Navorsings- en oorsigartikels

Die effek van subletale dosisse mangaan op die getal witbloedseltipes van die bloukurper *Oreochromis mossambicus*

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UITTREKSEL
Die toename in metaalione is 'n toenemende bron van besoedeling in die natuurlike strome van Suid-Afrika. Hierdie verskynsel is te wyte aan vinnig ontwikkelende industrieë, mynboubedrywighede en boerdery-aktiwiteite op die oewers van riviere. Metaalbesoedelde water het oor die algemeen 'n negatiewe invloed op die fisiologie van visse en ander akwatiese lewensvorme. In hierdie studie is die subletale effekte van mangaan op die getalle van verskillende tipes witbloedselle in vis ondersoek. Oreochromis mossambicus-*individue is aan niedodelike konsentrasies mangaan in 'n eksperimentele deurvloeisisteem blootgestel vir onderskeidelik akuut (96 uur) en kronies (28 dae) in water met 'n temperatuur van 23 °C ± 1 °C. Die resultate wat verkry is, het beduidende veranderinge in die teenwoordigheid van die verskillende tipes witbloedselle getoon (P < 0,05). Die leukositose en leukopenia wat opgemerk is, is 'n normale reaksie van die visse se fisiologie teen infeksies as gevolg van vreemde stowwe soos byvoorbeeld metaal-ione. Variasie in die getalle van die verskillende tipes witbloedselle is vasgestel, wat 'n aanduiding is van die effek van metaalbesoedeling op visse.*

ABSTRACT
***The sublethal effects of manganese on the differential white blood cell counts of* Oreochromis mossambicus**
Metal ions have become an increasing source of pollution in the natural waters of South Africa. This phenomenon is related to the increasing industrial, mining and agricultural activity along rivers. In general, metal ions have negative effects on the physiology of fish and other aquatic biota in metal polluted waters. In this study the sublethal effects of manganese were determined by exposing the freshwater fish, Oreochromis mossambicus, to this metal in an experimental flow-through system. The exposure times were divided in acute (96 hours) and chronic (28 days) exposures, both at 23 °C ± 1 °C. The differential white blood cell counts performed showed significant fluctuations (P < 0.05). This leucocytosis and leucopenia are normal reactions of the fish's body against infections of foreign substances, such as metal ions. It was concluded that the differential white blood cell counts can be used as indicators in detecting the effects of sublethal metal ion exposure to fish.

INLEIDING

Die vinnige bevolkingsgroei in Suid-Afrika asook 'n relatief goed ontwikkelde ekonomie, verhoog die vraag na water uit beskikbare damme en riviere.[1] Water van goeie kwaliteit is nodig, daarom is die gereelde monitering van waterbronne noodsaaklik sodat die toename in besoedelstowwe vroegtydig waargeneem kan word. 'n Verskeidenheid besoedelstowwe beland deur uitvloeisels van huishoudings, industrieë, myne en landboubedrywighede in natuurlike waterbronne wat die kwaliteit daarvan verswak.[2] Mangaan is 'n besoedelstof wat deur mynboubedrywighede in die oppervlakwater beland. Visse word direk en indirek hierdeur beïnvloed en word daarom deur verskeie navorsers as indikatororganismes in studies aangaande akwatiese besoedeling gebruik.[3,4,5] Die bloukurper, *Oreochromis mossambicus*, is 'n vissoort wat geskik bevind is om as eksperimentele organisme in varswaterbesoedelingstudies te gebruik.[5] *Oreochromis mossambicus* is daarom as eksperimenteringsorganisme gebruik in blootstellingseksperimente om die gevolge van mangaanbesoedeling op die hematologie van varswatervis te bepaal. Die visse is aan subletale konsentrasies van mangaan blootgestel om molekulêre en biochemiese versteurings deur hematologiese veranderinge te monitor. Die waarneming van die eerste afwykings van die standaardwaardes van hematologiese indikators is noodsaaklik, om te voorkom dat die dood weens besoedeling intree.[6]

