Toxicity and mutagenicity evaluation of water coagulated with *Moringa oleifera* seed preparations using fish, protozoan, bacterial, coliphage, enzyme and Ames *Salmonella* assays

WOK GRABOW[1], JL SLABBERT[1], WSG MORGAN[1] AND SAA JAHN[2]
[1]*National Institute for Water Research, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, South Africa*
[2]*German Agency for Technical Cooperation, P.O. Box 5180, D-6236 Eschborn 1, West Germany*

Abstract

Moringa oleifera seed preparations equivalent to the 200 mg/ℓ of cotyledon powder generally used for the coagulation of drinking-water in some rural areas had toxic effects on fish (guppies) (*Poecilia reticulata*), protozoa (*Tetrahymena pyriformis*), bacteria (*Escherichia coli*) and inhibited the enzyme acetylcholinesterase. The preparations had no effect on coliphages or the enzymes lactic dehydrogenase or invertase, nor did they display mutagenic effects in Ames *Salmonella* mutagenicity assays. Concentrations as low as the equivalent of 5 mg/ℓ cotyledon powder affected the oxygen uptake rate of *T. pyriformis*, 30 to 40 mg/ℓ induced aberration in locomotor behaviour patterns of guppies, while the 96-h LC_{50} for guppies was 196 mg/ℓ. *Salmonella* mutagenicity assays yielded negative results for batch and serial extracts representing the equivalent of up to 1 000 mg/ℓ cotyledon powder. Toxicity was limited to seed cotyledons; pericarps (shells) had no effect. The results suggest that toxic effects were due to the antimicrobial constituent 4(α-L-rhamnosyloxy) benzyl isothiocyanate, a glycosidic mustard oil. In view of complex associations of the toxin with organic matter, instability and the mechanism of action, it would, however, not appear to constitute a human health risk, at least not in the concentrations generally involved in the utilization of the seeds for nutritional, medicinal or water purification purposes.

Introduction

The tree *Moringa oleifera* Lam. is native to the sub-Himalayan region of India, and is today cultivated for various purposes in many countries of the tropical belt such as the Sudan, Malagasy and Sri Lanka. In rural areas of the Sudan, powdered seeds of the tree are traditionally applied as coagulants for drinking-water treatment (Jahn and Dirar, 1979). The coagulating activity has been ascribed to polypeptides acting like cationic polymers (Barth *et al.*, 1982). The seeds are also used in folk medicine and as food (Jahn and Dirar, 1979; Jahn, 1981). Although no adverse effects on the health of humans have yet been associated with the consumption of the seeds or water coagulated with powdered seeds (Jahn, 1981), the isolation of 4(α-L-rhamnosyloxy) benzyl isothiocyanate from the seeds raised questions regarding potential toxicity (Eilert *et al.*, 1981; Barth *et al.*, 1982). This mustard oil glycoside has anti-microbial activity (Eilert *et al.*, 1981; Jahn, 1981; Barth *et al.*, 1982), and powdered seeds administered orally to rats in doses of 5 g/kg body mass had detectable effects on cells of the stomach and liver (Barth *et al.*, 1982). Although no acute toxicity was evident, the results warrant further investigation (Barth *et al.*, 1982).

This paper deals with an evaluation of toxic and mutagenic effects of seeds and coagulated water using a variety of biological assays.

Materials and methods

Seed preparation

Seeds harvested in the Sudan were used. Seed powder was ground from whole seeds, seed cotyledons (seeds without pericarps), or pericarps (shells) by means of a pestle and mortar (Jahn and Dirar, 1979).

Coagulation experiments

Seed powder was suspended directly in water, except in the case of one set of protozoan assays dosed in the form of a milky filtrate prepared by suspending 5 g of cotyledon powder in 50 mℓ of distilled water, shaking manually for 5 min, standing for 10 min, followed by filtration through a coarse cloth, and used within the hour. Coagulation experiments were carried out with water collected from the Apies River about 50 m downstream of the inflow of treated sewage from the Daspoort purification works (Grabow *et al.*, 1981), water from the Moreletta River and distilled water. In different samples the turbidity of Apies River water varied from 10 to 20 NTU (nephelometric turbidity units) and that of Moreletta River water from 10 to 90 NTU. Jar tests were carried out using 500 mℓ of water in 1 ℓ glass beakers and a multiple stirrer unit. After the addition of the desired dose of coagulant, water was stirred for 5 min at 100 r/min. The stirrers were then removed and the beakers left standing to settle. Samples for analysis were removed from the supernatant (decanted after 1 h and kept at room temperature) at various time intervals. Experiments were done at room temperature (20 to 25 °C).

