Applied Soil Ecology 108 (2016) 62–71

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effect of cobalt and silver nanoparticles and ions on *Lumbricus rubellus* health and on microbial community of earthworm faeces and soil

Livia Vittori Antisari[a], Serena Carbone[a], Antonietta Gatti[b], Sara Ferrando[c], Michele Nacucchi[d], Fabio De Pascalis[d], Chiara Gambardella[c,e], Luigi Badalucco[f], Vito Armando Laudicina[f,*]

[a] Dipartmento di Scienze Agrarie, Alma Mater Studiorum-Università di Bologna, Italy
[b] Nanodiagnostics srl, Modena, Italy
[c] DISTAV, University of Genova, Genova, Italy
[d] ENEA-Technical Unit Technology of Materials, Brindisi, Italy
[e] ISMAR-CNR, Genova, Italy
[f] Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Palermo, Italy

ARTICLE INFO

Article history:
Received 22 May 2016
Received in revised form 22 July 2016
Accepted 31 July 2016
Available online xxx

Keywords:
Earthworm faeces
Microbial community structure
Microbial respiration
Earthworm health
Phospholipid fatty acids

ABSTRACT

The aim of this study was to investigate the impact of silver and cobalt, supplied both as ions and nanoparticles (Ag^{+}, Co^{2+}, AgNPs, CoNPs) through contaminated food to earthworms (*Lumbricus rubellus*), on their health as well as on microbial community of both soil and earthworm faeces. Earthworms and microbes were exposed to the contaminants in laboratory microcosms with artificial soil. Contaminants were supplied once a week for 5 weeks by spiking them on horse manure. The accumulation of CoNPs and Co^{2+} in earthworm tissues was two and three times greater than AgNPs and Ag^{+}, respectively. Except for AgNPs, contaminants significantly affected microbial community structure of earthworm faeces by increasing G- bacteria, thus also increasing the bacteria/fungi ratio while decreasing the G+/G- bacteria ratio. Such shift was also reflected on soil microbial community, thus suggesting a close relationship between microbial community of soil and of earthworm faeces. Neither of the Co treatments affected soil microbial basal respiration whereas they increased the microbial biomass specific respiration or metabolic quotient, suggesting some stress induction on soil microorganisms. Earthworm health was strongly affected as revealed by the reduced fluidity of fatty acids extracted from the body tissues. In addition, the histological investigations, after the depuration period, showed positive results about the NPs toxicity. In particular, TUNEL-positive nuclei in epidermis and in peritoneum, suggest the presence of toxicosis.The ESEM-EDS technique revealed the presence of Ca-P spherules (calcification) between mouth and clitellum of earthworms fed with Co^{2+} contaminated food.

1. Introduction

Metallic and ionic Ag has a long history of use as an antimicrobial agent in various industries, and is gradually being replaced by Ag nanoparticles (AgNPs) in agrochemicals, food preservatives and storage containers, textiles, personal-care products and laundry additives (Maynard et al., 2006). The use and disposal of materials containing AgNPs and Ag^{+} allow these potential contaminants to enter the environment. Another matter of interest as possible environmental contaminants are Co nanoparticles (CoNPs), which are used to produce magnetic polymer microspheres, for information storage and energy, as magnetic resonance imaging contrast agents, in cancer therapy and in anaerobic waste water treatment systems (Florencio et al., 1994; Magaye et al., 2012). The AgNPs and CoNPs forms merit study in natural environments, especially the soil ecosystem where they are subject to transformations like aggregation/agglomeration, redox reactions, dissolution, exchange of surface moieties, and reactions with biomacromolecules (Maurer-Jones et al., 2013). The multiple ways that these NPs may be transformed in the soil environment makes it difficult to predict their fate and toxicity to soil biota, which have a central role in sustaining soil fertility and productivity.

* Corresponding author at: Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Edificio 4, 90128 Palermo, Italy.
E-mail address: vitoarmando.laudicina@unipa.it (V.A. Laudicina).

http://dx.doi.org/10.1016/j.apsoil.2016.07.019
0929-1393/

Direct toxicity of Ag and Co to soil microorganisms is expected to reduce microbial activity and alter the biomass of key groups within microbial communities (Dinesh et al., 2012; Shah et al., 2014). However, soil microorganisms do not respond uniformly to NPs (Vittori Antisari et al., 2015), probably because some microbial groups and genera have mechanisms to sequester metallic and ionic compounds or alleviate cellular stress induced by metal exposure (Nies, 1999; Dinesh et al., 2012). The direct toxicity of Ag and Co to higher trophic groups in the soil food web is another factor that could impact microbial communities. For instance, earthworms are ecosystem engineers that consume and reorganize litter through the soil profile (Bernard et al., 2012; Blouin et al., 2013). Earthworm activities can also consistently stimulate bacterial growth, with higher bacterial populations in the earthworm gut and freshly-deposited faeces (Blouin et al., 2013). Earthworms consume fungal spores and defecate them in nutrient-rich faeces, a favourable microsite for microbial growth. As a consequence, during the passage through the digestive tract of the earthworms, ingested soil microorganisms can be selectively altered (Pedersen and Hendriksen, 1993; Sampedro and Whalen, 2007; Thakuria et al., 2010).

In a previous study (Vittori Antisari et al., 2015), we found that soil microbial biomass and activity is significantly affected by food contaminated with NPs. However, no indications emerged whether microbial community is altered following the passage through earthworms fed with contaminated food.