Daar is 'n verskeidenheid hematologiese indikators, soos hematokrit, rooibloedseltellings, glukosekonsentrasie en totale proteïenkonsentrasie wat gebruik kan word om die gevolge van blootstelling aan subletale konsentrasies van besoedelstowwe te bepaal.[7,8] Die gebruik van 'n groot aantal indikators is tydrowend en omslagtig. 'n Enkele betroubare hematologiese indikator sal daarom nuttig in besoedelingsmonitering gebruik kan word.[6,9,10] Die vlak van besoedeling kan dan vinnig bepaal en stappe geneem word om verdere besoedeling te voorkom. Navorsing in die verlede het getoon dat veranderinge in die totale getal, asook die getalle van die verskillende tipes witbloedselle by vis tydens blootstelling aan besoedelstowwe moontlik as 'n betroubare indikator van besoedeling benut kan word.[5] Hierdie studie is daarom uitgevoer om die bruikbaarheid van witbloedseltellings in die vinnige evaluering van waterkwaliteit te ondersoek. Inligting wat bekom word, kan dan moontlik ook in die kundigheidsverslag van die bestaande waterkwaliteitsindeks, RAUWater[29], opgeneem word.

MATERIAAL EN METODES

Oreochromis mossambicus (bloukurper) is aan subletale mangaanchloried-konsentrasies in 'n eksperimentele deurvloeisisteem blootgestel.[11] Hierdie subletale konsentrasies (slk) is bereken vanaf die LK_{50}-konsentrasie van mangaan as persentasies waar slk 10, slk 15 en slk 20 onderskeidelik 'n 10%, 15% en 20% konsentrasie van die LK_{50} is.[12] Die werklike mangaankonsentrasies ($MnCl_2.4H_2O$) in die water is bepaal met 'n atoomabsorpsie-spektrofotometer (Spectr. AA 10 plus, Varian). Die toegevoegde en werklike mangaankonsentrasie is in tabel 1 aangeteken. Nadat die bloed vanuit die koudale aorta versamel is, is die massa en die lengte van die visse onderskeidelik deur middel van 'n analitiese skaal (Sartorius, U6100) en 'n meetplank bepaal (tabel 2).

Tabel 1 Die subletale mangaankonsentrasies in die water toegevoeg tydens die blootstellingseksperimente (AAS mg/l: hierdie metinge is met die atoomabsorpsie-spektrofotometer (vlam-metode) gemaak - nadat die oplossing in die eksperimentele water gevoeg is)

Blootstellingsgroepe	Kontrole	SLK 10	SLK 15	SLK 20	Kontrole	SLK 10	SLK 15	SLK 20
Blootstellingstye	96 uur	96 uur	96 uur	96 uur	28 dae	28 dae	28 dae	28 dae
[Mn] mg/l	0	172	259	345	0	172	259	345
$MnCl_2$ toegevoeg, mg/l	0	594	990	1190	0	594	990	1190
Mn in water AAS mg/l	0	109	172	196	0	137	184	232

SLK = subletale konsentrasie, [Mn] = mangaankonsentrasie; $MnCl_2$ = mangaanchloried; AAS = atoomabsorpsiespektrofotometrie ; mg/l = milligram per liter

Tabel 2 Die lewende massa en lengte van *Oreochromis mossambicus* blootgestel aan subletale mangaankonsentrasies

Blootstellingsgroepe	Kontrole	SLK 10	SLK 15	SLK 20	Kontrole	SLK 10	SLK 15	SLK 20
Blootstellingstye	96 uur	96 uur	96 uur	96 uur	28 dae	28 dae	28 dae	28 dae
Massa (g)								
min/maks	46-96	45-81	45-88	43-86	42-99	40-81	44-82	41-90
$\pm S_d$	15	11	16	13	14	9	11	12
Gemiddeld	63	68	64	63	55	56	60	56
Lengte (mm)								
min/maks	120-201	147-192	140-187	145-187	136-186	140-178	147-181	142-189
$\pm S_d$	18	13	13	12	10	9	10	12
Gemiddeld	166	172	163	163	155	155	161	160

