Toxicity of cadmium, lead and lindane to Egeria radiata *Lamarck (Lamellibranchia, Donacidae)*

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Abstract

The toxicity of two heavy metals, cadmium and lead, and an insecticide, lindane (Gammalin 20), on the freshwater clam, Egeria radiata *Lam. was investigated in four static bioassay experiments each lasting for 96 hours. No mortality was recorded among clams in each of the control basins, indicating that all deaths recorded were most probably due to the effects of the toxicants. In all tests a dose-dependent mortality was evident.*

The 96 hours LC 50 values were 21.4 mg l^{-1} *for cadmium, 219 mg* l^{-1} *for lead, 145 mg* l^{-1} *for lindane and 200 mg* l^{-1} *or 40:160 mg* l^{-1} *ratio for cadmium and lead mixture, showing that cadmium was the most toxic of the three substances. These results indicate that the species has a higher degree of resistance to toxicants than previously published.*

Key words : Toxicity tests — *Egeria radiata* — Insecticide — Heavy metals — Mollusces — West Africa.

Résumé

Toxicité du cadmium, du plomb et du lindane pour *Egeria radiata* Lamarck (Lamellibranches, Donacidae)

La toxicité du cadmium, du plomb, et d'un insecticide, le lindane (Gammalin 20) a été testée sur le bivalve d'eau douce Egeria radiata *Lamarck au cours de quatre incubations de 96 heures chacune. Aucune mortalité n'a été notée dans les témoins. Dans tous les tests l'effet de la concentration est apparu.*

Les LC 50 pour 96 heures ont été de 21,4 mg l^{-1} *de Cd, 219 mg* l^{-1} *de Pb, 145 mg* l^{-1} *de lindane et 200 mg* l^{-1} *d'un mélange de Cd et Pb (40:160 mg* l^{-1} *respectivement). Ces résultats soulignent la toxicité du cadmium, mais montrent également que* E. radiata *est plus robuste que ne l'indiquaient d'autres tests publiés antérieurement.*

Mots clés : Test de toxicité — Egeria radiata — Insecticides — Métaux lourds — Mollusques — Afrique de l'Ouest.

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INTRODUCTION

The clam, *Egeria radiata* Lamarck [= *Galatea paradoxa* (Born, 1778)] (Lamellibranchia, Donacidae) occurs in fresh water in the lower reaches of some large West African rivers (PURCHON, 1963). An FAO species identification sheet shows that its range extends from the Gulf of Guinea to the Congo; but despite this wide range it is only from Ghana and Cameroun that literature is available to show its occurrence in terms of commercial use. For example, while LAWSON (1963) examined the economic organization of the *Egeria* fishing industry on the river Volta, Ghana, POPLE (1966) compared the *Egeria* fishery of the Sanaga river, Cameroun, with that in the Volta river. WHYTE (1981) highlighted the threat posed to the *Egeria* industry in the Volta as a result of the closure of the Akosombo dam in 1964. PURCHON (1963) dealt with the biology of *E. radiata* from river Volta, and ascribed the death of several specimens in the aquarium during laboratory observations, to either accumulations of metallic poisons or chlorine added to the water supply at the University of Ghana. Further investigation was, however, not done to establish the toxic element.

Although no toxicity report is available on *E. radiata* (apart from PURCHON's observation), some information is available on Asiatic clams (*Corbicula* spp.). INGRAM (1959) reported that a concentration of 750 $mg\,l^{-1}$ copper sulphate was required to kill *Corbicula* in 48 hr exposures. CHANDLER and MARKING (1975) found that Asiatic clams were more resistant than the native clams *Sphaerium* sp., *Elliptio* sp., and *Plectomerus* sp., to the lampricide 3 — trifluoromethyl — 4 — nitrophenol (TFM) and more resistant than the native clam *Magnonais* sp. to antimycin (MARKING and CHANDLER, 1978). CHANDLER and MARKING (1979) exposed the Asiatic clam, *Corbicula manilensis* to 20 chemicals and obtained the highest 96 — hr LC50 value of 1,450 $mg\,l^{-1}$ for the disinfectant calcium hypochlorite, with the piscicide antimycin being the most toxic, with a 96 — hr LC50 of 0,065 $mg\,l^{-1}$, and inferred that none of the chemicals had the potential for controlling unwanted populations of the clams. BROWN and NEWELL (1972, *in* ANDERSON *et al.*, 1974) exposed the mussel, *Mytilus edulis* to copper and zinc in the form of their sodium citrate salts and found that copper sodium citrate (500 ppm) caused a 50 % depression in respiratory rate of the whole animal as compared to unexposed controls and sodium citrate-exposed controls.