The aim of this study was to investigate the impact of Ag and Co fed to earthworms (*Lumbricus rubellus*) both as nanoparticles and ions through contaminated food on their health as well as on microbial community of both soil and earthworm faeces. The hypotheses to be tested were that the supply of Ag and Co can alter the microbial community of earthworm faeces and that such alteration in turn can induce changes in the soil microbial biomass, activity and community structure. Furthermore, also earthworms can be directly affected by contaminants so inducing a disorder in their tissues. Finally, we hypothesised that Ag and Co could remain within the body of earthworms also after a depuration period, thus negatively affecting their health. In the first part of the experiment, biochemical soil properties, such as microbial biomass, its activity and community structure, and parameters linked to earthworms stress were investigated in order to contribute to the identification and validation of biomarkers affected by contaminant exposure. Phospholipid fatty acids (PLFAs) and total fatty acids (FAs) were used as biomarkers of shifts in soil microbial community structure and of earthworms stress, respectively. PLFAs were also determined on earthworm faeces to find out possible relationships between microbial communities within soil and earthworm faeces.

Finally, in the second part of the experiment, earthworm health after a period of depuration was assessed by measuring various parameters and using advanced techniques, such as apoptosis frequencies (TUNEL test), Ag and Co contents in earthworm body tissues (ICP-OES), scanning electron microscopy coupled with X-ray probe for microanalysis (ESEM-EDS), and X-ray computed microtomography (X-ray micro-CT).

2. Materials and methods

2.1. Nanoparticles and ions

AgNPs and CoNPs were purchased from Polytech & Net GmbH Germany (AGS-WM1000C) in form of solution (1000 ppm) and from Nanostructured & Amorphous Materials Inc. (USA), in form of powder, respectively. The CoNPs vacuum-sealed bag was opened in a nitrogen-controlled atmosphere cabinet, weighed and mixed with milli-Q water.

The hydrodynamic diameter and zeta potential of nanoparticle fresh suspension were measured by the technique of Photon Correlation Spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, UK). The analyses were performed thrice at 25 °C with an angle of 90° (Table 1). Ionic treatments were prepared from standard solution bought from CPI International (USA): Ag (cod. 4400-1000511), Co (cod. 4400-1000131). The solutions were prepared using high purity metal (99.9%), sub-boiling in distilled nitric acid and diluting in 18 MΩ milli-Q water.

2.2. Experimental design

Mature (with clitellum, two months old) earthworms of the species *L. rubellus* were obtained from a local synchronized culture and weighed individually (average earthworm weight 323 ± 67 mg; n = 200). Ten mature earthworms were randomly placed in a box containing 500 g of an artificial soil and allowed to acclimate for two weeks. Soil moisture was maintained at 60% of water holding capacity and temperature at 25 °C. Twenty boxes, each with 10 earthworms, were prepared.

The artificial soil was prepared by mixing neutral sphagnum peat and forest soil (Epileptic Cambisol; sandy clay loam texture; total organic carbon 41.9 g kg^{-1}; total N 3.2 g kg^{-1}) at 1:1 v/v ratio. It had pH 6.9 (in 0.01 M $CaCl_2$; 1:2.5 w/v), cation exchange capacity (CEC) 35.2 $cmol_{(c)}$ kg^{-1}, total organic carbon (TOC) 167.5 g kg^{-1}, total nitrogen (TN) 6.8 g kg^{-1}; microbial biomass carbon (MBC) 863.2 mg kg^{-1}; microbial biomass nitrogen (MBN) 95.7 mg kg^{-1}.

After the acclimation, earthworms were exposed to AgNPs, Ag^{+}, CoNPs or Co^{2+} via their diet for five weeks. The control treatment differed from the four metal treatments only for earthworms being fed with uncontaminated food. Four boxes per treatment were set-up. Earthworms were fed once a week with ground horse manure (0.5 g dry weight of manure per worm per week). Manure from a non-medicated horse was spiked, 24 h prior to feeding, with a water solution of NPs or ions (as nitrates) to reach the 65% of WHC. The concentration of both NPs and ions was 10 mg of contaminant kg^{-1} dry horse manure. Therefore, each earthworm was exposed to 25 μg of contaminants during the five weeks, i.e. 0.5 μg per gram of soil. The amount of contaminants used in this study was of the same order of our previous and other similar works (Coutris et al., 2012; Dinesh et al., 2012; Vittori Antisari et al., 2015). After the five weeks of exposure to contaminated food (first part), the surviving earthworms of two boxes were transferred to Petri dishes for two days, in order to empty their guts by defecation, and then prepared for further analysis. Faeces produced during the two-days of

Table 1
Main characteristics of Ag and Co nanoparticles (NPs) used to contaminate the horse manure supplied to the earthworms.

Material	Purity (%)	Nominal size (nm)	SSA (m^2 g^{-1})	Morphology	Average hydrated diameter (nm)	Z-potential (mV)	Ratio of occupied volume (cm^3 mL^{-1})	Notes
AgNPs[a]	ND	1–10	–	irregular	60.3	−32.5	13 E-5	Solution in water
CoNPs	99.8%	28	40–60	spherical	102	24.6	77 E-5	Powder: partially passivated w/ [oxygen] about 10%

[a] AgNPs were coated with polyvinyl pyrrolidone (PVP); SSA is the specific surface area.