SNAIL SURVIVAL AS BIOINDICATOR OF CADMIUM CONTAMINATED SOILS UNDER SEMI-REALISTIC FIELD CONDITIONS

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ABSTRACT
Juvenile brown garden snails (*Helix aspersa* Müller) were exposed to Cd-contaminated soils for 60 days under semi-realistic field conditions. The soils were contaminated with increasing concentrations of cadmium chloride (0–2900 mg kg^{-1} Cd^{2+}/solution $CdCl_2$). The snails were housed in terrariums and were fed exclusively with nettle leaves. The experiments were carried out in Timisoara on 1000 snails.The survival rates differed significantly for different Cd treatments. Only higher cadmium concentrations (> 1000 mg kg^{-1} Cd^{2+}/solution $CdCl_2$) had a significant influence on survival curves of snails exposed to Cd-contaminated soils. The maximal death rate reached 81.00±9.9% for snails exposed to soils contaminated with 2900 mg kg^{-1} Cd /solution $CdCl_2$. We found dose-dependent survival rates, whereas the half maximal effective concentration was 1365 mg kg^{-1} Cd^{2+}/solution $CdCl_2$. Our results suggested that longer-term studies are required for assessing the real potential of snail survival rate as bioindicator of Cd-contaminated soils in field conditions.

Keywords: Helix aspersa, soil, cadmium, survival

INTRODUCTION

Cadmium (Cd) is the most dangerous pollutants among heavy metals (SHAHRTASH ET AL., 2010). Generally, it has no known physiological function in live organisms (TRAUB AND HOFFMAN, 2006), excluding diatoms for which a Cd-based enzyme plays an essential role in regulating atmospheric carbon (LANE ET AL., 2005). Naturally-occuring Cd in soil ranges between 0.01 and 1.1 mg/kg, depending on the type of parent rocks (EL FALAKY ET AL., 1991; SCOULLOS ET AL. 2001). The antropogenic sources of Cd includes industrial emissions and the application of sewage sludge and fertilizers to farm land (SATARUG ET AL., 2003; CHEN ET AL., 2007; PERALTA-VIDEA ET AL., 2009). Cd is regarded as the most mobile heavy metal in soils (PUEYO ET AL., 2003). However, this heavy metal accumulates in soil only in small amounts, but it is easily absorbed by plants and animals (VELTMAN ET AL., 2007). Recent studies found that cadmium pollution has contaminated many agricultural areas (CESUR AND KARTAL, 2007; TANG ET AL., 2011). Therefore, soil Cd concentration may pose serious threat to human health by uptake of Cd from vegetables grown on contaminated soils (ZHAI ET AL., 2008).
Among other terrestrial invertebrates used as bioindicator organisms of soil pollution, land snails are recognized for their outstanding ability to concentrate high amounts of Cd in their body (BERGER AND DALLINGER, 1989; GOMOT DE VAUFLEURY AND KERHOAS, 2000; NOTTEN ET AL., 2006). This is because of specific Cd-sequestering metallothioneins (Cd-MT) that are involved in Cd detoxification (DALLINGER ET AL., 2001; HISPARD ET AL., 2008). *Helix aspersa* (syn. *Cornu aspersum* and *Cantareus aspersus*), known by the common name brown garden snail, is the most often employed land snail as bioindicator

species in environmental monitoring studies. It was found that animals of similar size (weight and/or height) are convenient biological indicators for metallic pollution (COUGHTREY AND MARTIN, 1977). This species ecophysiological particularities are well known, and it is easily reared both in the laboratory and commercially (GARCIA ET AL., 2006). In addition, exposure to Cd-enriched food inhibited snail feeding and growth in a dose-dependent manner (LASKOWSKI AND HOPKIN, 1996). Therefore, GOMOT (1997) considers *Helix aspersa* a reliable Cd-pollution bioindicator, equally efficient with earthworms and much more sensitive than collembolas.

Standardized ecotoxicogical tests (ISO 15952, 2006) are currently available for assessing the effects of pollutants via digestive and cutaneous exposure on survival and growth of snails, usually young *Helix aspersa* Müller. Generally, these investigations were performed in specialized laboratory, under a long photoperiod, 18 h L/24 h, at 20 ± 2°C with a hygrometry of 80–95% (GOMOT DE VAUFLEURY AND PIHAN, 2000). Not only that such laboratory toxicity tests fail to predict effects under variable field conditions, but their applicability is limited only to the species being tested. Contact with the Cd-contaminated soil is essential in digestive and epithelial transfer of Cd from soil to snail (COEURDASSIER, 2002). However, little information exists concerning the soil Cd toxicity under field and/or semi-realistic conditions. Contextually, this study aims to evaluate the sensitivity of snails to Cd exposure via contaminated soil, and to examine the potential usefullness of survival rates as endpoints in assessing the long-term ecotoxicological impact of Cd-polluted soils under semi-realistic field conditions.