SLK = subletale konsentrasie; g = gram; mm = millimeter; $\pm S_d$ = ± standaardafwyking; min/maks = minimum of maksimum

Tabel 3 Geselekteerde waterkwaliteitsveranderlikes van die boorgatwater

pH	7.65
Temperatuur (°C)	23°C ± 1°C
Ammonium (NH_4, mg/l)	0.04
Stikstof as $NO_3 + NO_2$ (mg/l)	1.97
Fluoried (F, mg/l)	0.6
Totale alkaliniteit as $CACO_3$ (mg/l)	48
Natrium (Na, mg/l)	3.5
Magnesium (Mg, mg/l)	7.5
Silikon (Si, mg/l)	10
Fosfate (PO_4, mg/l)	0.025
Sulfate (SO_4, mg/l)	16
Chloriede (Cl, mg/l)	5.5
Kalium (K, mg/l)	2.05
Kalsium (Ca, mg/l)	12
Konduktiwiteit (Ec, mS/m)	15.1
Totale opgeloste stowwe (TDS, mg/l)	114.5

Bloed is ook van kontrolegroepe vis tydens hierdie blootstellingseksperimente versamel. Die kontrolegroepe is onder dieselfde laboratoriumtoestande aangehou (23 °C ± 1 °C; 12:12 uur dag:nag) as die visse wat aan mangaan blootgestel is, maar geen mangaan is by die water gevoeg nie. Boorgatwater van goeie gehalte[5] is gebruik om die eksperimentele deurvloeisisteme te vul (tabel 3). Bloed van die kontrolegroepe is gebruik om standaardwaardes te verkry om die afwykings deur mangaanblootstelling veroorsaak, aan te dui. Gedurende die twee weke van akklimatisering is die kontrolevisse elke tweede dag gevoer, maar 48 uur voor die toksisiteitstoetse begin is, is voeding gestaak.[5]

Tien visse is aan akute blootstelling (96 uur) en 10 visse aan kroniese blootstelling (4 weke) onderwerp. Die blootstelling is drie keer herhaal. Voeding is ook 48 uur voor die toksisiteitstoetse begin, gestaak. Die visse is nie gevoer tydens die akute blootstelling nie, maar tydens kroniese blootstelling wel elke tweede dag, behalwe die laaste 96 uur van die kroniese blootstelling. Die voeding tydens die kroniese behandeling voorkom verhongering, wat 'n effek op die metabolisme kan hê, maar het geen invloed op die verskille tussen akute en kroniese resultate nie.[13,14]

Nadat die vis versigtig met 'n skepnet gevang is, is die bloed onmiddellik vanuit die koudale aorta getrek en 'n druppel op 'n gewasde voorwerpglasie gedrup. 'n Bloedsmeer is volgens 'n standaardmetode voorberei.[5,15] Elke bloedsmeer is na kleuring in vier segmente verdeel en 50 witbloedselle per segment is met 'n mikroskoop (Olympus Vanox) onder die olie-vergroting (×1000) getel. 'n Totaal van 200 witbloedselle is dus op grond van hul algemene struktuur en affiniteit vir die kleurstof, geklassifiseer en in 'n tabel as 'n spesifieke seltipe genoteer (bv. limfosiet of monosiet). Die klassifisering van die witbloedselle is gebaseer op die klassifikasiesisteem wat deur Gey van Pittius (1991) gebruik is tydens 'n studie gedoen op *Tilapia sparrmanii*. Nadat die persentasies van die onderskeie witbloedselle bereken is, is die data met behulp van 'n statistiese program (Statgraphics) op 'n rekenaar geprosesseer. Variansie-analise (Student-T) is gedoen om statistiese beduidenheid tussen gemiddeldes te bepaal. Verskille in die waardes is as statisties beduidend aanvaar as P = 0.05.

RESULTATE

Tydens hierdie studie is gevind dat die limfosiete beduidend afgeneem het (P < 0.005) na die 173 mg/l, 259 mg/l en die 345 mg/l akute mangaanblootstellings (tabel 4). Dieselfde resultate is verkry na die 259 mg/l en 345 mg/l kroniese mangaanblootstellings (tabel 4).

