Pakistan J. Zool., vol. 37(2), pp. 127-132, 2005.

Selenium Toxicity in the Early Life Stages of African Catfish, *Clarias gariepinus* (Burchell)

E.E. OTI*

Department of Fisheries, Michael Okpara University of Agriculture, Umudike-PMB 7267, Umuahia, Abia State, Nigeria

Abstract.- Embryos of four stages of development and the newly hatched larvae of *Clarias gariepinus* (Burchell) were exposed to sublethal concentrations of selenium (0.5 – 10 µg/ml) to establish the toxic levels at different stages. Embryo mortality was negligible at all selenium concentrations. Following hatching, mortality among the larvae sharply increased at concentration of 3 µg/ml or greater irrespective of the embryonic at the time of exposure stage. For most treatment over 90% of the larvae died within 10 days of hatching. The mortality rate for larvae exposed to selenium after hatching was slightly less.

Key words: Selenium, early life stages, *Clarias gariepinus*.

INTRODUCTION

Selenium is particularly obtained as a by-product of copper refining. It is a non-metalic element, with a ubiquitous distribution in nearly all materials in the earth crust. It is a gray list substance which have a deleterious effects on the aquatic environment (EEC, 1990) and in natural waters are present in solution (Philips and Rainbow, 1994).

Selenium concentrations in natural waters depends largely on the occurrence of seleniferous soils. Average concentrations for selenium in natural waters are less than 10 µg/l, but can reach several hundred micrograms per liter in certain areas of both temperate and tropical region, of the world (Menzer and Nelson, 1975).

Environmental redistribution of selenium through man's activities is due to manufacturing processes such as copper smelting, lead, zinc, phosphate and uranium mining and processing; manufacturing solar batteries, paints and vamishes, fungicides, insecticides and insect repellent. Selenium is used medicinally as an antidandruff agent (Murphy, 1975). Selenium is an essential element. Selenium toxicity was recognized for more than a century before it was known that higher animals actually require this element in trace amounts. Evidence indicates that selenium is a component of the enzyme glutathione perioxides which is important in degrading small amount of H_2O_2 formed in metabolism. Thus it may protect certain biological structures (membranes lipid for example from oxidative disintegration (Rotruct *et al*., 1973). Selenium causes blind stagger (alkali diseases) in horses, reduces white muscle diseases in calves and lambs and improves health while rats fed selenium diets developed liver necrosis (Krehl, 1973). Selenium deficit diets cause liver necrosis in rats and mice, pancreatic fibrosis, exudative diathesis and alopecia in chicks and cardiac myopathy in pigs, turkey and lambs (Casarett and Doull, 1975). Selenium accumulates in certain plants in sufficient quantities to produce selenium toxicity in livestock. The concentration of selenium in food stuffs provides another source of exposure. Sea foods especially shrimps, meat, milk products and grains provide the largest amounts in the diet. Selenium toxicity occurs when intake exceeds the excretory capacity (McConnel and Portman, 1952; Schroedere *et al*., 1970).

Acute selenium poisoning produces central nervous system effects and sometimes convulsion. Chronic exposure symptoms include gastrointestinal disorders, nervousness, liver and spleen damage, anemia and mucosal irritation. Several reports are available on the toxicity of selenium. Blind staggers in livestock (Moxan and Rhian, 1943), fetal toxicity and tetratogenic effects in humans (Robertson, 1970) and hepatic cirrhosis in rodents (Muth, 1967). Its concentration in the aquatic environment is associated primarily with leaching from the

*E-mail: eeotll@yahoo.com
0030-9923/2005/0002-0127 $ 4.00/0

surrounding drainage system and the pH of water. Bjerregard (1982) noted that selenium promoted the accumulation of cadmium in the gills of the shore crab *Carcinils maenas* although the precise mechanism involved in such interaction is not known (Philips and Rainbow, 1994). Summarizing the selenium content of the ground and surface water in the United States, Larkin and Davidson (1967) reported a median value of 1 pg/l with occasional value up to 9000 µg/l.