Seed extracts

Extracts were prepared by shaking seed suspensions manually for 5 min or stirring them magnetically for 24 h, followed by filtration through a membrane (pore size 0,45 μm). In the former case distilled water was used as solvent; in the latter, distilled water or methanol was used for batch extractions and dichloromethane, acetone, methanol and distilled water (in this sequence) for serial extractions. Dichloromethane and methanol extracts were evaporated to dryness and the residues resuspended in desired volumes of acetone and distilled water, respectively. When necessary, extracts were diluted in distilled water.

Counts of faecal coliform bacteria

Faecal coliform bacteria, as well as *Escherichia coli*, were counted by means of membrane filtration using M-FC agar without rosolic acid (Grabow *et al.*, 1981). In some experiments water was seeded with *E. coli* E25 (Grabow and Prozesky, 1973).

Counts of coliphages

Coliphages were counted by means of a modified double-layer-agar plaque assay using *E. coli* C603 as host (Grabow *et al.*, 1984).

Protozoan toxicity assay

A *Tetrahymena pyriformis* assay in which oxygen uptake rate serves as the test parameter, was used (Slabbert and Morgan, 1982). Oxygen uptake was measured at a constant temperature (27 °C) by means of a biological oxygen monitoring system connected to a potentiometric recorder. Each sample (0,25 mℓ) was injected into a test chamber containing 4,75 mℓ of an appropriate *T. pyriformis* cell suspension, while oxygen uptake was recorded continuously before (reference), during (mixing) and after sample addition (test). Tests were done on coagulated water (membrane filtered before testing) as well as seed extracts. Since the assay involved a 20-fold dilution of test samples, coagulation experiments were carried out with concentrations of seed preparations which compensate for this dilution factor. The effect of a test sample was established by comparing the test uptake rate with the reference uptake rate, expressing all results in relation to a standard reference oxygen uptake rate of 8%/min. Tests were carried out in triplicate. Students' t-test was used to establish whether average results differed significantly at the $P = 0,05$ level.

Fish toxicity assay

Predetermined volumes of a manually prepared seed extract (standing for 10 min before filtration) were added to dechlorinated tap water to establish dilutions equivalent to 40, 60, 100, 250, 400 and 600 mg/ℓ of cotyledon powder. Dechlorinated tap water was used for the control. The test organisms were two-week old guppies (*Poecilia reticulata*) which had not been fed for 48 h before the test. Five fish were placed in each dilution and the number of dead fish in each concentration after 96 h recorded. The criteria for death were the cessation of all movement, especially gill movement, and no reaction to gentle prodding. The data were used to establish the 96-h LC_{50} according to the method of Litchfield and Wilcoxon (1949). Fish dying in the higher concentrations were histopathologically examined to establish possible causes of death.

The effect of exposure to seed extracts upon locomotor behaviour patterns of guppies was investigated employing an automatic biomonitoring system, where activity was continuously assessed by means of ultrasonic echoes (Morgan *et al.*, 1982). Sensor fish were exposed to increasing concentrations of the seed extract to determine the threshold level of detection. Fish were exposed to each concentration for a period of 24 h, and their activity levels recorded and compared to the normal behaviour pattern. After this period of exposure, sensor fish were allowed to resume normal activity before being exposed to successively higher concentrations. The experimental series was concluded when more than 60% of the sensor fish had elicited a response by exhibiting activity levels over and above those to be expected under normal conditions, within 24 h of initial exposure.

Enzyme assays

Enzyme detector strips, impregnated with either acetylcholinesterase, lactic dehydrogenase or invertase, were dipped into extracts equivalent to 50 to 2 500 mg/ℓ of cotyledon powder and then placed in contact with similar strips impregnated with their specific substrates, being indoxylacetate, pyruvate and sucrose, respectively. Indoxylacetate turns to a blue colour when hydrolyzed, whereas N-methylphenazonium methosulphate (PMS) and 2-(4-indophenyl)-3-(4-nitrophenyl)-5-phenyl-tetrazolium chloride (INT) were used as chromogenic agents to indicate the degree of hydrolysis of pyruvate and sucrose. The intensity of colour development for each of the detector strips was compared with that formed when similar strips were dipped into deionized water, as controls, and the level of inhibition determined.