Available information on *E. radiata* from the Cross River system includes osmotic and ionic regulation (ODIETE, 1979), studies on the cruciform muscle complex (ODIETE, 1981), and proximate composition (IFON and UMOH, 1987). Recently, MOSES (1990) has worked on several aspects of the biology of this species, with information on its range of occurrence in the Cross River system, where it has since been commercially exploited. Due to the significance of this clam industry to the economic and food needs of the locality, and the present high level of fishing which may lead to over-exploitation if not regulated, there is need for more studies of various aspects of its biology in the river system.

Presently, no toxicity report is available on this species and with the recent establishment of a newsprint manufacturing factory at Oku Iboku (Fig. 1), and the new agricultural thrust in the area, there is the possibility of discharging toxic substances into the river, and these may have deleterious impacts on the *Egeria* populations.

This paper presents the findings from laboratory bioassay experiments conducted to ascertain the susceptibility or resistance of *E. radiata* to the toxicants cadmium (Cd), lead (Pb) and lindane (Gammalin 20), as alternatives to the pulpmill effluents which could not be procured. The tests deviate, however, from the use of distilled water in conventional bioassay experiments, and make use of water and sand from the same river at the location where the clams were collected. The aim was to simulate as much as possible the natural conditions under which the clams exist, the responses of which would be of value in interpreting future tests.

STUDY AREA

The Cross River system (7°30′ and 10°00′ E) (4° 15′ and 7° 20′ N) (Fig. 1) is principally Guinean in character although north of latitude 7°00′ N the channels of the tributaries pass through derived savanna which partly alternates with high forests (MOSES, 1987). According to ENPLAN (1974) the soil of the floodplain area of the river (6° 45′ and 5° 08′ N) consists of alluvium developed from sediments deposited by the river. Perhaps this feature, together with the meandering reaches and braided channel further south (see MOSES, 1987) determine the high productivity of the Cross River system which covers an area of 54,000 km² with 40,000 km² lying within Nigeria while 14,000 km² is in the Federal Republic of Cameroun.

The *Egeria* bed extends from Akpatre Efe, its lower limit (about 35 km from the river mouth) with a mean dry season salinity of 1.25 ‰ at high tide and 1.0 ‰ at low tide, to around Umon Island, its

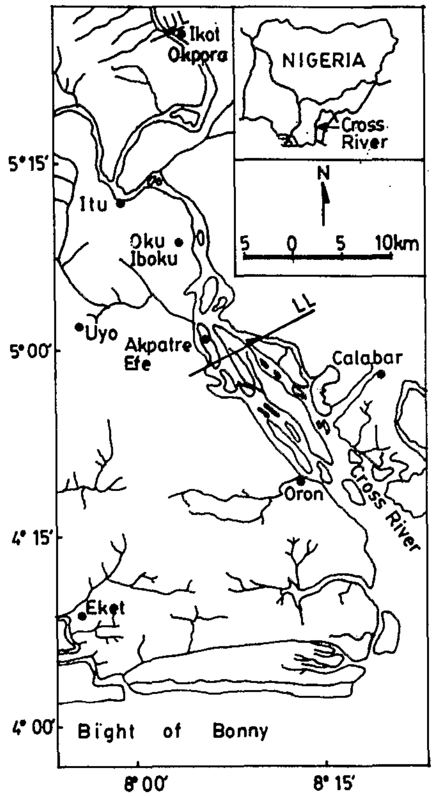

FIG. 1. — Map of the lower reaches of the Cross River system showing the range of occurrence of the clam, *Egeria radiata* : LL = Lower limit of occurrence; UL = Upper limit of occurrence. Inset : map of Nigeria showing the position of the Cross River.
Situation et carte de la basse vallée de la Cross River, indiquant la zone de présence du bivalve Egeria radiata. *LL = limite aval, UL = limite amont.*

upper limit (Fig. 1) (about 150 km from the river mouth) with pure fresh water (MOSES, 1990), as evidenced by a salinity of 0.05 ‰ at Oku Iboku location (Table I). Since the lower limit of occurrence of this clam experiences tidal regimes, it may be improper to describe the species as a "fresh water" clam. MOSES' data also indicate that clams occur predominantly in coarse grade (Md∅ = − 1.5 to − 0.06) and medium grade (Md∅ = − 0.07 to + 0.89) sandy bottoms with the highest percentage in the latter. Very few clams are reported to have been collected from silty deposits especially where these contained a lot of vegetable debris. Our observations show that clams collected from silty substrata are darker than those from sandy areas.

