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Effects of Lead Pollution at Industrial Contaminated Sites on Sentinel Juvenile *Achatina achatina*

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Abstract We investigated juvenile *Achatina achatina* snails exposed as sentinels in plastic cages for 12 weeks to compare lead pollution at dump sites of abandoned battery factory (Niger Delta, Nigeria). Results indicated 0.56, 20.37, 200.42 and 1200.30 µg/g soil lead at control, storage, dried effluent and waste dump sites, respectively. There were significant ($p < 0.05$) depression in snail growth with increasing level of lead pollution. Snails were tolerant of all levels of lead pollution with no mortalities. This novel approach provides a basis for use of snail data in environmental pollution assessment of industrial sites.

Keywords Edible snail · Lead accumulation

Lead can reach terrestrial environments via natural processes including weathering of rocks (Hodson and Langan 1999), plants rooting in contaminated dredged sediment, or by anthropogenic activity as in the applications of sewage sludge to soil, applications of fertilizers to soils, industrial discharge and atmospheric deposition (Chapman et al. 2003). Agricultural soils have a median Pb content of 11 µg/g (Holmgren 1993). Exposure to and uptake of this non-essential element have consequently increased (Tong et al. 2000). Macrofauna are an important part of the soil environments. Snails are involved in many aspects of organic matter decomposition, partial regulation of microbial activities, nutrient cycles and crumbly structure (Cortet et al. 1999). Metals in the soil can be taken up by plants and transferred to higher trophic level by means of herbivory (Beeby and Richmond 2003; Hopkin 1989). Ecotoxicological effects of pollutants at contaminated sites are dependent on the exposure and bioavailability of compounds, uptake and metabolism, intracellular concentration, mode of toxic action and balance between toxicity and protective cellular responses (Fent 2004).

Terrestrial snails are suitable to investigate transfer of metals from the environment to the herbivore level (Notten et al. 2005). Used as sentinels, snails are the representative primary consumers in the terrestrial ecosystem (Naeem et al. 1994). Terrestrial snails are well known for their accumulating capacities. Previous studies have investigated accumulation and effects of metals in land snails *Helix aspersa* (Regolis et al. 2006; Gimbert et al. 2006), *Cepaea nemoralis* (Jordaens et al. 2006; Notten et al. 2006), *Achatina achatina, Archachatina marginata* and *Limicolaria aurora* (Ebenso and Ologhobo 2007, 2008; Ebenso et al. 2006). This study investigated effects of elevated levels of Pb pollution against *A. achatina* as sentinels on contaminated soil sites.

Materials and Methods

The design of the experiment was completely randomized. The experimental area was the abandoned Sunshine Batteries Limited (SBL), Ukana, within latitude 5°80′N and longitude 7°41′E of Essien Udim Local Government Area, Akwa Ibom State, Niger Delta, Nigeria. Factory's major raw materials being Pb. Four soil sites were selected within the experimental area, viz: entrance as control, storage dump, dried effluent, and waste dump, respectively.

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Solid wastes were deposited at the waste dumpsite. The storage of sludge in drums was kept at the storage dumpsite. Liquid effluents were discharged into the Atan stream. Air pollution was ignored in this study. The experimental sites had an annual precipitation 1,300 mm, temperature 26 ± 2°C, relative humidity 80–90% and photoperiod 12 h light:12 h darkness.

A total of 120 juvenile *A. achatina*, with mean fresh weight of 32.00 ± 0.50 g, from unpolluted laboratory reared species. The snails were randomly assigned to 4 treatment sites of 10 snails replicated three times. The microcosms were plastic snaileries $0.24 \times 0.24 \times 0.12$ m^3, with mosquito netting on the lid to allow for light, air and protect snails against predators. The snails, as sentinels, were transferred to bottomless microcosms (suitable for contact with soil and vegetation), set up on the sites to detect presence of pollution. During the experimental period of 12 weeks (April–June, rainy season), the snails only consumed food (soil and vegetation) in situ and ad libitum.