Information on the acute biological effects of this element is generally limited to birds and other mammals although its effects on the fishes have been reported by few workers especially on temperate species (Ellis *et al*., 1937; Weir and Hine, 1970; Nimi and Laham, 1975; Huckabee and Griffth, 1974). Selenium exposure to 11-day old chick reveals that nerve fibers were sensitive to selenite than the glial cells and neuron. Other signs were decreased cellular migration, diffused cellular growth pattern and increased vacuolization and degeneration. The cytotoxic effects were protected by vitamin E (Oberseiner and Sharma, 1978). The purpose of this research is to study the impact of selenium on several embryonic and early larval stages of *Clarias gariepinus* (Burchell) in order to establish the toxic level of different life stages of their development.

MATERIALS AND METHODS

The procedures for maintaining and breeding *C. gariepinus* have been described (Omoregie and Ufodike, 1991). Females were bred at 5-10 day intervals to ensure low embryo mortality and uniform hatching rate. Following spawning the eggs were examined and the unfertilized and abnormality developed eggs removed. Groups of 50 embryos each were placed in 60x15 mm. Petri dishes with aluminum covers 1, 2, 5, 7 and 27 hr after fertilization and exposed to the appropriate selenium concentrations. These time intervals correspond to the two to eight cell ovum (stages 3-5), late blastula (state 10), blastoderm enveloping yolk (stage 15), and initiation of heart beat (stage 21) of the normal embryonic development of this species as described by Hisaoka and Battle (1958). A fifth group of larvae, ranging in age from 2 to 8 h after hatching, were also exposed to selenium. There were 10 replicates for treatment.

Selenium concentrations of 0.5, 1, 3, 5 and 10 µg/ml were prepared using selenium dioxide and reconstituted water. The water was derived by passing domestic water through an activated charcoal filter, a mixed-bed demineralizer, and a glass wool filter. The pH was adjusted to 7.0 and the total hardness to 40-50 mg/l (as $CaCo_3$) according to Marking and Hogan (1967). Dissolved oxygen levels ranged from 6.6 to 6.9 mg/l. Water temperatur was 26°C. Selenium content was determined fluorometrically according to Lott *et al*. (1963). The excitation and emission wavelength used were 400 and 535 nm, respectively. Less than 5% of the selenium in solution at each concentration was either lost or taken up by the embryos and larvae.

Dead eggs and larvae were removed daily and the media changed. The hatched larvae were maintained in the same selenium concentrations and were denied food during this period. Criteria for death used were the development of an opaque coloration by the embryos and a failure of larvae to respond to tactile stimulation. Those manifesting an extremely weak response to the touch stimulus at the end of the observation period (less than 15% of the total mortality) were also counted as dead.

Hatching and mortality among embryos were expressed as a percentage of the sample size and larval mortality as a percentage of the larvae hatched, after adjustment for mortality among the controls. A skewed distribution, similar to that observed for the natural embryos mortality and larval hatching for this species (Niimi and LaHam, 1975), required that a geometric mean do not necessarily total 100%. The mortality of embryos that died during the first 24 h of exposure and those that died before hatching or did not hatch during the observation period were calculated for each treatment. Mortality among the controls as well as those treated, sharply increased 312 h (13 days) after hatching. This was probably attributed to factors such as food deprivation. Because the influence of such factors is not consistent with the objectives of this study, the results beyond 240 h (10 days) were not included. Attempts to extend the period of observation by feeding the newly hatched

larvae infusoria were not successful. Fouling f the media by dying organisms increased larval mortality.

RESULTS

The hatching of *C. gariepinus* embryos was not affected by exposure to selenium concentrations of 0.5-10 µg/ml. Approximately 41-52% of the embryos of all groups hatched 120 h (5 days) after fertilization, an additional 20-30% hatched during the following 24 h (Table I).

Table I.- Hatching of *C. gariepinus* (Burchell) embryos exposed to different selenium concentrations of several stages of development. The values shown represent the geometric mean of 10 replicates for each treatment and of 62 replicates for the controls.

