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Effects of Lead Pollution Against Juvenile *Achatina achatina* Fed on Contaminated Artificial Diet

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Abstract We investigated juvenile *Achatina achatina* snails by feeding graded levels of inorganic lead metal contaminated artificial diet in plastic snaileries in the laboratory. Snails were tolerant of all levels of lead contamination with no mortalities. Results indicated significant ($p < 0.05$) transfer of lead from diet to snail with high positive ($r^2 = 0.98$) relationship. Our data suggests that decreased feed intake and growth were found at elevated lead levels. Tissue lead accumulations were lower than dose in artificial diet.

Keywords Edible snail · Lead accumulation · Contaminated diet

Until recently, indications provided by contaminants analyzed in animals sampled in the field yielded generalized information. To compensate for this, animals (from standardized cultures) are now used in the laboratory to evaluate specific outcomes (Cortet et al. 1999). In the laboratory studies, mostly an artificial type of food was used (Gomot-de Vaufleury and Kerhoas 2000). Contaminants are taken up by snails through diet, which serve both as food and bait (Ebenso 2004). Besides, elevated metal concentrations in the food can directly influence consumption, growth, reproduction and survival (Russell et al. 1981).

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Land snails are herbivores and detritivores. Land snail biological cycle is fully controlled and easily reared in the laboratory (Gomot-de Vaufleury 2000). Snails satisfy all conditions of a good biological indicator (Hopkin 1993). Snails can also be useful bioindicators within their niche (Ebenso 2006). This study concerns feeding of inorganic Pb contaminated diet to evaluate the effects of Pb on juvenile *Achatina achatina* under controlled laboratory conditions.

Materials and Methods

The design of the experiment was completely randomized. The experiment was conducted in the laboratory with conditions of temperature 24 ± 2°C, relative humidity 90% and photoperiod 12 h light:12 h darkness.

A total of 210 juvenile *A. achatina,* with mean fresh weight of 32.00 ± 0.50 g, were sourced from unpolluted laboratory reared species. The snails were randomly assigned to artificial dietary treatment (ingredients were milled into mash form) (Table 1) of seven experimental doses using three replicates of ten snails. Experimentation was conducted in microcosms (plastic snaileries), according to methods of Ebenso and Ologhobo (2008a, b). Microcosms were placed on laboratory benches to detect presence of Pb pollution through oral route, using dose-dependent contaminated diet for 12 weeks. These snails received diet ad libitum. Snails were sprinkled with water twice weekly to maintain humidity.

Nominal Pb doses were 0, 50, 100, 250, 500, 750 and 1,000 μg/g, respectively (Table 2). Solutions of 0.1 M of pre-defined doses were prepared from $PbCl_2$ (Analar

Table 1 Composition of *Achatina achatina* artificial diet

Ingredient	%
Maize	29.00
Brewer's dried grain	10.00
Wheat offal	4.00
Palm kernel cake	2.20
Groundnut cake	10.00
Soya bean meal	31.40
Fish meal	4.00
Oyster shell	8.05
Bone meal	1.10
Vitamin premix	0.25
	100.00

grade), using calculated (actual) Pb^{2+} weights of 0, 67, 130, 335, 671, 1,007 and 1,343 µg/g (Table 2). The diet was enriched (wetted) with corresponding dose solution for each treatment during each new feeding session. On weekly basis previous uneaten artificial diets were collected and weighed to arrive at weekly feed intake, snail weight and shell thickness were measured according to methods of Ebenso (2003).

Samples of snails and diets were analyzed for Pb, using Atomic Absorption Spectrophotometer (AAS) as outlined by Ebenso and Ologhobo (2008a).

All data collected from each treatment for all parameters considered were subjected to ANOVA using SAS (1999). The means were separated using Duncan Multiple Range Test.

Results and Discussion

The analyzed Pb doses in Table 2 correspond to environmentally realistic soil Pb of previous field study of Ebenso and Ologhobo (2008a). Snails in the present study did not die, this compares with previous field studies of Ebenso and Ologhobo (2008a, b). Reports of Pawert et al. (1996) studying collembolan indicated comparatively high tolerance, which were also reflected by its remarkably low mortality during exposure. Contrary to studies of Ebenso (2004), no neurotoxic effects were observed in the present study. Neurotoxic effects may result in alteration in feeding behaviour (Bailey 1989).

In Table 2, decrease in feed intake resulted in depressed growth (weight gain and shell thickness). Swaileh and Ezzughayyar (2001) reported that Pb contaminated diet significantly reduced growth and feeding rates of *Helix engaddensis*. According to Gomot-de Vaufleury (2000), these results appear to be paradoxical, in that, a strong decrease in feed intake does not seem to bring about a decrease in growth. On the other hand, Laskowski and Hopkin (1996) recorded no reduction in growth from Pb even at high dose (30,000 µg/g). However, Russell et al. (1981) reported a sharp decrease in food intake (from 100 µg/g) that is closely related with a reduction in growth. Further, reduced consumption results in a decrease in energy entering the animal's body. Besides, according to Walker et al. (2001), accumulation and detoxification of metals within soft tissues generally implies an additional energy cost, in combination, these affect growth (Notten et al. 2006).

As in previous study (Ebenso and Ologhobo 2008a, b), Pb accumulation in Table 2 increased with increasing Pb concentration in diet. The positive significant ($p < 0.05$) relationship $Y = 0.08x + 0.20$ of snail and diet in the present study indicated transfer of Pb from diet to snail, the coefficient of determination ($r^2 = 0.98$) was high. This relationship compares with reports of Notten et al. (2005). Gimbert et al. (2006) suggested that metal transfer along the food chain is important, demonstrated by the internally cross-validated variances (Q^2), which is always close to coefficient of determination (r^2).

Table 2 Lead metal in diet and tissue of *Achatina achatina*

Parameter	Diet							SEM
Nominal Pb, µg/g	0	50	100	250	500	750	1000	
Calculated Pb, µg/g	0	67.00	130.00	335.00	671.00	1007.00	1343.00	
Analyzed Pb, µg/g	1.33 g	70.98 f	134.61 e	339.40 d	674.86 c	1009.22 b	1344.39 a	0.04
Growth performance								
Feed intake, g	35.73 ab	35.79 a	34.45 c	35.28 c	34.73 d	34.31 e	32.64 f	0.11
Weight gain, g	24.44 a	23.75 c	23.98 b	23.74 c	22.80 d	22.15 e	21.47 f	0.01
Feed conversion ratio	1.46 d	1.51 b	1.48 c	1.49 c	1.52 b	1.55 a	1.52 b	0.01
Shell thickness, mm	0.17 a	0.17 a	0.15 c	0.16 b	0.15 c	0.15 c	0.14 d	0.02
Tissue lead accumulation								
Pb µg/g	2.48 g	37.61 f	63.40 e	110.37 d	320.00 c	517.10 b	621.45 a	0.04

abc… means followed by different letters are significantly different by Duncan Multiple Range Test $\alpha = 0.05$

Gomot-de Vaufleury and Pihan (1997) observed that, the capacity for bioaccumulation of metals by slugs and snails demonstrated both in the field and laboratory studies makes these invertebrates excellent accumulation bioindicators. In conclusion, the findings of our study revealed that dose-dependent laboratory toxicological experiments could be useful to monitoring accumulation effects of environmental Pb pollution, with similar bioavailabilty.

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