Ames *Salmonella* mutagenicity assays

Plate incorporation and spot tests using tester strains TA98 and TA100 in the presence or absence of liver preparations were carried out as described by Grabow *et al.* (1980). Assays were done in fivefold, and included positive, negative and sterility controls. Toxic effects of test material on tester strains were evaluated by the methods of Grabow *et al.* (1980). Residues of extracts and seed powder were tested in dichloromethane suspensions which eliminated the possibility of contamination.

Results

Effects of coagulation on bacterial counts in different waters

Counts of seeded *E. coli* or naturally occurring faecal coliform bacteria in various waters coagulated with different quantities of cotyledon powder, are listed in Table 1. Results represent the average of three counts. Unseeded Moreletta and distilled water had faecal coliform counts of 0/100 mℓ. In the absence of coagulant, counts of seeded *E. coli* increased slightly over 24 h in Moreletta water, while those of naturally occurring faecal coliforms in unseeded Apies water decreased marginally. Counts decreased during the first 60 min after the addition of coagulant in all waters. In distilled and Moreletta water *E. coli* counts continued their downward trend and after 24 h counts were much lower, with the highest, and dose-related, reduction in distilled water. In contrast to seeded *E. coli* in distilled and Moreletta water, the counts of naturally occurring faecal coliforms in Apies water displayed dose-related increases after an initial reduction. Reduction of both seeded *E. coli* and naturally occurring faecal coliform counts during the first 60 min was less when coagulant prepared from whole seeds was used, while ground pericarps had virtually no effect on *E. coli* or faecal coliform counts.

Effect of coagulation on coliphage counts in Apies River water

Average results of three counts (Table 2) showed an increase of 3 % in controls (water without coagulant) during the first hour but a 38% reduction during the subsequent 23 h. In coagulated water coliphage counts displayed a dose-related reduction after both 1 and 24 h. The reduction during 1 and 24 h was slightly higher in coagulated water than in controls. As in the case of *E. coli* and faecal coliforms, water treated with ground whole seeds yielded lower reduction, and ground pericarps virtually no reduction of coliphage counts during the first 60 min.

Tetrahymena pyriformis toxicity assays

Toxicity was detected in Moreletta and distilled water coagulated by means of the milky cotyledon filtrate or the direct addition of

TABLE 1
COUNTS OF SEEDED *ESCHERICHIA COLI* OR NATURALLY OCCURRING FAECAL COLIFORM BACTERIA IN DIFFERENT WATERS COAGULATED WITH *MORINGA OLEIFERA* COTYLEDON POWDER

Time	Count/mℓ								
	Seeded *E. coli* in distilled water			Seeded *E. coli* in Moreletta River water			Naturally occurring faecal coliforms in Apies River water		
	Coagulant (mg/ℓ)			Coagulant (mg/ℓ)			Coagulant (mg/ℓ)		
	0	200	1 000	0	200	1 000	0	200	1 000
0 min	14×10^5	14×10^5	14×10^5	12×10^5	12×10^5	12×10^5	74×10^2	74×10^2	74×10^2
5 min	14×10^5	75×10^4	89×10^4	12×10^5	12×10^5	12×10^5	74×10^2	68×10^2	52×10^2
60 min	16×10^5	24×10^4	17×10^3	12×10^5	56×10^4	68×10^4	73×10^2	44×10^2	39×10^2
24 h	11×10^5	0	0	82×10^5	20×10^2	31×10^2	43×10^2	43×10^4	68×10^3

TABLE 2
COUNTS OF NATURALLY OCCURRING COLIPHAGES IN APIES RIVER WATER COAGULATED WITH *MORINGA OLEIFERA* COTYLEDON POWDER

Time (h)	Coliphages at various concentrations of coagulant (mg/ℓ)					
	0		200		1 000	
	Count/mℓ	% Reduction	Count/mℓ	% Reduction	Count/mℓ	% Reduction
0	500		500		500	
1	515	3*	270	46	245	51
24	319	38	159	41	137	44

* % Increase

TABLE 3
OXYGEN UPTAKE RATE OF *TETRAHYMENA PYRIFORMIS* CELLS EXPOSED TO MORELETTA OR DISTILLED WATER COAGULATED WITH *MORINGA OLEIFERA* COTYLEDON POWDER OR COTYLEDON FILTRATE