Selenium (conc.) (µg/ml)	Time of exposure	% Hatch, hours after fertilization (Hours)				
		<72	96	120	144	>168
0.5	1 h	0	2	37	38	12
	2.5 h	2	7	50	19	5
	7 h	0	3	42	30	8
	27 h	0	2	36	29	12
1	1 h	0	4	54	25	11
	2.5 h	0	4	41	30	9
	7 h	<1	5	41	21	9
	27 h	0	4	56	17	8
3	1 h	2	13	43	26	0
	2.5 h	2	10	45	15	4
	7 h	<1	7	37	26	9
	27 h	2	14	36	12	4
5	1 h	<1	2	47	34	6
	2.5 h	0	3	40	25	5
	7 h	0	5	41	34	6
	27 h	0	2	54	23	11
10	1 h	<1	4	35	24	9
	2.5 h	0	>2	53	32	13
	7 h	0	4	46	22	9
	27 h	1	2	51	17	12
Control		<1	4	48	28	8

Mortality among embryos exposed at different stage of development was not appreciably influenced by increasing selenium concentrations. Generally, less than 3% of the embryos exposed either died, or did, not, hatch, during the period of observation (Table II). The mortality after hatching was dependent on the selenium concentration and the duration of exposure. Larval mortality after 96 h exposure at concentrations of 3 and µg/ml was less than 10%, increasing thereafter to approximately 50% at 240 h. The mortality at 10 µg/ml increased at faster rate, approaching 90% at 168 h exposure and 99.50% at 240 h. Concentrations of less than 3 µg/ml did not influence larval mortality.

A pronounced enlargement of the abdominal region was the only anomaly consistently observed. This condition was demonstrated by approximately 40% of the larvae at death and was first indicated by a convex flexure of the vertebral column following which there was a gradual distension of the abdominal region. Death usually occurred within 2-3 days. The frequency of this condition did not appear to be influenced by the selenium concentration or the stage of development when exposed.

DISCUSSION

The toxicity of selenium concentration of 3 µg/ml or greater determined for young *C. gariepinus* is in agreement with the toxic level of 2-5 µg/ml for adult goldfish (*Carassius auratus*) reported by Ellis *et al*. (1973). A 7-day LC_{50} value of 12 µg/ml determined for goldfish (Weir and Hine, 1970) would also be consistent with these observations. These toxic concentrations are well above most of the selenium levels of the ground and surface waters in the United States cited by Larkin and Davidson (1967), and the water quality standard of 10 µg/ml for selenium established by regulatory agencies (United States Public Service, 1962; Environment Canada, 1972; APHA, 1980).

The newly hatched larvae were the most susceptible to selenium toxicity of all the life stages examined in the present study. This is consistent with similar observations on the effects of other elements such as Zinc (Skidmore, 1965; Annune *et al*., 1991) and mercury (Akiyama, 1970). No suitable explanation can be suggested for the viability of *C. gariepinus* embryos in selenium concentration upto 10 µg/ml although 3 µg/ml was lethal to the larvae. In a similar study, Huckabee and Griffith (1974) reported that selenium levels of 1-5

Table II.- Mortality of embryos and larvae of *C. gariepinus* exposed to different concentrations of selenium at several stages of development. The values shown represent the geometric mean of 10 replicates for each treatment. Mortality rate among the embryo and larval controls was generally less than 4 and 1% respectively.