Die monosiete het beduidend toegeneem (P < 0.005) na die 173 mg/l en 259 mg/l akute blootstelling, sowel as na die 345 mg/l kroniese mangaanblootstelling (tabel 4). Na die 259 mg/l en 345 mg/l akute en kroniese mangaanblootstelling het die neutrofille beduidend toegeneem (P < 0.005) (tabel 4).

Die eosinofilgetalle het beduidend toegeneem (P < 0.005) na die 173 mg/l akute blootstelling, maar het beduidend afgeneem (P < 0.005) na die 345 mg/l akute blootstelling (tabel 4). Na die 345 mg/l kroniese blootstelling het die eosinofilgetalle beduidend afgeneem (P < 0.005)(tabel 4).

Tabel 4 Die gemiddelde witbloedseltipe-persentasies van *Oreochromis mossambicus* na subletale mangaanblootstelling

Blootstellingsgroepe	Kontrole	SLK 10	SLK 15	SLK 20	Kontrole	SLK 10	SLK 15	SLK 20
Blootstellingstye	96 uur	96 uur	96 uur	96 uur	96 uur	96 uur	96 uur	96 uur
n	10	10	10	10	10	10	10	10
Limfosiet %								
Mean ±S_d	84 ± 4	41 ± 12	63 ± 12	35 ± 14	84 ± 4		51 ± 13	45 ± 16
Min/maks	80 - 90	21 - 58	41 - 81	17 - 63	80 - 90	N/B	38 - 79	21 - 75
P	*	P < 0.005	P < 0.005	P < 0.005	*		P < 0.005	P < 0.005
Monosiet %								
Mean ± Sd	3 ± 3	45 ± 17	9 ± 5	7 ± 7	3 ± 3		3 ± 3	17 ± 9
Min/maks	0 - 17	12 - 67	4 - 19	2 - 23	0 - 17	N/B	0 - 10	4 - 33
P	*	P < 0.005	P < 0.005	*	*		*	P < 0.005
Neutrofil %								
Mean ± S_d	10 ± 5	10 ± 6	25 ± 11	61 ± 10	9.5 ± 4.6		44 ± 13	39 ± 13
Min/maks	3 - 18	2 - 32	12 - 44	40 - 75	3 - 18	N/B	20 - 57	21 - 61
P	*	*	P < 0.005	P <0.005	*		P < 0.005	P < 0.005
Eosinofil %								
Mean ± S_d	2 ± 1	5 ± 4	3 ± 2	1 ± 1	2 ± 1		1 ± 1	1 ± 0
Min/maks	0 - 5	2 - 12	0 - 7	0 - 2	0 - 5	N/B	0 - 4	0 - 2
P	*s	P < 0.005	*	P < 0.005	*		*	P < 0.005

SLK = subletale konsentrasie; * = P > 0.05; N/B = nie beskikbaar; n = aantal visse; min/maks = minimum of maksimum

BESPREKING

Witbloedseltellings kan as indikators gebruik word met die opspoor en evaluering van die subletale effekte van gifstowwe op visse omdat dit op infeksies, patogene, spanning en besoedelstowwe reageer.[16,17,18] Besoedelstowwe soos metale kan die normale fisiologiese prosesse in visse versteur. 'n Groot aantal stowwe is bekend daarvoor dat dit die getalle van die witbloedselle laat wissel. Lood (Pb) veroorsaak 'n toename in die getal basofille, limfosiete, monosiete, eosinofille en trombosiete van die baber *Ameiurus nebulosus*, terwyl kwik (Hg) verhoogde trombosietgetalle in *Barbus conchonius* veroorsaak.[19,20] Vorige navorsing deur Agrawal en Srivastava (1980) asook Wepener *et al.* (1992) het getoon dat subletale mangaankonsentrasies 'n variasie in die onderskeie getalle witbloedselle veroorsaak.