Water	Coagulant*	Oxygen uptake rate (%/min) at time intervals (min)				
		1	2	3	4	5
Distilled	Control (no coagulant)	7,17	7,17	7,17	7,17	7,17
	Milky filtrate	7,31	6,93	6,51	6,28	6,03
	Powder	6,99	5,37	4,18	3,35	2,58
Moreletta	Control (no coagulant)	7,31	7,31	7,31	7,31	7,31
	Milky filtrate	7,34	7,03	6,69	6,17	6,08
	Powder	7,07	5,70	4,25	3,18	2,51

*Equivalent of 200 mg/ℓ cotyledon powder after addition to cells

TABLE 4
OXYGEN UPTAKE RATE OF *TETRAHYMENA PYRIFORMIS* CELLS EXPOSED TO MEMBRANE-FILTERED *MORINGA OLEIFERA* COTYLEDON SUSPENSIONS

Concentration of cotyledon powder*	Oxygen uptake rate (%/min) at time intervals (min)				
	1	2	3	4	5
0	7,18	7,18	7,18	7,18	7,18
200	7,20	6,82	6,59	6,28	6,02
1 000	6,20	4,06	2,03	1,22	1,02

*Concentration in test chamber (mg/ℓ)

the cotyledon powder. Tests were carried out 60 min after the addition of coagulant. The toxic effects of coagulated Moreletta and distilled water did not differ significantly (Table 3). However, the water was less toxic when coagulated using the milky filtrate than by direct addition of an equivalent quantity of the powder. In the former case, there was a moderate reduction in oxygen uptake rate noticeable from 2 min onward. In the case of cotyledon powder the reduction in oxygen uptake rate was already detectable after 1 min, and after 5 min it was pronounced. Water tested 5 min after the addition of coagulant yielded similar results. The higher oxygen uptake rate in the case of the Moreletta water without coagulant (compared with distilled water) may be ascribed to dissolved salts in the water (Slabbert *et al.*, 1983).

Dose-related toxicity was detected by addition of extracts prepared by membrane filtration of manually shaken cotyledon suspensions to test chambers. A concentration equivalent to 1 000 mg/ℓ of cotyledon powder (20 000 mg/ℓ before addition to cells) caused a reduction in oxygen uptake rate which was noticeable after 1 min and extensive after 5 min (Table 4). The equivalent of 200 mg/ℓ of cotyledon powder (4 000 mg/ℓ before addition to cells) reduced oxygen uptake rate to a lesser extent, recorded from 2 min onward. Suspensions standing for up to 60 min prior to filtration and testing, yielded similar results.

Batch water extracts even up to the equivalent of 500 mg/ℓ of cotyledon powder, displayed a marginal effect only (Table 5). In contrast, the water extracts preceded by serial solvent extractions were more toxic and even the 5 mg/ℓ extract inhibited oxygen uptake rate. Batch methanol extracts, as well as the dichloromethane, acetone and methanol extracts of serial solvent extractions, had no significant effect in concentrations of up to 200 mg/ℓ.

There was a reduction in the toxicity of coagulated water with time (Table 6). Test samples taken from coagulated Moreletta water after storage for 24 h, had a similar but slightly less toxic effect than those taken 60 min after coagulation, while samples tested after 48 h were much less toxic.

In toxicity assays similar to those done on cotyledon preparations, whole seeds in similar concentrations yielded results along the same lines, but of lesser magnitude than cotyledons, while pericarps were not toxic.

Fish toxicity assays

The 96-h LC_{50} for guppies exposed to dilutions of the seed extract was the equivalent of 196 mg/ℓ of cotyledon powder. Lethal concentrations caused the respiratory folds of the gill lamellae to stick together and there was a loss of mucous cells. There was evidence of destruction of the respiratory epithelium in higher concentrations with the production of multiple haematomas. The damage to the respiratory organs would result in diminished oxygen uptake and impairment of the salt balance. Death probably ensued due to asphyxiation.

