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Novel egg life-stage test with *Folsomia candida* – A case study with Cadmium (Cd)

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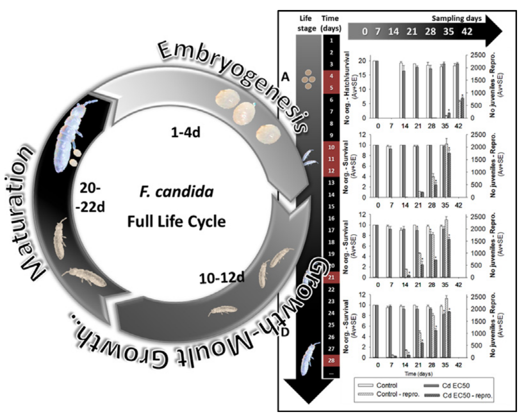
HIGHLIGHTS

- The standard collembolan test is based on exposure of one life stage (juveniles).
- An egg stage test has been here developed and optimized.
- Lower Cd impact on reproduction from pre-exposed eggs (compared to juveniles).
- Cd seems to affect reproduction via exposure of adults.
- It is recommended to test different life stages in a combined approach.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxicity of pollutants is known to have a different impact depending on the organisms' life stage. Standard tests are often based on one life stage, i.e. effects could be underestimated. We aimed here to develop and optimize a test system using eggs of *Folsomia candida* (4–5 days) instead of the juveniles (10–12 days old) required by the OECD standard test guideline No. 232 (2009). Accordingly, the exposure time and thus the test duration was extended. Tests with "standard" juveniles (10–12 days old) and, adults (21 and 28 days old) were also performed. Cadmium (Cd) was used as test substance. The extension to the test guideline starts as follows: 1) synchronization of eggs in a thin soil layer on plaster of Paris, 2) selection of viable eggs, 3) burying these eggs in groups of 5 in soil. Afterwards, the test procedure will follow the standard procedure as described in the OECD standard test. Cadmium caused ca. 50% effects on reproduction at 60 mg Cd/kg soil dry weight (DW) when exposing juveniles or adults. There was no significant impact of Cd on the eggs, the hatching process or the latter life stages until ca. 250 mg Cd/kg DW (Cd is stable during this exposure period). Hence, Cd seems to affect reproduction before egg laying, i.e., during egg formation or during juvenile-adult stages. In order to clarify whether other chemicals do act in a similar way testing with different chemicals is highly recommended. Testing of different life stages does provide insight on the mechanisms and effects of contaminants and offers important insight.

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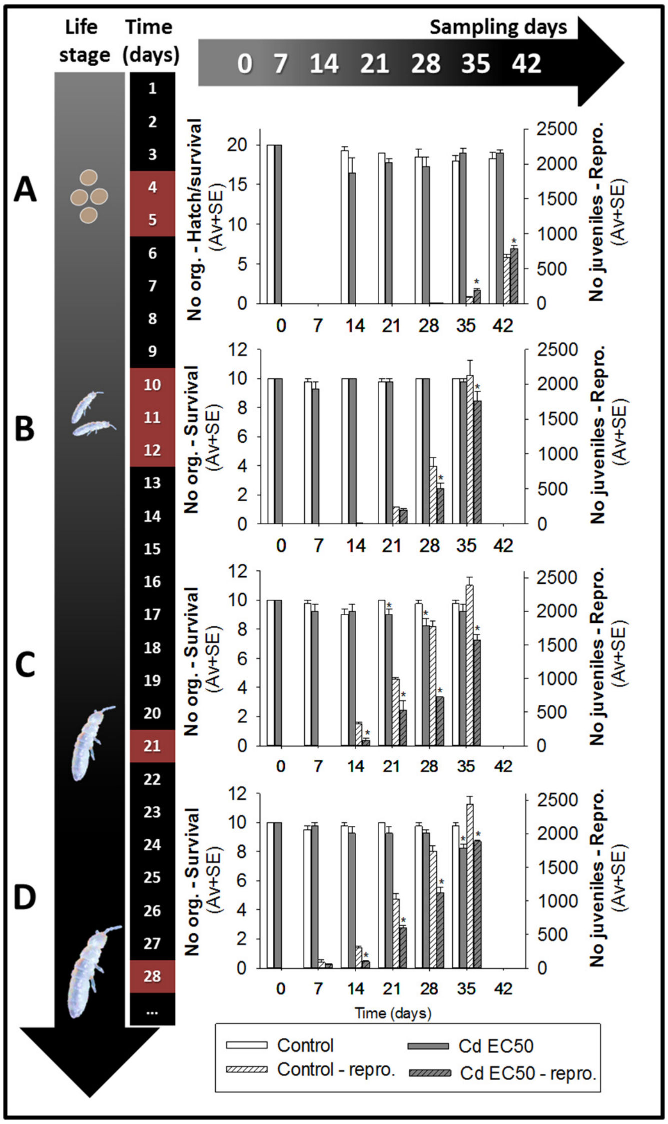

Fig. 1. Hatching success, survival and reproduction of *Folsomia candida* when organisms were exposed as A) eggs, B) 10–12 d C) 21 d and D) 28 d old organisms to 60 mg Cadmium/kg d.w., which has been determined to be the EC50 reproduction for *F. candida*,) in LUFA 2.2 soil. Open columns refer to hatching/survival numbers and striped columns to the number of juveniles. For day 0 columns represent the number of introduced eggs/organisms. Values are expressed as average ± standard error (AV + SE). *p < 0.05: corresponds to statistically significant differences between the control and the treatment (Dunnets').