Beduidende limfosietafnames (limfopenia) is 'n aanduiding van infeksie in vis. Hierdie afnames is in die verlede waargeneem tydens blootstelling aan metale soos koper (Cu) en kwik (Hg).[18,20] In vorige navorsing het limfopenia selfs by *Carassius auratus*[21] voorgekom as gevolg van koueskok. Die limfosietafname wat plaasgevind het tydens die akute en kroniese blootselling aan mangaan, kan veroorsaak word deur verhoogde kortikosteroïedsekresie, 'n reaksie wat volg op stimulasie van die hipotalamus.[20,22] Kortisol verlaag die aantal limfosiete in sirkulerende visbloed. Na gelang hierdie situasie voortduur, word groter dosisse kortisol gesekreteer, wat atrofie in limfweefsel veroorsaak. Dit het 'n afname in die produksie van T-limfosiete en teenliggaampies tot gevolg.[23] Vislimfosiete is immuno-kompeterend, daarom kan 'n afname in die aantal limfosiete as gevolg van mangaanblootstelling die werking van die immunologiese verdedigingsisteem inhibeer.[24] Die limfosietafname in vis by mangaanblootstelling kan dus wees as gevolg van die afname in die aantal onvolwasse limfosiete.[22]

Die toename van die monosietgetalle kan die gevolg wees van moontlike skade wat die mangaan die kieue, lewer en niere aandoen.[25] Hierdie skade aan bogenoemde weefsels bevorder die fagositiese reaksies wat deur die monosiete uitgevoer kan word.[4,8,26] Die monosiete val die vreemde gifstowwe en bakterieë aan en vernietig dit. Hierdie selle is in die bloed egter onvolwasse, maar sodra dit die beskadigde weefsels binnedring, begin dit swel en tot makrofage omvorm.[10] Makrofage is fagosiete wat lisosome besit en effektiewe proteolitiese ensieme bevat.[10,23] Die toename in die aantal neutrofille gaan gepaard met die toename van die monosietgetalle. Daar word vermoed dat hierdie selle ook na mangaangeaffekteerde weefsels migreer om fagositiese funksies te verrig.[10,27,28]

Eosinofille word beskou as 'n skaars leukosiet in visbloed.[10] In hierdie navorsing is min eosinofille ook na die mangaanblootstelling geïdentifiseer. Blootstelling aan koper veroorsaak 'n toename in eosinofilgetalle,[5] terwyl blootstelling aan mangaan 'n afname in eosinofille toon. Jakowska (1956) het toenemende eosinofilgetalle by ontsteekte areas in weefsel gevind. Daar kan dus afgelei word dat mangaan visweefsel waarskynlik beskadig. Sodoende migreer eosinofille na die beseerde of beskadigde weefsel. Die funksie van eosinofille by metaalbeskadigde weefsel is egter onbekend en inligting daaromtrent is onvolledig.

In vorige navorsing[10] is bevind dat die limfosiete van visse moontlik dieselfde rol as die limfosiete van hoër vertebrate speel aangesien dit immuno-kompeterende selle is. Volgens dié navorsing kom immunoglobulien (Ig) voor op die oppervlak van membrane van limfosiete van verskeie visspesies. Hierdie verskynsel kan 'n meganisme inhou waardeur limfosiete geïnduseer word om in teenliggaam-produserende selle of voorlopers daarvan te omvorm. Mangaan in niedodelike hoeveelhede onderdruk dus die immuunsisteme van die bloukurper en verlaag sodoende die vis se verdedigingsmeganisme teen patogene wat altyd teenwoordig is. Die wisselende getalle van die verskillende witbloedselle as gevolg van mangaanblootstelling kan dus as 'n belangrike indikator gesien word om die niedodelike effekte van metaalione in visse te identifiseer. Dit is dus duidelik uit die studie dat mangaanbesoedeling die aantal verskillende witbloedseltipes in die bloukurper affekteer. Daar moet egter in gedagte gehou word dat die resultate slegs bruikbaar sal wees as faktore soos byvoorbeeld ouderdom, geslag, seisoen, dieet en temperatuur bekend is of konstant gehou word.[5]