MATERIAL AND METHOD

Chemicals

The stock solution of cadmium chloride ($CdCl_2$ 99.999% pure) was purchased from Sigma-Aldrich Chemie GmbH **(**Buchs,Switzerland). For all metal treatments, nine ascending concentrations of Cd were used to contaminate the soil. The nominal concentrations were 250, 500, 1000, 1250, 1500, 1750, 2000, 2500, and 2900 mg kg^{-1} $Cd^{2+/}$sol. $CdCl_2$. In contrast, the control group was not exposed to Cd-contaminated soil. Each test included two replicates per concentration with each replicate jar containing 50 snails.

Maintenance of Animals

Juvenile garden snails (*Helix aspersa* Müller) approx 4-month old were purchased from a specialized snail farm (Edimpe Auto S.R.L., Muntenii de Sus, Vaslui county, Romania) in April 2011. All the experiments were conducted outdoor in Timisoara (Timis county, Romania). The thermal and pluvial regime of this area allows a proper snail development during between April and June, when 33 percent of the annual rainfall takes place, the average diurnal temperature usually does not exceed 25°C, and the mean nocturnal temperature does not decrease below +8°C (NICA, 2009). Before experimental phase 1200 animals were acclimatized to these conditions during 20 days.

To test the samples homogenity, the shell height and the snail weight were measured and compared statistically. Shell height was compiled from BURA (2004), and was performed with a digital caliper (YT 7201, Yato Electronics Co. Ltd, Guangzhou, China) to the nearest 0.01 mm. All snails were weighted by using an analytical balance (model TP-214, Denver Instrument Gmbh, Göttingen, Germany) to the nearest 0.1 mg. The most homogenous 1000 juveniles were transfered in terrariums/plastic boxes (length: 50 cm, width: 20 cm, height: 25 cm, volume: 0.025 m^3, surface area available for the snails to

move across: 0.10 m^2), 50 juveniles per each terrarium. Each box was covered with a lid built of glass fiber net (φ mesh = 0.50 cm), mounted on an aluminium frame.
The soil, red turf (code 01-F2-61-A), was purchased from a specialized trader (Iza S.R.L., Timisoara, Romania). The soil had the following physico-chemical properties: pH: 5.50 – 6.50; ash content: < 5.00%; nitrites: 5.50%; nitrates: 8.50%; phosphor: 16.00%; potasium: 18.00%; magnesium: 0.65%; boron: 0.03%; copper: 0.12%; iron: 0.90%; manganese: 0.16%. zinc: 0.04%; chlorium: 0.30%; molybdenum: 0.20%. Before being introduced into the plastic boxes, the soil (500g/plastic box) was contaminated with cadmium chloride solutions, and homogenized for a proper dispersion of Cd ions. A sponge (10x10 cm) soaked with double-distilled water (spectroscopic pure) was placed at the bottom of each terrarium to maintain humidity at 100%. The snails were fed ad libitum with fresh nettle leaves. The nettle leaves were collected daily from the same place, and rinsed in double distilled water to wash off potential air pollutants. To limit food contamination through soil contact, in each terrarium the nettle leaves were placed inside a watch glass (φ = 10.00 cm). Every day, the cages were checked to monitor juveniles fitness, whereas dead specimens, excrements, and uneaten food were removed. The experimental phase lasted 60 days.

Statistical Analysis
Statistical analysis was provided by using the Statistica 10 software package, free trial version. For each sample, the shell height and the body weight were checked to see if they meet the normality assumptions (Shapiro-Wilcoxon test, n = 50).
All the samples were tested for equal variances of shell height and body weight (Bartlett's test, n = 1000). A One Way Analysis of Variance (Anova, df = 9) was carried out to test for significant differences in shell size and snail weight among different samples. For survival rate tests, EC50 value was calculated according to formula:
$y = b_0 - b_0/[1 + (x/b_2)^{b1}]$
In this mathematical model, x is the pollutant level ($x \geq 1$) and y is the organism response, in terms of the percent of maximum responsiveness. The parameter b_0 denotes the expected response at saturation, b_2 is the concentration for a half-maximal response, and b_1 determines the slope of the function. Breslow's test (also known as Gehan's generalised Wilcoxon test) was used to compare the survival distribution of all samples. Post hoc analysis performed statistical comparisons between the survival curves of control and Cd-exposed groups (Log-rank test).

RESULTS

The average fresh weight of snails was 2.06 g (range 1.432 – 2.622 g). The mean shell height was 1.923 cm (range 1.840 – 1.995 cm). Statistical tests for normality showed that neither shell height nor snail weight departed with statistical significance from the normality assumption (Shapiro-Wilcoxon test, p > 0.67). Analysis of variance demonstrated that variances are equal across all samples both for shell height and snail weight (Bartlett's test, p > 0.25). There were no statistically significant differences between group means for shell height and snail weight as determined by One Way Analysis of Variance (ANOVA, p > 0.55**).** Therefore, it was concluded that our samples meet the assumption of homogenity.
Total survival rate (%) revealed a direct relationship between soil Cd concentration and juveniles death rates (*Figure 1*). Our results showed that survival curves were significantly different among different Cd treatments and the control groups (Breslow`s test, p < 0.001).