All sites were randomly sampled three times within the microcosms. The top 15 cm of soil, excluding the litter layer, was sampled with a soil cores (diameter 10 cm), and hand sorted to remove roots a litters. Samples of the predominant vegetation (*Raphanus sativus)*, were randomly cut within microcosm at 3 cm above the soil and taken to the laboratory and dried to constant weight. Sample weights were multiplied by area of microcosm to calculate total weight of vegetation at the beginning of each week. This procedure was repeated at the end of each week, to arrive at the weekly feed intake (Heady and Heady 1982). Shell thickness was randomly measured (once a week) to the nearest 0.01 mm using micrometer screw gauge and the weekly mean was taken as an estimate of shell thickness, according to Ebenso (2003).

The soil samples were oven dried at 60°C for 4 days, then sieved prior to further analyses. Soil pH, particle size analysis and organic carbon were done according to methods of Bouyoucos (1951), clay content was determined by methods of Konert and Vandenberghe (1997). Cation Exchange Capacity (CEC) according to methods of Black (1967). The plants were rinsed in distilled water to wash off potential air pollutants. The leaves were separated, oven-dried at 60°C for 2 days and milled. The aliquot samples were divided into two parts. One part for proximate analyses, according to AOAC (1995). The second part for Pb content analyses.

Atomic Absorption Spectrophotometer (AAS) was used to analyze for Pb. AAS was calibrated using standard reference materials, as described by Hopkin (1990). A 1 g sample was digested in 2 ml 4:1 HNO_3 (65%) and HCl (37%) at 140°C for 7 h. The sample volume was made up to 10 ml with distilled water. All digestion procedures included 3–5 control blanks. All soil samples were analyzed for Pb on a Perkin Elmer Flame AAS1100. A 0.1 g plant sample was digested (as above) and plant samples were analyzed for Pb content using on Perkin Elmer Graphite Furnace AAS 2100. Foot tissue samples of snail oven-dried at 60°C for 2 days and digested in 2 ml HNO_3 (70%) and analyzed for Pb content as for plant samples.

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

Achatina achatina were all healthy throughout the experimental period. No snail aestivated and no snail died. Mortality can be considered as a first pollution alarm (Gomot de Vaufleury and Pihan 2000). Biota can and do adapt to wide range of metal concentrations without necessarily incurring any fitness costs (Barata et al. 2002).

The high percentage of sand recorded (Table 1) indicates the well-drained nature of the soil, and the sandy origin of the parent rock. According to Pederson et al. (2000), in such well drained soil, animals living in air-filled pores in the soil and in the litter layer on the top soil can accumulate metals both from water phase of the soil, from ingesting the soil itself and from food. By contrast, Scheifler et al. (2002) reported that, the well-drained nature of the soil results in leaching of metals to deep soil layers and ground water, thus may have decreased the quantity of metals at soil surface and less available for uptake by plants and snails.

The increasing acidity due to elevated Pb pollutions in soil resulting in proportionate decreasing values of organic carbon, P and low Ca in the present study, according to Scheifler et al. (2003) this is characteristic of low fertility. Similarly, Allen (2002); Ge et al. (2000) concluded that values of pH and organic carbon are important in determining the mobility, solubility and toxicity of metals in soil ecosystems.

Elevated soil Pb pollution was associated with increasing values of clay content (Table 1). This observation is supported by reports of Chapman et al. (2003) that most metals in soils are found as absorbed on clay colloidal complexes. Soil Pb at the sites in this study, may have been in ionic forms in wet soil conditions (experimental period was during rainy season). Ge et al. (2000) concluded that, soluble forms of metals in soil solution are only present at low concentrations; hence metal bioavailability in soil is low. In addition, inorganic forms of metals tend to decline along the food chain (USEPA 2002). Biodilution clearly occurs for metals such as Pb (Chen and Folt 2000).