TABLE 5
OXYGEN UPTAKE RATE OF *TETRAHYMENA PYRIFORMIS* CELLS EXPOSED TO WATER EXTRACTS OF *MORINGA OLEIFERA* COTYLEDON POWDER

Water extract	Concentration*	Oxygen uptake rate (%/min) at time intervals (min)				
		1	2	3	4	5
Control (dist. water)	0	7,15	7,15	7,15	7,15	7,15
Batch	500	7,80	7,60	7,13	6,79	6,40
Serial**	5	6,95	6,95	6,95	6,95	6,95
	200	6,41	4,60	2,80	2,00	1,62

*Concentration in the test chamber (mg/ℓ)
**Serial liquid extract in which water was preceded by dichloromethane, acetone and methanol

TABLE 6
OXYGEN UPTAKE RATE OF *TETRAHYMENA PYRIFORMIS* CELLS EXPOSED TO MORELETTA RIVER WATER STORED FOR VARIOUS PERIODS AFTER COAGULATION WITH *MORINGA OLEIFERA* COTYLEDON POWDER*

Storage time**	Oxygen uptake rate (%/min) at time intervals (min)				
	1	2	3	4	5
Control***	7,25	7,25	7,25	7,25	7,25
60 min	7,29	6,20	4,45	3,26	2,30
24 h	7,30	6,41	4,95	3,80	2,99
48 h	7,70	6,89	6,40	6,38	6,39

*Concentration in test chamber equivalent to 200 mg/ℓ
**Stored at room temperature (20-25 °C)
***Uncoagulated water

Figure 1 depicts the effect of serial concentrations of seed extract on diurnal activity patterns of guppies. Concentrations of 10 and 20 mg/ℓ produced no adverse effects, whereas fish exposed to 30 mg/ℓ exhibited increased activity rates although the normal diurnal pattern was maintained. A 40 mg/ℓ concentration produced an alarm response in all sensor fish, locomotory activity being maintained at a higher level than that to be expected under normal conditions for the whole period of exposure, indicating a possible avoidance reaction on the part of the sensor fish. In a continuous automatic biomonitoring system, therefore, the seed extract would be detected at a level between 30 and 40 mg/ℓ, approximately 0,2 of the 96-h LC_{50}.

Enzyme inhibition assays

Exposure of enzymes to 200 mg/ℓ of cotyledon powder inhibited the activity of acetylcholinesterase, but not that of lactic dehydrogenase or invertase.

Ames *Salmonella* mutagenicity assays

Concentrations of up to 1 000 mg/ℓ of cotyledon powder had no detectable mutagenic effect on the tester strains used, both in the presence or absence of liver preparations. Higher concentrations could not be tested because of toxic effects on the tester strains. Assays on serial as well as batch extracts and on distilled or Moreletta water coagulated with cotyledon powder, likewise yielded negative results. Mutagenic activity was also not detected in similar preparations of whole seeds or pericarps.

Discussion

The reduction in counts of *E. coli* and faecal coliforms in water treated with *M. oleifera* seed preparations due to coagulation and sedimentation, the antibacterial effect of seed preparations, and the increase in faecal coliform counts in coagulated water as a result of regrowth (Table 1), are in agreement with data reported by Jahn and Dirar (1979), Jahn (1981) and Barth *et al.* (1982). This agreement confirms the similarity of materials and techniques used in the present and earlier studies.

The reduction in counts of coliphages as a result of coagulation and sedimentation (Table 2) is in line with the data on bacteria. An important difference, however, is that the survival of coliphages was not significantly affected by cotyledon preparations, even in concentrations as high as the equivalent of 1 000 mg/ℓ of cotyledon powder (Table 2).

The highly sensitive response of *T. pyriformis* and guppies (Tables 3 to 6 and Figure 1) represent another outstanding feature of the toxic effect of *M. oleifera* seeds. The almost instantaneous effect on oxygen uptake by *T. pyriformis* and the histopathological picture of damage to the respiratory epithelium of guppies, would seem to indicate that the primary site of action of the toxicant is the cell membrane, resulting in, for instance, disruption of the permeability barrier. This appears to be in agreement with the inflammatory effect on cells of the gastrointestinal tract and liver of rats to which powdered seeds were administered in massive doses (Barth *et al.*, 1982). An effect on cell membranes is also supported by the resistance of coliphages and the limited effect on bacteria where the cytoplasmic membrane