SUMMARY

For this investigation ten *Oreochromis mossambicus* were exposed to sublethal manganese concentrations in an experimental flow-through system. These sublethal concentrations (slc) were determined from the LC_{50} concentration of manganese as percentages, for example 10, 15, 20 which are 10%, 15% and 20% of the LC_{50}. The exposure values as well as the actual manganese concentration are represented in Table 1. The actual manganese concentration in the water was determined by means of atomic absorption spectrophotometry (Spectr AA 10 plus, Varian). During the exposure experiments, control groups were also kept under the same experimental conditions without the manganese being added. These control fish set a baseline of values during this study to detect the effects of manganese on the fish. Ten *Oreochromis mossambicus* were exposed in periods of three acute exposures (96 hours) and three chronic exposures (4 weeks). Although the fish were not fed during the acute exposure, feeding took place every second day during the chronic exposure, except the last 96 hours, to prevent starvation. After the exposure the fish were carefully netted and blood was drawn from the caudal aorta.

Directly after extraction, a drop of blood was placed on a washed and defatted slide. The slide was prepared for differential white blood cell counts with Giemsa-Romanowsky stain 15 and an Olympus Vanox Microscope (under oil immersion, ×1000) was used to examine the stained blood slides. Each slide was divided into four segments and 50 leucocytes per segment were counted. The 200 leucocytes were classified according to their general structure and affinity to the dye. The data was processed on an IBM compatible computer using a Statgraphics statistical programme. Independent Student's T-tests were performed to prove probability hypothesis. Differences in mean values were accepted as being statistically significant if $P \leq 0.05$. The leucocytes of *Oreochromis mossambicus* were divided into two categories (1) agranulocytes (lymphocytes and monocytes) and (2) granulocytes (neutrophils and eosinophils).

The lymphocytes decreased significantly ($P < 0.005$) after the 173,2 mg/l, 259 mg/l and 345 mg/l acute exposures and resulted in the same decline after the two chronic exposures (Table 4). Monocytes increased significantly ($P < 0.005$) after the 173,2 mg/l and 259 mg/l acute exposures, as well as the 345 mg/l chronic manganese exposure (Table 4). After the 259 mg/l and 345 mg/l acute and chronic exposures the neutrophils increased significantly ($P < 0.005$) (Table 4). There was a significant increase ($P < 0.005$) in eosinophils after the 173,2 mg/l acute exposure and a significant decrease ($P < 0.005$) after the 345 mg/l acute exposure (Table 4). The eosinophils resulted in a significant decrease ($P < 0.005$) after the 345 mg/l chronic exposure (Table 4).

The different concentrations of circulating blood-cell types are important parameters in detecting and evaluating the sublethal effects of toxicants on fish.[18] The increase in the number of leucocytes (leucocytosis) and the decrease in the number of leucocytes (leucopenia) are normal reactions of the fish body against infections of foreign substances.[17] Therefore these substances, such as metals, can alter the normal physiological processes in fish. In response to a variety of surrounding stimuli, white blood cell numbers are known to fluctuate. Several effects have been reported on the exposure of fish to sublethal concentrations of manganese.[9,8] Exposure to sublethal manganese concentrations resulted in variation in the different types of leucocytes. The four types of leucocytes, i.e., lymphocytes, monocytes, neutrophils and eosinophils were previously described in *Oreochromis mossambicus*. No basophils were identified in the blood smears of *Oreochromis mossambicus*, during the present investigation and that of Nussey (1994). Any kind of stress usually increases the susceptibility of fish to infections and diseases. This susceptibility is enhanced by the suppression of the immune system by stressors such as metals (in this case manganese). When the immune system is challenged, the lymphocytes' ability to differentiate and produce antibodies in response to antigenic stimuli is reduced. This reduction thus decreases the fish's defense against pathogens that enter the body. Lymphopenia, after acute and chronic sublethal manganese exposures, could be due to increasing levels of corticosteroid secretion by stimulation of the hypothalamus.[20,22] The corticosteroid, cortisol, decreases the number of lymphocytes in fish blood. As this stress situation continues, larger doses of corticol could cause atrophy of the lymphoid tissues, decreasing the production of T-lymphocytes and anti-bodies.[23] The fish lymphocytes are immuno-competent.[24] Therefore reducing numbers in these cells, as the result of manganese exposure, may be responsible for generating the stimulation of the immunological defense. Lymphopenia thus occurs due to the decreasing numbers in small lymphocytes.[22] An increase in the number of monocytes was found in the blood of *Ameiurus nebulosis* after chronic lead exposure.[19] The same results were found in *Oreochromis mossambicus* after acute and chronic manganese exposure. The increase in the number of monocytes could be as a result of required phagocytic response in the manganese affected, and possibly damaged tissues such as the