Table 1 Chemical properties at battery factory sites

Parameter	Control	Storage dump	Dried effluent	Waste dump	SEM
Soil					
Sand (%)	85.50 b	79.41 d	84.17 c	88.59 a	0.2
Silt (%)	6.19 c	8.29 a	6.81 b	6.39 c	1.1
Clay (%)	8.27 c	9.21 b	14.29 a	14.59 a	0.09
Bulk density (g/cm^3)	1.25 c	1.68 b	1.59 b	2.20 a	0.07
pH H_2O	6.29 a	5.38 b	5.21 b	4.42 c	0.09
Organic carbon (%)	3.45 a	1.84 b	1.49 c	1.39 d	0.02
Total nitrogen (%)	0.29 a	0.17 b	0.13 b	0.06 c	0.02
Av. phosphorus (μg/g)	172.65 a	102.38 c	122.26 b	120.45 b	0.01
Ex. calcium (cmol/kg)	0.83 a	0.82 a	0.74 b	0.55 c	0.03
CEC (cmol/kg)	3.32 c	4.41 b	5.33 a	5.37 a	0.07
Base saturation (%)	68.01 a	56.61 b	55.72 c	38.45 d	0.23
Mg (μg/g)	1.03 d	1.21 c	2.45 b	3.57 a	0.11
Fe (μg/g)	102.43 d	128.35 c	141.49 b	174.53 a	0.10
Cd (μg/g)	0.03 d	0.05 c	0.08 b	1.10 a	0.004
Ni (μg/g)	0.08 b	0.06 b	0.09 b	1.20 a	0.01
Pb (μg/g)	0.56 d	20.37 c	200.42 b	1200.30 a	0.04
Plant *Raphanus sativus*					
Dry matter (%)	83.67 d	84.15 c	86.15 b	88.32 a	0.05
Crude protein (%)	10.36 a	10.34 a	10.23 a	9.35 b	0.04
Crude fibre (%)	14.43 d	15.60 c	17.14 b	18.37 a	0.10
Ether extract (%)	6.67 b	6.25 c	6.87 b	7.51 a	0.04
Ash (%)	3.18 d	4.32 c	6.27 b	9.38 a	0.04
NFE (%)	52.14 a	48.61 b	44.57 c	43.24 d	0.08
Gross energy (Kcal/g)	3.44 d	4.04 c	4.36 b	4.43 a	0.01
Calcium (%)	0.64 a	0.55 b	0.48 c	0.39 d	0.02
Pb (μg/g)	0.45 d	7.07 c	29.87 b	68.35 a	0.04

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

With uptake of Pb by *R. sativus* from polluted sites (Table 1), Clark et al. (2000) would classify *R. sativus* in the present study as an "indicator" due to its proportional uptake of Pb. A bioindicator can act as early warning systems of pollution whose effects are possibly reversible. Plant metal tolerance uptake mechanism includes sequestration, binding, selective secretion, and leaf fall to rid plants of excess metals. Plant Pb pollution was low and with significant ($p < 0.05$) positive log-log relationship $Y = 0.74x + 0.65$ between leaf and total soil metal concentrations. The coefficient of determination ($r^2 = 0.92$) is very high. These results suggest a high transfer of Pb from soil to leaf in the food chain, this showed that leaf is able to access the non-liable soil Pb pool, which is usually thought to be low bioavailable according to Ge et al. (2000). Total soil metal concentrations, however, are not good predictor of metal bioavailability in plants (McLanghlin et al. 2000).

In the present study, elevated levels of Pb pollution resulted in thinner shells compared with snails in control site. This is contrary to reports of Jordaens et al. (2006). These authors found no evidence for elevated levels of shell Pb in *C. nemoralis* from polluted sites, despite polluted sites having much higher Pb level compared with control site. This is intriguing because it is well known that Pb affects shells of *H. aspersa* (Beeby et al. 2002). Factors that influence shell traits include the concentration of Ca in the environment, food and the soil pH (Lewis and Magnuson 1999).