Selenium (conc.)	Stage of development when expected	Embryos		Larvae			
		% Mortality after 24 h exposure	% Mortality[a] at hatch	24 h	96 h	165 h	240 h
0.5 µg/ml	1 h	<2	1	0	<2	<2	<1
	2.5 h	<1	<1	0	<1	<1	<1
	7 h	0	<2	0	0	<1	<1
	27 h	<2	<1	<2	<1	<2	<1
	Larva			0	1	1	1
1 µg/ml	1 h	<1	1	0	0	<1	<1
	2.5 h	0	<1	<2	<1	<1	<1
	7 h	<2	<1	0	<1	<1	<1
	27 h	<1	2	0	<2	<1	<1
	Larva			0	<1	<1	1
3 µg/ml	1 h	<1	1	0	7	59	97
	2.5 h	2	2	0	8	59	90
	7 h	<2	2	0	6	56	94
	27 h	1	2	1	7	53	95
	Larva	0	0	0	<1	11	29
5 µg/ml	1 h	<1	2	<2	8	56	96
	2.5 h	<2	4	<1	8	49	93
	7 h	<1	1	2	5	70	97
	27 h	0	2	<1	3	53	90
	Larva			<2	1	19	86
10 µg/ml	1 h	<2	2	<1	32	82	97
	2.5 h	0	1		24	87	100
	7 h	<1	3	<1	27	90	88
	27 h	<2	3	<2	45	88	98
	Larva			<1	8	74	92

[a]Includes viable embryos not hatched during the period of observation.

µg/ml and lead levels of 5 µg/g (Oladameji and Olagunmeta, 1987) had effect on the hatchability of carp eggs (*Cyprinus carpio*); however, the viability of the larvae following hatching was not examined. Huckabee and Grifith (1974) have also shown that although the presence of 1 mg/L of both selenium (added as SeO_2) and mercury (added as $HgCl_2$) resulted in a reduction in hatch of eggs of Carp (*Cyprinus carpio*) of only 0.4 and 0.6 percent, respectively, of mixture of the two each at concentration of 1 mg/L (the lowest concentration tested, but unrealistically, high in relation to those reported to occur in surface waters) was markedly more than additive in toxic effects, resulting in reduction in that of over 80%. Their results suggest that the effect is due to the interaction of the sulphahydryl group (SH) similar to the finding of this research. In this experiment 90% of mortality was recorded. Kim *et al*. (1977) reported lower percentage mortality in *Semotilus atromaculus* exposed to 3 mg/L of selenium for 48 h contrary to the funding of this investigation. Helsinger *et al*. (1979) reported that in the presence of equimolar concentrations of selenium dioxide the 48-h LC_{50} to goldfish was 0.8 times less than the corresponding value in its absence and 0.7 times less when exposure to mercury and selenium followed a 24-h pretreatment with selenium. In this research, the experimental fish was not pretreated and this therefore could have accounted for the variation in

their result contrary to the finding of this research. Ingersoil *et al*. (1990) also reported similar toxicity to daphinds.

A short hatching time of 4-5 days after fertilization for *C. gariepinus* did not allow this criterion to be effectively used as a reliable toxicological indicator. The present study could demonstrate on differences in hatching rates among treatments.

The limited information presently available on selenium does not allow an accurate assessment of its biochemical role in the aquatic environment. However, the accumulating information on selenium does suggest its action may be analogous to that of mercury. There is evidence that does suggest an accumulation of selenium by aquatic organisms. The selenium level in the muscle of Great lakes fishes (Pakkala *et al*., 1972) is considerably higher than the selenium levels of the waters in the Great lakes region (Goulden and Brooksbank, 1974). More so, the uptake of selenium by phytoplankton, zooplankton, an fishes demonstrated (Sandholm *et al*., 1973), and more importantly, the methylation of inorganic selenium salt into organoselenium compounds by micro-organisms has been established (Fleming and Alexander, 1972). These similarities with mercury would strongly suggest a greater of selenium in an organic state than as an inorganic salt, and the probable existence of a selenium cycle in the aquatic states would be desirable for its confirmation. Further studies on its distribution in and rates of uptake by aquatic organisms and other food chains would be necessary before the schematics of a cycle can be suggested as have been done for several elements found in the earth crust.

REFERENCES

AKIYAMA, A., 1970. Acute toxicity of two organic mercury compounds to the teleost, *Oryzias latipes*, in different stages of development. *Bull. Jap. Soc. Sci. Fish*, **36**: 563-570.

ANNUNE, A.A., OLADADIMEJI, A.A. AND EBELE, S., 1991. Acute toxicity of zinc to the fingerlings of *Clarias lazera. J. Aquat. Sci*., **6**: 19-22.