gills, liver and kidneys.[4,25,26] Monocytes attack and destroy invading toxins and bacteria. These cells are immature in the blood but once they enter the damaged tissues, they begin to swell and transform to macrophages.[10] These macrophages are powerful phagocytes consisting of lysosomes that contain proteolytic enzymes.[10,23] The increase in the number of neutrophils corresponds with the increasing number of the monocytes. As previously mentioned, it is suspected that these cells also migrate to the affected tissues and might have phagocytic actions.[10,27] Monocytes and neutrophils might play an important part in the teleost inflammatory response. Vessels adjacent to necrotic lesions in fish usually contained many polymorphonuclear leucocytes and macrophages. Both these leucocytes were found to be phagocytic. Eosinophils are very rare leucocytes in fish blood.[10] However, stress and hormones possibly affect the nature of eosinophils in fish.[10] An increase occurred in the number of eosinophils in *Oreochromis mossambicus* after copper exposure.[5] After sublethal manganese exposure the decreased numbers in the blood of *Oreochromis mossambicus* could be in response to the effect of the manganese on the fish, as it was found that increasing numbers of eosinophils were detected at inflammatory sites.[27] Thus, the eosinophils migrated to the site of injury as tissue damage occurred due to manganese exposure.

It was concluded that sublethal manganese exposure suppresses the immune system and reduces the defence mechanism of the body against pathogens that might be present. Thus, the different numbers of circulating white blood cell types are important parameters for use, in detecting the sublethal effects of metal ions on fish.