Weight gain and feed intake of snails at polluted sites were lower than control (Table 2). Notten et al. (2006) studying *C. nemoralis* agreed that, on the one hand, it could be due to snails actually detecting metals in food, while on the other hand, decreased food consumption could be due to internal toxicity of the snails, but regardless of the mechanism, reduced consumption on polluted leaves is likely to affect general snail fitness. These authors further observed that snails at polluted smelter locations are chronically exposed to high metal levels in the food and decreased consumption is a continuous issue. Alternatively, according to Laskowski and Hopkin (1996a) the solubility

Table 2 *Achatina achatina* at battery factory sites

Parameter	Control	Storage dump	Dried effluent	Waste dump	SEM
Growth performance					
Feed intake (g)	32.17 a	32.05 b	31.05 c	28.05 d	0.03
Weight gain (g)	22.36 a	21.31 b	20.80 c	18.49 d	0.02
Feed conversion ratio	1.44 c	1.50 b	1.49 b	1.52 a	0.01
Shell thickness (mm)	0.16 a	0.15 b	0.15 b	0.13 c	0.03
Lead accumulation					
Pb (μg/g)	1.76 d	12.20 c	91.38 b	468.50 a	0.13

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

of Pb in the gut of snails may be much lower; hence less will be assimilated by the hepatopancrease. Laskowski and Hopkin (1996b), Gomot (2000) recorded no reduction in growth from Pb even at very high dose (30,000 μg/g).

The highest feed utilization was recorded by snails at control site (Table 2), likewise *R. sativus* contained highest crude protein and least crude fiber (Table 1). These observations are contrary to reports of Hamzat et al. (2007), in which snails on diets with least crude fibre responded with the least feed consumption. The Pb pollutants in snail foot tissue (Table 2), are above the maximum permissible concentration (MPC) of 1.5 μg/g in bivalve mollusks, oyster shells, mussels and clams (Dietary lead 2007).

Considering Pb pollution in Tables 1 and 2, the transfer of Pb from leaf to snail had a significant ($p < 0.05$) log-log relationship $Y = 1.07x + 0.47$ with $r^2 = 0.95$. According to Notten et al. (2005), the elevated snail Pb indicates transfer of Pb from leaf to snail. On the other hand in Tables 1 and 2, the transfer of Pb from the soil to snail had a significant ($p < 0.05$) positive log-log relationship $Y = 0.73x + 0.5$ with $r^2 = 0.94$, hence there was additional transfer route directly to snail via the soil. These routes could be through the ingestion of soil and snail cutaneous contact with soil at the sites. However, an earlier contrary observation was reported by Beeby and Richmond (2003) that there was no robust relationship between free Pb concentrations in soil and snail. Snail picked from the wild, from environments prone to pollutants can be of concern to public health (Udosen 2000).

According to Dallinger and Rainbow (1993), snails survive at sites polluted by metals, this resistance results from their ability to retain and inactivate toxic metals, either through intracellular compartmentalization and excretion, or through protein binding and storage of metals for a long time. The most efficiently regulated metal is Pb, with excreted concentrations exceeding those in diet (Laskowski and Hopkin 1996a). Reports of Beeby and Richmond (1987) suggested that Pb assimilation and excretion is under control of a physiological mechanism that is able to adapt to high concentrations of this metal in the environment.

Heavy metals are potential threat to terrestrial biota, and can cause a variety of acute and chronic effects in humans (Parmeggiani 1983). It can therefore be suggested from the results of this study that, such snails could provide important background information for studies of environmental pollution assessement.

References

AOAC (Association of Official Analytical Chemist) (1995) Official methods of analyses, 20th edn. Washington

Allen HE (2002) Bioavalibility of metals in terrestrial ecosystems: importance of partitioning for bioavailibility to invertebrates, microbes and plants. SETAC Press, Florida

Barata C, Baird DJ, Mitchell AD (2002) Among and within population, variability in tolerance to cadmium stress in natural pollutions of *Daphnia magna*: implications for ecological risk assessment. Environ Toxicol Chem 21:1058–1064. doi:10.1897/1551-5028(2002)021<1058:AAWPVI>2.0.CO;2

Beeby A, Richmond L (1987) Adaptation by an urban population of the snail *Helix aspersa* to a diet contaminated with Pb. Environ Pollut 46:73–82. doi:10.1016/0269-7491(87)90146-1

Beeby A, Richmond L (2003) Do the soft tissues of *Helix aspersa* serve as a quantitative sentinel of predicted free lead concentrations in soils? Appl Soil Ecol 22:159–165. doi:10.1016/S0929-1393(02)00130-0

Beeby A, Richmond L, Herpe F (2002) Lead reduces shell mass in juvenile garden snails *Helix aspersa*. Environ Pollut 120:283–288. doi:10.1016/S0269-7491(02)00151-3

