained may be higher than those expected when earthworms are in the environment. There are no data reported for U_{total} in landfill residue using earthworms, but there is for absorption and accumulation in landfill leachate. Uptake and accumulation of leachate contaminants into earthworms not only poses a risk to earthworm directly but transfer bioaccumulated contaminants such as Hg [17], Cd, Cu, Cr, Ni, Pb and Zn through the food chain [18]. In addition, earthworms living on metalliferous soil are biomonitors of Cd, Cu, Pb, Zn [19].

A species is considered a biomonitor when the Bioaccumulation Factor (BAF) is greater than 1. As displayed in Table II, in samples 1, 2 and 4 this factor was near 2, i.e. this means that the difference in U_{total} concentration is circa 1,900 times compared with control soil. These values may indicate that *E. fetida* is a hyperaccumulator of the U_{total}.

In sample 3, the BAF was about 1. This may have occurred due to the low Cu concentration (Table I). The Cu is an essential element to the physiological functions linked to the production of hemoglobin, metalloproteins, iron metabolism and found in muscles. Cu deficiency may damage reproduction and interfere in the uptake U by the earthworm. In addition, Ca assist in cation exchange in *E. fetida* organism, as this constituent was depleted in the soil there were limited exchanges with U [20]. In *E. fetida* digestive system, enzymatic action and microorganisms, such as bacteria, fungi, actinomycetes, algae and protozoa, activities are greatly stimulated, before gastric contents are excreted [21].

These BAF demonstrate that *E. fetida* is a biomonitor and can be used bioacumulator/hyperaccumulator of U_{total} in landfill residue.

Table II. U_{total} in residues, muscles, and BAF

Samples	Soil/Residues U_{total} (µg g^{-1}) (n=3)	Muscle U_{total} (µg g^{-1}) (n=3)	BAF
0 (Control)	0.804±0.002	0.800±0.001	1.0
1	3.45±0.06	6.5±0.1	1.9
2	5.81±0.03	11.2±0.2	1.9
3	3.52±0.01	3.1±0.2	0.9
4	4.13±0.06	7.6±0.3	1.8

4. Conclusions

Quantitative determination of major and minor constituents of landfill residue was successfully implemented. *E. fetida* worm was unveiled as a promising biomonitor organism, due to its capacity of hyperaccumulating U_{total} in muscles. It must be remarked that low concentrations of some essential elements, notedly Ca and Cu, are probable causes of uptake reduction. U concentrations in earthworms are a measure of the "ecologically significant" fraction of U within a given microhabitat, this indicates that the values obtained in this study may be higher than earthworms living in the environment. Although several studies have been carried out, we have established data base method to assess U levels in landfill residue, according to our knowledge. This study showed the adaptation of earthworm to residue media, which is useful for further research on ecotoxicology using microplastic, as well as for biomonitoring and evaluation soil contamination from metals.