The survival rates of snails exposed to high Cd-contaminated soils (1000–2900 mg kg^{-1} $Cd^{2+/}$sol. $CdCl_2$.) were significantly higher than those of control snails (Logrank test, $0.05 < p < 0.001$). In contrast, no statistical difference were found between the survival curves of snails exposed to lower doses of Cd via soil (250–500 mg kg^{-1} $Cd^{2+/}$sol. $CdCl_2$.) and the control group ($p > 0.55$, Log rank test).

Because of data redundancy (i.e. duplication of data as a result of equal survival rates) several algorithms were used to compute the derivatives of the loss function. The Rosenbrock Pattern Search method found the best curve fitting function that acounted for 87.593% of the variance of snail response to Cd exposure via soil (function loss: 0.002, R = 0.9351). It was found that the half-maximal effective concentration (EC_{50}) of Cd toxicity for soil exposure resulted from the watering of soil with 1365 mg kg^{-1} $Cd^{2+/}$sol. $CdCl_2$ (*Figure 1*).:

$$y = 0.1615 - 0.1615/[1 + (x/1365.4278)^{6.597}]$$

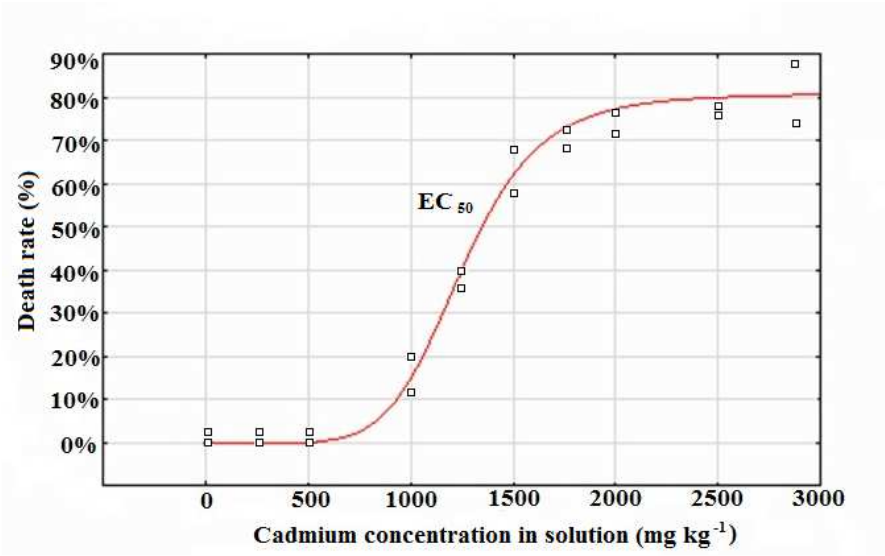

Figure 1. Dose-response curve between soil Cd concentration and snail death rate.

GOMOT-DE VAUFLEURY ET AL. (2006) conducted experiments under laboratory conditions to assess Cd toxicity on juvenile *Helix aspersa* growth and survival rates. It was found that Cd-exposure via food (EC_{50} = 68–139 mg kg^{-1}) was six fold more toxic than exposure via contaminated soil (EC_{50} = 534–877 mg kg^{-1}). When comparing to our results it was inferred that such differences resulted from different experimental conditions (i.e., duration, dose, microclimate). COEURDASSIER ET AL. (2002) used two models to expose juvenile *Helix aspersa* to Cd-contaminated substrate (0, 100, 500, 1000 mg kg^{-1}): in direct contact with the substrate or separated from substrate with a perforated plate, thus avoiding tegumentary contact but allowing substrate ingestion. Experiments were performed for 4 weeks under laboratory conditions. The results showed that epithelial contact doubles the rate of Cd transfer via soil to juvenile snails than simple soil ingestion. Generally, elevated Cd bioaccumulation in the snail body, growth inhibition, and decreased food consumption are used to assess Cd toxicity on snails. LASKOWSKI AND HOPKIN (1996) reported high survival rates to *Helix aspersa* juvenile and adult specimens (5.00–8.33%) that were fed with Cd-enriched diet (0.32–145.00 mg kg^{-1}) for four weeks. The same authors suggested that longer exposure would certainly induce lower survival rates as GOMOT-DE VAUFLEURY ET AL. (2006) and the present study have demonstrated it. The applicability of the present approach in ecotoxicology is currently rather theoretical since such heightened

Cd concentrations in soil are rarely expected to occur in the natural environments. Therefore, further studies must approach this problem by using lower Cd concentration and longer time of exposure.

CONCLUSIONS

Total survival rate revealed a direct relationship between soil Cd concentration and juveniles death rates. Survival curves were significantly different among different Cd treatments and the control groups. Our results suggested that longer-term studies are required for assessing the real potential of snail survival rate as bioindicator of Cd-contaminated soils in field conditions.

ACKNOWLEDGMENTS

The present work was funded by project "Post- doctoral School Of Agriculture And Veterinary Medicine Posdru/89/1.5/S/62371", co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007–2013.

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