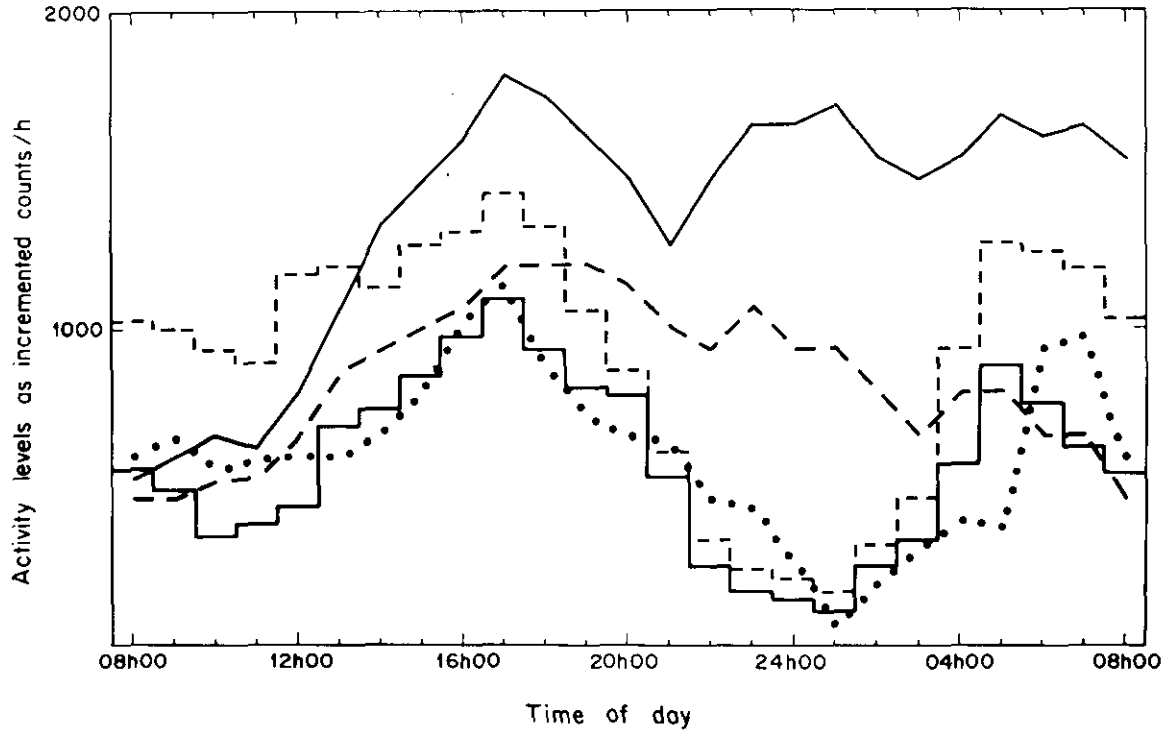

Figure 1
Average diurnal activity patterns of 5 guppies (Poecilia reticulata) exposed to 20 mg/ℓ (dotted line), 30 mg/ℓ (broken line) and 40 mg/ℓ (continuous line) of Moringa oleifera cotyledon powder in tap water. Normal activity patterns, averaged over 7 days, are presented as a histogram (continuous line), together with upper 95% confidence limits (broken line).

may be protected by the cell wall to a large extent.

The negative results of *Salmonella* mutagenicity assays on various seed preparations and extracts indicate an absence of mutagenic activity, which implies that the seeds are not likely to have carcinogenic properties (Grabow *et al.*, 1980). However, it is possible that mutagenic activity was obscured by the toxic effect, even though this is largely ruled out by the negative results of spot tests. The failure to detect mutagenic activity and the resistance of coliphages support the view that the primary target of the toxicant is the cytoplasmic membrane rather than nucleic acid, protein synthesis machinery or structural compounds.

It would appear that only one toxic compound is involved because the toxic effect could not be separated into different fractions by various batch and serial solvent extractions. Inhibition of the enzyme acetylcholinesterase suggests that the toxin's mechanism of action may resemble that of organophosphorous compounds, which inhibit the enzyme by covalently binding to serine hydroxyl groups (Mahler and Cordes, 1966). Since the enzymes invertase and lactic dehydrogenase were not affected by the toxin, the mechanism of action would not seem to be related to either that of heavy metals which inhibit invertase by the formation of covalent metal salts, or that of compounds which inhibit lactic dehydrogenase by acting as competitive inhibitor for the substrate (Mahler and Cordes, 1966).