APHA (American Public Health Association, American Works Association and Water Pollution Control Federation), 1980. *Standard method for the examination of water* 15th Edn. American Public Health Association Washington DC, pp. 1076.

BJERREGARD, P., 1982. Accumulation of cadmium and selenium and their mutual interaction in the shore crab, *Corcinus maenas* (L.). *Aquat. Toxicol*., **2**: 113-125.

BRUMBAUGH, W.G. AND WALTHER, M.J., 1991. Improved selenium the cover from tissue with modified sample decomposition. *J. Assoc. Anal. Chem*., **3**: 576-577.

CASARETT, J. AND DOULL, J., 1975. *Toxicology: The basic science of poisons*. 2nd Edition. MacMillan Publishing Co. Inc. New York, pp. 778.

EEC, (European Economic Commission), 1990. *Proposal for a council directive caused by certain dangerous substances discharged into the aquatic environment of the community 00/c55/09*, EEC Brussels.

ELLIS, M.M., MOTLEY, H.L., ELLIS, D.M. AND JONES, R.O., 1973. Selenium poisoning in fishes. *Proc. Soc. exp. Biol. Med*., **36**: 519-522.

ENVIRONMENT CANADA, 1972. *Guideline for water quality objectives and standards, a preliminary report prepared by the working group on water quality criteria of the subcommittee on water quality.* Inter-departmental Committee on Water Department of the Environment Inland Waters Branch. Tech. Bull. No. 67.

FLEMING, R.E. AND ALEXANDER, M., 1972. Dimethyselenide and demethyltelluride formation by a strain of Penicillin. *Appl. Microbial*., **24**: 424-429.

GOULDEN, R.D. AND BROOKSBANK, P., 1974. Automated atomic absorption determination of arsenic, antimony, and selenium in natural waters. *Anal. Chem*., **46**: 1431-1436.

HELSINGER, J.F., HANSEN, C.O. AND KIM, J.H., 1979. Effects of selenium dioxide on the accumulation and acute toxicity of mercuric chloride in golf fish. *Arch. environ. Contam. Toxicol*., **8**: 297-283.

HISAOKA, K.K. AND BATTLE, H.I., 1958. The normal developmental stages of the zebrafish *Brachydanio rario* (Hamilton – Buchanan). *J. Morphol*., **102**: 311-327.

HUCKABEE, J.W. AND GRIFFITH, N.A., 1974. Toxicity of mercury and selenium to the carp (*Cyprinus carpio*). *Trans. Am. Fish. Soc*., **103**: 822-825.

INGERSOIL, C.G. AND DWYER, F.J. AND MAY, T.W., 1990. Toxicity of inorganic and organic selenium on *Daphnia magna*. *Environ. Toxic. Chem*., **9**: 1171-1181.

KIM, J.H., BIRK, B. AND HEISINGER, J.G., 1977. Protective action of selenium against mercury in northern creek chusb. *Bull. environ. Contam. Toxicol*., **17**: 132-136.

KREHL, W.A., 1973. In: *Modern nutrition in health and disease*. 5th ed. (eds. R.S. Goodhart and M.E. Hills), Lea Philadelphia.

LARKIN, H.W. AN DAVIDSON, D.F., 1967. The relation of the geochemistry of selenium to its occurrence in soils. In: *Symposium: Selenium in biomedicine* (eds. O.H. Muth, J.E. Oldfield and P.H. Weswig), pp. 27-56. Avi Publ. Co. Inc. Westport. Conn.

LOTT, P.F., CUKOR, P., MORIBER, G. AND SOLGA, J., 1923. Diaminonaphthalene as a reagent for the determination of milligram to submicrogram amounts to selenium. *Anal. Chem*., **35**: 1159-1163.