TABLE I

Mean values of measured physicochemical characteristics of the river water
Valeurs moyennes des conditions physico-chimiques dans la rivière

Characteristics	Mean value
Water temperature (°C)	31.0
pH	7.4
Dissolved oxygen (mg l^{-1})	5.1
Free carbon dioxide (mg l^{-1})	5.7
Total alkalinity (mg l^{-1})	35.0
Salinity (‰)	0.05

MATERIALS AND METHODS

Collection and acclimation

The clams, always buried in the sandy bottom, are located with the feet by the fishermen, with subsequent collection with the hands into basins or other containers. Specimens for the present study were obtained from Itu (5° 13′ N; 7° 59′ E) (Oku Iboku location) (Fig. 1) directly from the fishermen at site, together with water and sand. In order to reduce transportation stress, they were always placed in basins containing the river water and sand, and brought to the laboratory. They were then immediately removed and placed in plastic basins of approximately 10 litres capacity, each with enough quantity of sand (7 cm deep) for the clams to burrow into, and 4 litres of the river water. They were left in this condition without food, to acclimate to the laboratory conditions. The same arrangement (exclusive of the very water and sand used in acclimation) was used in the actual experiments. Active clams (3.06 − 5.34 cm in length, and 48.0 − 89.5 g in weight) were used for the experiments. Mean values of the measured physico-chemical characteristics of the river water at the time of collection are shown in table I.

Concentrations of toxicants

Initial test-runs revealed that clams were resistant to 600 $\mu g\, l^{-1}$ cadmium as 3 Cd SO_4. 8 H_2O, 1 200 $\mu g\, l^{-1}$ lead as Pb $(NO_3)_2$, and 1 500 $\mu g\, l^{-1}$ lindane, as no mortality was recorded at the end of a 96-hr exposure for each test. Consequently, higher concentrations (in mg l^{-1}) were used. These were : 5, 10, 15, 20, and 25 for cadmium; 150, 200, 250, 300 and 350 for lead; and 50, 100, 150, 200, and 250 for lindane. The toxicity of a mixture of cadmium and lead (ratio 1 : 4 in weight) was also examined, with the following concentrations : 25, 50, 75, 100 and 125 $mg\,l^{-1}$.

Stock solutions containing 1 000 mg Cd and Pb l^{-1} were prepared using AR — grade cadmium tetraoxosulphate VI and lead trioxonitrate V and dilutions made from these. For lindane, 0.03 cm^3 was calculated as the volume of the original solution of 6.2M strength (an emulsifiable concentrate) which when diluted to 1 dm^3 gave 50 $mg\,l^{-1}$ of the toxicant. Other concentrations were prepared by stepwise multiplication of 0.03 cm^3 for the appropriate quantities of the stock solution.

For each toxicant, five test concentrations were prepared as indicated above, plus a control with no toxicant. Ten clams were exposed to each treatment in static toxicity tests. The medium was not renewed and only temperature was monitored during the period. The general behaviour and mortality of the clams were checked and recorded every twelve hours. Clams were considered dead when they failed to respond to touch or to close their valves when pried open using a scalpel, and were removed from the basins. Data obtained were analysed on a log-probit graph and the LC 50 values calculated. Presumable "safe" concentrations of each toxicant were determined by the method recommended by SPRAGUE (1971).

TABLE II

Mortality of *E. radiata* exposed to varying concentrations of toxicants in 96 hours static tests

Mortalité de E. radiata *après 96 heures d'exposition à diverses concentrations de produits toxiques*

	Concentration ($mg\,l^{-1}$)	% Mortality
Cadmium (Cd)	Control	0
	5	0
	10	20
	15	30
	20	50
	25	70
Lead (Pb)	Control	0
	150	0
	200	40
	250	50
	300	80
	350	90
Cd - Pb (mixture)	Control	0
	Cd + Pb	
	5 20	0
	10 40	10
	15 60	20
	20 80	30
	25 100	40
Lindane	Control	0
	50	0
	100	10
	150	50
	200	100
	250	100

RESULTS

At the end of each test, a slight reduction in temperature from 28.0 °C at the beginning of the test, to 27.2 °C in the end, with an average of 27.6 °C was observed, with no apparent adverse effect on the animals. Behavioural responses were marked by a general reduction in such activities as burrowing and withdrawal of the feet and siphons when touched. These reduced activities were more pronounced in the last two concentrations of lindane. The inability to withdraw the feet into the valves agrees with the observation of ONUOHA (1985) on the brackishwater periwinkle, *Tympanotomus* sp. exposed to crude petroleum in static bioassay.