Black CA (1967) Methods of soil analysis. ASA monograph 2. American Soil Sci Assoc, Madison

Bouyoucos GH (1951) A calibration of the hydrometer method for making mechanical analysis of soils. Agron J 43:434–438

Chapman PM, Wang F, Janssen CR, Goulet RR, Kamuade CN (2003) Conducting ecological risk assessments of inorganic metals and metalloids: current status. Human Ecol Risk Assess 9(4):641–691. doi:10.1080/713610004

Chen CY, Folt CL (2000) Bioaccumulation and diminution of arsenic and lead in freshwater food web. Environ Sci Technol 34:3878–3884. doi:10.1021/es991070c

Clark J, Fairbrother A, Kapustka LA (2000) Acclimation and adaptation of terrestrial organisms to metals in soil. Int Copper Assoc, New York

Cortet J, Gomot-de Vaufleury A, Poin-sot-Balaguer N, Gomot L, Texie C, Cluzeau D (1999) The use of invertebrate soil fauna in monitoring pollution effects. Eur J Soil Biol 35:115–134. doi:10.1016/S1164-5563(00)00116-3

Dallinger R, Rainbow PS (1993) Ecotoxicology of metals in invertebrates. Lewis Publishers, Boca Raton

Dietary lead (2007) Dietary lead. www.food.gov.uk/multimedia/pdf

Ebenso IE (2003) Egg weight and shell thickness as affected by dietary calcium. Global J Agric Sci 2(1):1–4

Ebenso IE, Bassey FN, Ologhobo AD (2006) Antifeedant activity of different organic solvents crude extract of *Euphorbia hirta* latex on edible tropical land snail *Limcolaria aurora*. J Agric Soc Sci 4(2):151–158

Ebenso IE, Ologhobo AD (2007) Bioaccummulation assessement of juvenile edible tropical land snails exposure to zinc. Bio-Sci Res Bull (India) 23(2):77–84

Ebenso IE, Ologhobo AD (2008) Edible land snail shell thickness as bioindicator of environmental lead metal pollution. Pollut Res (India) 27(2):75–76

Fent K (2004) Ecotoxicological effects at contaminated sites. Toxicology 205:223–290. doi:10.1016/j.tox.2004.06.060

Ge Y, Murray P, Hendershot WH (2000) Trace metal speciation and bioavailibility in urban soils. Environ Pollut 107:137–144. doi:10.1016/S0269-7491(99)00119-0

Gimbert F, de Vaufleury T, Douay F, Scheifler R, Coeurdassier M, Badot PM (2006) Modeling chronic exposure to contaminated soil: a toxicokinetic approach with the terrestrial snail *Helix aspersa*. Environ Int 32:866–875. doi:10.1016/j.envint.2006.05.006

Gomot A (2000) Standardized growth toxicity testing (Cu, Zn, Pb and Pentachlorophenol) with *Helix aspersa*. Ecotoxicol Environ Saf 46:41–50. doi:10.1006/eesa.1999.1872

Gomot de Vaufleury A, Pihan F (2000) Growing snails used as sentinels to evaluate terrestrials environment contamination by trace elements. Chemosphere 40:275–284. doi:10.1016/S0045-6535(99)00246-5

Hamzat RA, Omole AJ, Babatunde BB, Adejumo MO, Longe OG (2007) Comparative value of kola testa, corn-bran and rice-bran with maize for mature African giant land snail *Archachatina marginata*. Nigerian J Anim Prod 34(2):265–276

Heady HF, Heady EB (1982) Range and wildlife management in the tropics. Longman, London

Hodson ME, Langan SJ (1999) Considerations of uncertainty in setting critical leads of acidity of soils: the role of weathering rate determination. Environ Pollut 106:73–81. doi:10.1016/S0269-7491(99)00058-5

Holmgren GSS (1993) Metals in agricultural soil. J Environ Qual 22:335–348

Hopkin SP (1989) Ecophysiology of metals in terrestrial invertebrates. Elsevier Applied Science, New York