LITERATUURVERWYSINGS

1. Department of Water Affairs. (1991). *Water Quality Management Policies and Strategies of South Africa.* (Pretoria, SA) p. 32.
2. Lloyd, R. (1992). *Pollution and Freshwater Fish* (Fishing News Books Publishers, London, England) p.176.
3. Latif, M.A., Khalaf, A.N., Khalid, B.Y. (1982). Bioaccumulation of copper, cadmium, lead and zinc in two cyprinid fishes of Iraq, *J. Biol. Sci.*, 2(13), 45-64.
4. Gey van Pittius, M. (1991). *Die effek van swaarmetale by variërende pH op lewerensieme en bloedstolling by Tilapia sparrmanii (Cichlidae)* (M.Sc.-tesis, Randse Afrikaanse Universiteit, SA).
5. Nussey, G. (1994). *The effect of copper on the blood coagulation and general haematology of Oreochromis mossambicus (Cichlidae)* (M.Sc.-thesis, Randse Afrikaanse Universiteit, SA) p. 132.
6. Hesser, E.F. (1960). Methods for routine fish hematology, *Progve. Fish-Cult.*, 22, 164-171.
7. Blaxhall, P.C., Daisley, K.W. (1973). Routine haematological methods for use with fish blood, *J. Fish Biol.*, 5, 771-781.
8. Wepener, V., Van Vuren, J.H.J., Du Preez, H.H. (1992) Effect of manganese and iron at neutral and acidic pH on the hematology of the banded tilapia (*Tilapia sparmanii*), *Bull. Environ. Contam. Toxicol.*, 49, 613-619.
9. Donald, P., Patt, D.I., Patt, G.R. (1969). *Comparative Vertebrate Histology* (Harper and Row Publishers, New York) p. 226-253.
10. Ellis, A.E. (1977). The Leucocytes of fish: A review, *J. Fish Biol.*, 11, 453-491.
11. Barnhoorn, I.E.J. (1996). *Effects of manganese on the haematology of Oreochromis mossambicus and the bioaccumulation of metals in Labeo umbratus.* (M.Sc.-thesis, Randse Afrikaanse Universiteit, SA).
12. Seymore, T. (1990). *Bioaccumulation of metals in Barbus marequensis from the Olifants River, Kruger National Park and lethal levels of manganese to juvenile Oreochromis mossambicus* (M.Sc.-thesis. Randse Afrikaanse Universiteit, SA) p. 282.
13. Hepher, B. (1988) *Nutrition of Pond Fishes* (Cambridge University Press, Cambridge, England) p. 64, 247.
14. Grobler, E., Van Vuren, J.H.J., Du Preez, H.H. (1989). Routine oxygen consumption of *Tilapia sparmanii* (Cichlidae) following acute exposure to atrazine, *Comp. Biochem. Physiol.*, 93(C), 37-42.
15. *Puchkov, N.V. (1964). In *Techniques for the Investigation of fish physiology,* Monson, S. ed. (Jerusalem) p. 11-15.
16. Hibiya, T. (1982). *An Atlas of Fish Histology - Normal and Pathological Features* (Tokyo Kodansha Ltd., Stuttgart, Gustav Fisher Verslag, Germany) p. 147.
17. Heath, A.G. (1987). *Water Pollution and Fish Physiology* (CRC Press Inc., Boca Raton, Florida, USA) p. 245.
18. Dick, P.T., Dixon, D.G. (1985). Changes in circulating blood cell levels of rainbow trout, *Salmo gairdneri* Richardson, following acute and chronic exposure to copper, *J. Fish Biol.*, 26, 475-481.
19. Dawson, A.B. (1935). The hemopoietic response in the catfish, *Ameiurus nebulosus*, to chronic lead poisoning, *Biol. Bull.* (Woods Hole, Mass.) 68, 335-346.
20. Gill, T.S., Pant, J.C. (1985). Mercury-induced blood anomalies in the freshwater teleost, *Barbus conchonius* Ham, *Wat. Air Soil Pollut.*, 24, 165-171.
21. Bennet, M., Neville, C.B. (1975). Effects of cold shock on the distribution of leucocytes in goldfish, *Carassius auratus*, *J. Comp. Physiol.*, 8, 213-216.
22. Srivastava, A.K., Agrawal, S.J. (1979). Hematological anomalies in a freshwater teleost, *Colisa fasciatus*, on acute exposure to cobalt, *Acta. Pharmacol. Toxicol.*, 44, 197-199.
23. Guyton, A.C. (1991). *Textbook of Medical Physiology* (W.B. Saundres Company, Philadelphia, USA) p. 1014.
24. Ellis, A.E. (1976). Leucocytes and related cells in the plaice *Pleuronectes platessa*, *J. Fish Biol.*, 6, 143-156.
25. Wepener, V. (1990). *Die effek van swaarmetale by variërende pH op die bloedfisiologie en metaboliese ensieme van Tilapia sparmanii (Cichlidae)* (M.Sc.-tesis, Randse Afrikaanse Universiteit, SA) p. 119.
26. Van der Merwe, M. (1992). *Aspects of heavy metal concentration in the Olifants River, Kruger National Park, and the effect of copper on the haematology of Clarias gariepinus (Clariidae).* (M.Sc.-thesis, Randse Afrikaanse Universiteit, SA).
27. Hines, R.S., Spira, D.T. (1973). Ichthyophthiriasis in the mirror carp. II. Leukocyte response. *J. Fish Biol.*, 5, 527-534.
28. Jakowska, S. (1956). Morphologie et nomenclature des cellules du sang des teleosteens. *Revue Hemat.*, 11, 519-539.
29. Nussey, G. (1998). *Metal ecotoxicology of the upper Olifants River at Selected localities and the effect of copper and zinc on fish blood physiolgy.* (M.Sc.-thesis, Randse Afrikaanse Universiteit, SA).