In all the tests, no mortality was recorded in the control basins, indicating that the period of acclimation was satisfactory. Absence of "natural mortality" in the control basins also indicates that all deaths recorded were most probably due to the toxic effects of the pollutants used, apparently corroborating PURCHON's (1963) prediction.

Cadmium toxicity

Table II illustrates the results of clam exposures to varying concentrations of the toxicants. For cadmium it is evident that the clams were resistant to 5.0 $mg\,l^{-1}$ concentration. The periodic monitoring showed that for the first 24 hours no mortality was recorded in the other concentrations, and was gradual when it commenced 12 hours later in 10,15 and 20 $mg\,l^{-1}$ concentrations, but increased in the last 24 hours in the last concentration. The 21.4 $mg\,l^{-1}$ LC 50 value for cadmium (Table III) shows that it was the most toxic of the three substances. This value is lower than 37.0 $mg\,l^{-1}$ for the fiddler crab, *Uca pugilator* (O'HARA, 1973), but higher than 14.0 $mg\,l^{-1}$ for the crab, *Paragrapsus quadridentatus* (ASHANULLAH, 1976).

Lead toxicity

The results of the experiment on lead toxicity are shown in table II. In the 96 hours exposure period,

TABLE III

Egeria radiata. LC 50 values and probable safe levels for clams exposed to cadmium, lead and lindane for 96 hours
Concentrations léthales LC 50 après 96 heures d'exposition, et calcul des seuils probables d'inocuité ($mg\,l^{-1}$)

Toxicant	LC50 ($mg\,l^{-1}$)	"Safe levels"
Cadmium (Cd)	21.4	2.14 -0.214
Lead (Pb)	219.0	21.90 -2.19
Cd-Pb (mixture)	200 (40:160)	20.0 -2.0
Lindane	145.0	14.5 -1.45

lead was not toxic to the clams at 150 $mg\,l^{-1}$ concentration. In 200 and 250 $mg\,l^{-1}$ concentrations, mortality was gradual, commencing 36 hours after exposure. In these two concentrations mortality was neither gradual nor fast, as the number of dead clams alternated between 1 and 2, after 24 hours exposure. Mortality was almost total in the highest concentration (350 $mg\,l^{-1}$) and the only "survivor" was so weak that it might not have lasted 1 hour longer. A concentration of 219 $mg\,l^{-1}$ was obtained as the LC 50. BRYAN (1976) indicated that lead concentrations exceeding 8 000 ppm in the sediments and 1 000 ppm in *Nereis* have been observed in parts of the Gannel estuary, south-west England.

Cadmium and lead (mixture) toxicity

A comparison of the toxicity of cadmium and lead (singly) and that in the mixture concentrations (table II) reveals that for cadmium in both the single and mixture experiments, no mortality was recorded among clams exposed to 5 $mg\,l^{-1}$ concentration. Mortality in the other mixture concentrations was approximately halved. In the experiment with lead only, no mortality was recorded in the least concentration (150 $mg\,l^{-1}$) used and it is plausible to imagine that mortality in the mixture concentrations was primarily due to cadmium which toxicity appeared to have been reduced in the presence of lead. The much longer (48 hours) period before mortality started in the mixture concentrations lends credence to the observed reduction in cadmium toxicity. This apparent reduction in cadmium toxicity in the presence of lead is at variance with the toxic unit concept on the effects of mixtures of toxicants (see e.g. HERBERT and VAN DYKE, 1964; SPRAGUE and RAMSAY, 1965; BROWN, 1968; NIMMO and BAHNER, 1976) which assumes that all poisons contribute in a similar way to the overall toxicity of a mixture. However, BROWN (1968) noted that *a priori*, it is illogical to expect poisons of different toxicological properties and having different concentration-response curves to contribute in this manner. Our observation agrees with that of NEGILSKI *et al.* (1981) in which metals acted in an interactive manner in paired mixtures of zinc, cadmium and copper on the shrimp *Callianassa australiensis*. They however, pointed out that several factors influence and complicate prediction of the effects of mixtures of toxicants.