Hopkin SP (1990) Species-specific differences in the net assimilation of Zn, Cd, Pb, Cu and Fe by the terrestrial isopods *Onicus asellus* and *Porcello scaber*. J Appl Ecol 27:460–474. doi:10.2307/2404294

Jordaens K, De Wolf H, Vandecastelle B, Blust R, Backeljau T (2006) Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis*. Sci Total Environ 363:285–293. doi:10.1016/j.scitotenv.2005.12.002

Konert M, Vandenberghe J (1997) Comparison of laser grain size analysis with pipette and sieve analysis: a solution for the under estimation of the clay fraction. Sedimentology 44:523–535. doi:10.1046/j.1365-3091.1997.d01-38.x

Laskowski R, Hopkin SP (1996a) Accumulation of Zn, Cu, Pb and Cd in the garden snail *Helix aspersa*: implications for predators. Environ Pollut 91(3):289–297. doi:10.1016/0269-7491(95)00070-4

Laskowski R, Hopkin SP (1996b) Effect of Zn, Cu, Pb and Cd on fitness in snail *Helix aspersa*. Ecotoxicol Environ Saf 34:59–69. doi:10.1006/eesa.1996.0045

Lewis DB, Magnuson JJ (1999) Intraspecific gastropod shell strength variation among with temperate lakes. Can J Fish Aquat Sci 56:1657–1695. doi:10.1139/cjfas-56-9-1687

McLanghlin MJ, Zarcinas BA, Stevens DP, Cook N (2000) Soil testing for heavy metals. Commun Soil Sci Plant Anal 31(11–14): 139–144

Naeem S, Thompson LJ, Lowler SP, Lawton JH, Woodfin RM (1994) Declining biodiversity can alter the performance of ecosystems. Nature 365:734–737. doi:10.1038/368734a0

Notten MJM, Oosthoek AJP, Rozema J, Aerts R (2005) Heavy metal concentrations in a soil–plant–snail food chain along a terrestrial soil pollution gradient. Environ Pollut 135:178–190. doi:10.1016/j.envpol.2005.01.011

Notten MJM, Oosthoek AJP, Rozema J, Aerts R (2006) Heavy metal pollution affects consumption and reproduction of land snail *Capaea nemoralis* fed on naturally polluted *Urtica dioica* leaves. Ecotoxicology 15:295–304. doi:10.1007/s10646-006-0059-3

Parmeggiani L (1983) Encyclopedia of occupational health and safety. International Labour Organization, Geneva

Pederson MB, van Gestel CAM, Elmgaard N (2000) Effects of copper on reproduction of the collaboration species exposed through soil, food and water. Environ Toxicol Chem 19:1002–1010. doi:10.1897/1551-5028(2000)019<1002:COTMIS>2.3.CO;2

Regolis F, Gorbi S, Fathorini D, Telesco S, Notti A, Machella N, Boccheti R, Benedetti M, Piua F (2006) Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach. Environ Health Perspect 114(1):63–68

SAS (Statistical Analysis System) (1999) Users guide statistics, version 9. SAS Institute, New Carolina

Scheifler R, Brahim MB, Gomot-de Vauflenry A, Carnus JM, Badot PM (2003) A field method using microcosms to evaluate transfer of Cd, Cu, Ni, Pb and Zn from sewage sludge amended forest soils to *Helix aspersa* snails. Environ Pollut 122:343–350. doi:10.1016/S0269-7491(02)00333-0

Scheifler R, Gomot-de Vauflenry A, Badot PM (2002) Transfer of cadmium from plant leaves and vegetable flour to the snail *Helix aspersa*: bioaccumulation and effects. Ecotoxicol Environ Saf 53:148–153. doi:10.1006/eesa.2002.2216

Tong S, Von Schruding YE, Prapamentol T (2000) Environmental lead exposure. A public health of global dimensions. Bull World Health Organ 78(9):1060–1077

Udosen ED (2000) Levels of toxic metal in *Achatina achatina* from parts of Akwa Ibom state, Nigeria. J Environ Sci 12(1):68–74

USEPA (United States Environmental Protection Agency) (2002) Review of metal action plan. Report nr. EPA-SAB-LTR-001. Science Advisory Board, Washington