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References

[1] CEQG-Canadian Environmental Quality Guidelines, Canadian Council of Ministers of the Environment (2007).
[2] TITARA-Central de gerenciamento ambiental, http://www.cgatitara.com.br, Accessed 25 Nov (2019).
[3] M.E.F. Correia, "Potencial Utilização dos Atributos das comunidades de fauna de solo e de grupos chave de invertebrados como bioindicadores do manejo de ecossistemas," Documentos 157. EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária, Ministério da Agricultura e do Abastecimento. ISSN 1517-8798. Dez (2002).
[4] A.G.H.P.V. Elk, K. Segala, "Redução de emissões na disposição final. Mecanismo de desenvolvimento limpo aplicado a resíduos sólidos," 3:40 Rio de Janeiro: IBAM (2007).
[5] T. Leveque, Y. Capowiez, E. Schreck, T. Xiong, Y. Foucault, C. Dumat, "Earthworm bioturbation influences the phytoavailability of metals released by particles in cultivated soils," *Environmental Pollution*, vol. 191, pp. 199-206 (2014).
[6] A. Giovanetti, S. Fesenko, M.L. Cozzella, L.D. Asencio, U. Sansone, "Bioaccumulation and biological effects in the earthworm *Eisenia fetida* exposed to natural and depleted uranium", *J Environmental Radioactive*, vol. 101, pp. 509-506 (2010).
[7] T. Hirano, K. Tamae, "Earthworms and Soil Pollutants", *Sensors*, vol. 11, pp. 11157-11167 (2011).
[8] E. Kırıs, H. Baltas, "Sediment distribution coefficients (Kd) and bioaccumulation factors (BAF) in biota for natural radionuclides in eastern Black Sea coast of Turkey," *Microchemical J*, vol. 149, pp. 104044 (2019).
[9] J. Ivanova, R. Djingova, S. Korhammer, B. Markert, "On the microwave digestion of soils and sediments for determination of lanthanides and some toxic and essential elements by inductively coupled plasma source mass spectrometry," *Talanta*, vol. 54, pp. 567-574 (2001).
[10] S.N. Guilhen, M.E.B. Cotrim, S.K. Sakata, M.A. Sacapin, "Application of the fundamental parameter method to the assessment of major and trace elements in soil and sediments from," *Int. Eng. J.*, vol. 72, pp. 609-61 (2019).
[11] M. Nascimento, M.B.M. Monte, F.E. L. Loureiro, "Rochas e Minerais Industriais-Agrominerais Potássio," CETEM, 2ª edição (2008).
[12] F.E. Loureiro, M.B. Monte, M. Nascimento, "Rochas e Minerais Industriais-Agrominerais. Fósforo," CETEM (2005).
[13] E.V.S. Freitas, C.W.A. Nascimento, D.F. Goulart, J.P.S. Silva, "Disponibilidade de cádmio e chumbo para milho em solo adunado com fertilizantes fosfatados," *Revista Brasileira de Ciências do Solo*, vol. 33, pp.1899-1907 (2009).
[14] R.G. Reis, NORM: guia prático. ISBN: 978-85-922211-0-2 (2016).
[15] R.S. Amaral, W.E. Vasconcelos, E. Borges, S.V. Silveira, B.P. Mazzilli, "Intake of uranium and radium-226 due to food crops consumption in the phosphate region of Pernambuco e Brazil," *J of Environmental Radioactivity*, vol. 82, pp.383-393 (2005).
[16] I. M. Yamazaki, L.P. Geraldo, "Uranium content in phosphate fertilizers commercially produced in Brazil," *Applied Radiation and Isotopes*, vol. 59, pp. 133–136 (2003).
[17] C. He, K. Arizono, H. Ji, Y. Yakushiji, D. Zhang, K. Huang, Y. Ishibashi, "Spatial distribution characteristics of mercury in the soils and native earthworms (*Bimastos parvus*) of the leachate-contaminated zone around a traditional landfill," *Science of the Total Environment*, vol. 636, pp. 1565–1576 (2018).
[18] M.A. Jóźwiak, M. Jóźwiak, R. Kozłowsk, M. Żelezik, "Zooremediation of leachates from municipal residue using *Eisenia fetida* (SAV.)," *Environmental Pollution*. DOI: https://doi.org/10.1016/j.envpol.2019.07.039 (2019)
[19] J.E. Morgan, A.J. Morgan, "Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils," *Environmental Pollution*, vol. 54, pp. 123-138 (1988).
[20] T. Lukkari, M. Aatsinki, A. Vaisanen, J. Haimi "Toxicity of copper and zinc assessed with three different earthworm tests," *Applied Soil Ecology*, vol. 30, pp. 133–146 (2005).
[21] V.C. Rodrigues, V.C. Theodoro, I.F. Andrade, A. Inácio-Neto, F.V. Alves, "Produção de minhocas e composição mineral do vermicompostos e das fezes procedentes de bubalinos e bovinos," *Ciência Agrotécnica*, vol. 27, pp.1409-1418 (2003).