The behaviour and level of activity of the toxin in water would seem to be complex and subject to a number of variables. Toxicity appears to be reduced by the presence of organic and possibly also other compounds because coagulated Moreletta River water was less toxic to *E. coli* than coagulated distilled water (Table 1), water coagulated by means of a milky filtrate derived by shaking of a cotyledon suspension was less toxic to *T. pyriformis* than water coagulated by the direct addition of cotyledon powder (Table 3), and removal of organic compounds by serial liquid extraction increased the toxicity of cotyledon preparations (Table 5). The reduction in toxicity of coagulated water with time (Table 6) may be due to masking effects of proteins or bacterial growth, decomposition by bacteria, or instability of the toxin. The latter would seem to be in agreement with findings that the activity of the toxin is greatly reduced by boiling seeds for a short time in water (Eilert *et al.*, 1981).

Properties of the toxic effect revealed in this and earlier studies indicate that it is caused by the antimicrobial compound 4(α-L-rhamnosyloxy) benzyl isothiocyanate, a glycosidic mustard oil isolated from *M. oleifera* seeds (Eilers *et al.*, 1981; Barth *et al.*, 1982). Further evidence in this regard is that neither the latter toxin (Eilert *et al.*, 1981) nor the toxic constituent investigated in this study were methanol extractable.

There is no evidence that the toxin, which occurs in the cotyledon of *M. oleifera* seeds and is absent from the pericarp, may have short-term toxic, long-term chronic or carcinogenic effects on humans under the conventional conditions of utilization of the seeds for nutritional, medicinal or water treatment purposes (Jahn and Dirar, 1979; Jahn, 1981). Human exposure to doses of the toxin used in studies on micro-organisms, fish and rats is hardly possible. All indications are that potentially harmful effects of the toxin in coagulated water are eliminated in the gastrointestinal tract as a result of interference by organic matter and instability of the toxin.

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References

BARTH, W.H., HABS, M., KLUTE, R., MÜLLER, S. and TAUSCHER, B. (1982) Trinkwasseraufbereitung mit Samen von *Moringa oleifera* Lam *Chemiker-Zeitung* **106** 75-78.

EILERT, U., WOLTERS, B. and NAHRSTEDT, A. (1981) The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. *J. Medicinal Plant Res.* **42** 55-61.

GRABOW, W.O.K., COUBROUGH, P., NUPEN, E.M. and BATEMAN, B.W. (1984) Evaluation of coliphages as indicators of the virological quality of sewage-polluted water. *Water SA* **10** 7-14.

GRABOW, W.O.K., DENKHAUS, R. and VAN ROSSUM, P.G. (1980) Detection of mutagens in wastewater, a polluted river and drinking-water by means of the Ames *Salmonella*/microsome assay. *S. Afr. J. Sci.* **76** 118-123.

GRABOW, W.O.K., HILNER, C.A. and COUBROUGH, P. (1981) Evaluation of standard and modified M-FC, MacConkey, and Teepol media for membrane filtration counting of faecal coliforms in water. *Appl. environ. Microbiol.* **42** 192-199.

GRABOW, W.O.K. and PROZESKY, O.W. (1973) Drug resistance of coliform bacteria in hospital and city sewage. *Antimicrob. Agents Chemother.* **3** 175-180.

JAHN, S.A.A. (1981) *Traditional Water Purification in Tropical Developing Countries – Existing Methods and Potential Application.* German Agency for Technical Cooperation, Eschborn.

JAHN, S.A.A. and DIRAR, H. (1979) Studies on natural water coagulants in the Sudan, with special reference to *Moringa oleifera* seeds. *Water SA* **5** 90-97.

LITCHFIELD, J.T. Jr and WILCOXON, F. (1949) A simplified method of evaluating dose-effect experiments. *J. Pharm. Exp. Ther.* **96** 99-113.

MAHLER H.R. and CORDES, E.H. (1966) *Biological Chemistry* p. 256. Harper and Row, New York.

MORGAN, W.S.G., KÜHN, P.C., ALLAIS, B. and WALLIS, G. (1982) An appraisal of the performance of a continuous automatic fish biomonitoring system at an industrial site. *Wat. Sci. Technol.* **14** 151-161.

SLABBERT, J.L. and MORGAN, W.S.G. (1982) A bioassay technique using *Tetrahymena pyriformis* for the rapid assessment of toxicants in water. *Water Research* **16** 517-523.

SLABBERT, J.L., SMITH, R. and MORGAN, W.S.G. (1983) Application of a *Tetrahymena pyriformis* bioassay system for the rapid detection of toxic substances in wastewaters. *Water SA* **9** 81-87.