MARKING, L.L. AND HOGAN, J.W., 1967. Toxicity of Bayer 73 to fish. In: *Investigations in fish.* Wildlife Resources

Pub. 36., p. 13.
McCONNEL, K.P. AND PORTMAN, O.W., 1952. Excretion of dimethyl selenide by rats. *J. biol. Chem*., **195**: 277-282.
MENZER, R.E. AND NELSON, J.O., 1975. Water and soil pollutants: metabolic of)-ethyl in bean and corn plants. *J. Agric. Fd. Chem*., **19**: 351-356.
MOXAN, A.L. AND RHIAN, M., 1943. Selenium poisoning. *Physiol. Rev*., **203**: 305-337.
MURPHY, S.D., 1975. Pesticide. In: *Toxicology*. MacMillan Publishing Co. Ltd. New York, pp. 778.
MUTH, O.H., 1967. Symposium: Selenium. In: *Biomedicine*. 1st International Symposium, Oregon State University, 1966, Westport CT.
NIIMI, A.J. AND LAHAM, Q.N., 1975. Influence of breeding time interval on egg number, mortality and hatching of the zebrafish (*Brachydamio rerio*). *Can. J. Zool*., **52**: 515-517.
OBERSTEINER, E.J. AND SHARMA, R.P., 1978. Selenium cytotoxicity and its relation to viamin E.N. chick. *Proc. 1st Intl. Conf. Toxicol*., 628 p.
OMOREGIE, E. AND UFODIKE, E.B.C., 1991. Histopathology of *O. niloticus* exposed to actellic 25 EC. *J. aquat. Sci*., **6**: 13-18.
OLDADIMEJI, A.A. AND OLOGUNETA, R.T., 1987. Toxicity of water boreal lead to *Tilapia nilotica. Nigerian J. appl. Fish. Hydrobiol*., **2**: 19-24.
OLUAH, N.S. AND AMALU, C.C., 1998. Effect of heavy metal on plasma alanine amino transferase activity in catfish. *J. aquat. Sci*., **13**: 5-7.
PAKKALA, I.S., GUTFNMANN, W.H., LISK, D.J., BURDICK, G.E. AND HARRIS, E.J., 1972. A survey of the selenium content of fish from 49 New York State Waters. *Pestic. Monit. J*., **6**: 107-144.
PARRIKEZ, J., OSTADALOVA, I., KALOUSKOVA, J., BABICKY, A. AND BENS, J., 1971. The detoxifying effect of selenium interrelations between compounds of selenium and certain metals. In: *Trace elements in nutrition* (eds. W. Mertz and W.E. Cornatzer), pp. 85-122. Dekker, Inc. New York. N.Y.
PHILIPS, D.J.H. AND RAINBOW, S.P., 1994. *Biomonitoring of trace aquatic contaminants*. Chapman and Hall Publishers, London. pp. 371.
PUBLIC HEALTH SERVICE, 1972. *Public health service drinking water standards*, U.S. Department of Health, Education and Welfare Publ. No. 956. Washington, DC. p. 61.
ROBERTSON, D.S.E., 1970. Selenium: A possible tetratogen. *Lancet*, **1**: 518-519.
ROTRUCT, J.T., POPE, A.L., GANTHER, H.E., SWANSOR, A.B., HAFEMAN, D.G. AND HOEKSTRA, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, **179**: 588-590.
SANDHOLM, M.H., OKSANEN, E. AND PESONEN, L., 1973. Uptake of selenium by aquatic organisms. *Limnol. Oceanogr*., **18**: 496-499.
SCHROEDERE, H.A., FROST, D.V. AND BALASSA, J.J., 1970. Essential trace metals in man: Selenium. *J. Chron. Dis*., **23**: 227-243.
SKIDMORE, J.F., 1965. Resistance to zinc sulphate of the zebrafish (*Brachydanio rerio* Hamilton – Buchanan) at different phases of its life history. *Ann. appl. Biol*., **56**: 47-53.
US PUBLIC HEALTH SERVICES, 1962. *Public health service drinking water standards*. U.S. Government Printing Office, Washington, DC.
WEIR, P.A. AND HINE, C.H., 1970. Effects of various metals on behaviour of conditioned goldfish. *Arch. environ. Hlth*., **20**: 45-50.

(Received 20 September 2003, revised 18 August 2004)