Lindane toxicity

Results of lindane toxicity are shown in table II. No death occurred in the lowest concentration (50 $mg\,l^{-1}$) and it took 72 hours before a single clam died in the 100 $mg\,l^{-1}$ concentration. Conversely, mortality was total in the last two concentrations and toxicity highest in the highest concentration (250 $mg\,l^{-1}$), death ensuing within the first 12 hours of the test. The 96 hr LC 50 value was 145 $mg\,l^{-1}$, ranking it second in toxicity. No bioassay report on lindane toxicity is accessible, but reports on the Asiatic clams, *Corbicula* spp. show that they are highly resistant to a variety of toxicants, for example copper sulphate (INGRAM, 1959); TFM (CHANDLER and MARKING, 1975); antimycin (MARKING and CHANDLER, 1978); piscicides, lampricides, therapeutants, disinfectants and oxidizing agents (CHANDLER and MARKING, 1979).

DISCUSSION

There was pronounced variation in the toxicant concentration at which response (death) occurred, showing variation in tolerance of the clams to the different substances used. While they could tolerate 150 $mg\,l^{-1}$ lead, they were slightly susceptible to 10 $mg\,l^{-1}$ cadmium and 100 $mg\,l^{-1}$ lindane. These results portray the clams as being highly resistant, just as Asiatic clams, but contrary to PURCHON'S (1963) postulation that *Egeria radiata* being a recent addition to the freshwater fauna, its powers of self-regulation are less than those of a more long-established *Aspatharia*, another bivalve that was maintained in the same water that killed several specimens of *Egeria* in the aquarium. In light of the findings in the present study and those on Asiatic clams, it may well be that the mass mortality of *E. radiata* observed by PURCHON might not have been due to low powers of self-regulation but rather to high concentrations of unidentified toxic elements, especially if maintained without a sandy substrate. However, IMEVBORE (pers. communication) pointed out that the very high LC 50 values obtained in the present study are a result of the use of the river water (which possibly contains humic substances) for the tests.

Although this explanation is correct, it may not be the only reason (if at all) for the high concentrations of toxicants tolerated by *E. radiata* in this study, since ABAYCHI and MUSTAFA (1988) used a similar experimental set-up (sand and river water) for toxicity tests on the Asiatic clam, *Corbicula fluminea*, although the type of water in the Shatt Al-Arab River used has not been indicated.

A more plausible reason could be that since the study made use of static experimental arrangements, it could be reasonably expected that the quantity of particulate matter in the experimental basins (in the absence of circulation) was negligible, having settled into the sand. This would mean that the levels of the toxicants (especially lindane, an emulsion) in the dissolved state available to the clams were lower than indicated, thus leaving the clams relatively unperturbed. Moreover, metal bioaccumulation and excretion kinetics in mussels may be slow compared to the short term fluctuations in metal concentrations occurring in the water, indicating, according to FOWLER and OREGIONI (1976) that metal concentrations in mussels at a particular time will not reflect exactly those measured in water samples at the same time. *Egeria radiata* may also behave like the Asiatic clam, *Corbicula fluminea* which has the ability to accumulate and eliminate trace metals from its tissues in response to their increase and decrease in the ambient water (ABAYCHI and MUSTAFA, 1988). The inherent characteristic of lindane could be a contributory factor to its low toxicity on *E. radiata*. Further, HELLAWELL (1988) stated that the stability of lindane is less, relative to other organochlorine insecticides and accumulated residues are readily eliminated, making it less hazardous to wildlife.

CONCLUSION

This study has shown that *Egeria radiata* exposed to high concentrations of toxicants in static biossay tests using water and sand from the site of collection of the specimens, exhibited a high degree of resistance, contrary to PURCHON'S (1963) observation from which he concluded that the species had low powers of self-regulation. However, our present finding does not imply the discharging of high concentrations of toxicants into the river since most aquatic organisms may not withstand comparable concentrations for prolonged periods, and since more investigations need to be carried out to confirm or disprove our results.

ACKNOWLEDGEMENTS

We are grateful to Mr. B. S. MOSES for his kind permission for us to use aspects of his data prior to publication. We also acknowledge Professor A. M. A. IMEVBORE for his useful comments on the original report of this study, and Mr. R. P. KING for his suggestions on the draft manuscript. Our gratitude also go to Mr. N. A. UDOFIA for technical assistance.

Manuscrit accepté par le Comité de Rédaction le 20 février 1991

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