1. Introduction

Pollutants have a different impact depending on the life stage during which the tested organisms are exposed (Belanger et al., 2010; Bicho et al., 2015). Testing for hazard assessment has been based on standard guidelines where organisms' life stage or age is often optimized and synchronized, e.g. for *Folsomia candida* (Willem 1902; Collembola), 10–12 day old juveniles are used (OECD 232, 2009). This exposure regime may cover potential effects on more than one life stage, but the way the test is designed does not allow for the identification of effects at individual life stages (which can reveal increased sensitivity, among others, see e.g. Bicho et al., 2016), hence the need for refinement. The implications are currently not covered in the Environmental Risk Assessment (ERA) procedures of the European Union (EU). In detail, the sensitivity of organisms to contaminants may be higher in certain periods of their life cycle, especially early life stages (Tarazona et al., 2014). For instance, eggs of the springtail *F. candida* are not mobile but show high physiological activity due to embryo growth and development, whereas hatched juveniles are in active contact with pollutants, e.g. can avoid it in patchiness field contamination. However, juveniles are not effective migrators and cannot avoid toxic conditions, especially in the first days of life. For oligochaete worms, different sensitivity between juveniles and adults is known, e.g. regarding survival in earthworms (Kwak and An, 2015; van der Ploeg et al., 2011) or reproduction of enchytraeids (Bicho et al., 2015, 2017; Santos et al., 2017). Few studies testing alternative exposure regimes have been published, including different multigenerational approaches with collembolans (Amorim et al., 2017; Campiche et al., 2007; Ernst et al., 2016). For instance, Filser et al. (2013) exposed collembolan eggs to chemicals and reported eggs preliminary results, but hatching success was relatively low. Besides, differences in sensitivity of juvenile *F. candida* differing in age by just one day are known (Crouau and Cazes, 2003) and show the importance and inherent variability with age. Therefore, the optimization of a test to expose *F. candida* from egg stage is highly relevant for chemical risk assessment in the soil compartment.

Hence, the aim of this study was to optimize and develop a test system using eggs of *F. candida* (4–5 days) instead of the standard juvenile stage (10–12 days old). In addition, it was investigated which exposure regime would be relevant to cover the life cycle of the collembolan, from the egg stage till the reproduction phase. Further, in order to compare the recorded sensitivity of the egg stage with those from older life stages, adults with an age of 21 and 28 days were also tested. Cadmium was used as test substance since: 1. its toxicity to collembola in standard tests is well-known (Amorim et al., 2017); 2. it has embryotoxic (Stummann et al., 2008); 3. it is not an essential and is widespread in the environment due to anthropogenic activities (Son et al., 2011); 4. it is quite bioavailable to living organisms (Crommentuijn et al., 1997; van Gestel and Mol, 2003; Vig, 2003).

Table 1
Effects of cadmium (60 mg Cd/kg) on the reproduction of *Folsomia candida* in LUFA 2.2 natural soil based on tests using different life stages. Results are expressed as average (±SE) the number of organisms and the relative percentage of effects on organisms found in spiked soil compared to control soil.

Life stage	Eggs		Juveniles (standard)		Adults		Adults	
Age (days)	4–5		10–12		21		28	
Cd (mg/kg)	0	60	0	60	0	60	0	60
Exposure time (days)								
14	0	0	0	0	(0%) 322 ± 25	(75 ± 10%) 81 ± 33	(0%) 303 ± 29	(67 ± 4%) 101 ± 10
21	0	0	(0%) 238 ± 8	(16 ± 9%) 199 ± 21	(0%) 989 ± 22	(47 ± 15%) 523 ± 151	(0%) 1027 ± 80	(41 ± 3%) 605 ± 29
28	0	0	(0%) 829 ± 118	(39 ± 10%) 502 ± 82	(0%) 1780 ± 83	(59 ± 0%) 726 ± 5	(0%) 1738 ± 84	(35 ± 5%) 1123 ± 78
35	(0%) 85 ± 22	(−132 ± 25%) 197 ± 21	(0%) 2124 ± 210	(17 ± 6%) 1763 ± 130	(0%) 2388 ± 119	(34 ± 4%) 1570 ± 83	(0%) 2435 ± 123	(23 ± 1%) 1886 ± 16
42	(0%) 658 ± 47	(−20 ± 6%) 789 ± 42	–	–	–	–	–	–