Original Article

Preliminary Bacteriological Evaluation of Abattoir Waste water Treated With Moringa Oleifera Seed Powder

ASEMOTA, U.K*[1], ODUTAYO, O.I[2], OYEWOLE, I.O[2] AND ABUBAKAR S.[1]

[1]Biotechnology and Genetic Engineering Advanced Laboratory, Sheda science and Technology Complex (SHESTCO), P.M.B 186, Garki, Abuja. FCT. Nigeria
[2]Department of Biosciences and Biotechnology, Babcock University, Ilisan-Remo, Ogun state. Nigeria

Address for Correspondence

Biotechnology and Genetic Engineering Advanced Laboratory, Sheda science and Technology Complex (SHESTCO), P.M.B 186, Garki, Abuja. FCT. Nigeria

E-mail: Peaceaka2011@yahoo.com

ABSTRACT

Objective: The used of synthetic chemicals in water treatment is expensive and raises fears on health implications and environmental safety, the need to find alternatives that are safe health-wise and environmentally friendly gave rise to this study.

Methods: A preliminary bacteriological evaluation of abattoir waste water treated with powder obtained from *M. oleifera* was carried out. Isolation and biochemical techniques were employed to identify pathogenic organisms. The *M. oleifera* seeds were dried in the oven at 40^0C to constant weight. They were winnowed, shelled and grounded using sterile mortar and pestle. A sufficient powderwas obtained using a 400mm size sieve. Before treatment a 1ml aliquot of the waste water sample was serially diluted, the 10^7 and 10^8 were cultured on Nutrient agar, MacConkey agar and Eosin Methylene Blue agar and the plates were incubated at 37 ± 2^0C for 24 hours. Plates containing colonies ranging from 30 – 150 were counted and recorded at 24 hours interval for 3 days to obtain the Total Bacterial Count (TBC), Total Coliform Count (TCC), *Escherichia coli* Count (ECC) before and after treatment.

Results: Different concentrations of the powder (1.0g, 2.5g, and 5.0g) were used to treat 100ml of the waste water resulting in a gradual change in colour with increasing concentration and time. TBC, TCC, ECC fallen to ≈ 4.4 x 10^7, 3.2 x $10^5$and 1.6 x 10^4 respectively. After 48 hours, TBC, TCC, and ECC fallen to ≈ 2.2 x 10^7, 3.1 x 10^5 and 1.1 x 10^4 respectively. After 72 hours TBC, TCC and ECC fallen to 2.1 x 10^7, 1.7 x 10^5 and 1.5 x 10^4 respectively. On treatment with 2.5g, after 24 hours the TBC, TCC and ECC reduced to 2.3 x 10^7, 3.5x 10^5 and 1.1 x 10^4 respectively. After 48 hours TBC, TCC and ECC fallen to 2.1 x 10^7, 2.5 x 10^5 and 0.5 x 10^4 respectively. After 72 hours TBC, TCC, ECC fallen to 2.0 x 10^7, 1.5 x 10^5, and 0.3 x 10^4 respectively. After 24 hours of treatment with

5.0g, TBC, TCC and ECC fallen to 2.0 x 10^7, 2.9 x 10^5 and 0.9 x 10^4 respectively. After 48 hours, TBC, TCC and ECC fallen to 1.1 x 10^7, 2.4 x 10^5 and 0.2 x 10^4 respectively. At 72 hours TBC, TCC and ECC fallen to ≈ 1.0 x 10^7, 1.1 x 10^5 and 0.1 x 10^4 respectively. **Conclusion:** This investigation shows that the powder obtained from *M. oleifera* seed can also be used effectively in the management of abattoir waste water.

Keywords: Abattoir, Biochemical tests, *Moringa oleifera*, Seed powder, Waste water.

INTRODUCTION

An abattoir is a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animal processing and effective preservation, and storage of meat products for human consumption.[1] A large quantity of water is used for the washing of meat and cleaning of the environment, therefore generally abattoirs are located near a flowing river, the effluent is then discharged into the environment, and this constitutes is one of the greatest threats to human health and environmental safety. Abattoir waste have the potential to befoul the environment, or cause hazards to human health, and harm to living resources and ecological systems,[2] Bacteria from abattoir waste discharged into water columns can subsequently be absorbed to sediments and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns presenting long-term health hazards.[3]

Pathogens present in animal carcasses or shed in animal waste may include Rotaviruses, Hepatitis E virus, *Salmonella spp,E. coli* 0157:H7, *Yersinia enterocolitica*, *Campilobacter spp*, *Cryptosporium parvum* and *Giardia lambi*.[4] They can pollute surface waters, underground waters, abattoir market environment and consumables around the abattoir especially when abattoir wastes are not properly treated and disposed.[2]

Appropriate facilities to ensure safe disposal of abattoir wastes in a manner that will not constitute a potential hazard to public health, animal health and the environment is considered very essential. Most abattoirs in Nigeria have no facilities for waste treatment, wastes are either disposed on open dumps or are discharged into nearby streams, hence constituting an environmental menace.[5] Natural coagulants are cost effective while conventional water purification systems using imported chemicals are prohibitively expensive.[6]

Moreover the continued use of chemicals in water treatment raises fears on health implications and environmental safety, for example, chlorine is known to produce trichloromethane, a cancer precursor,[7] while aluminium sulphate has been linked to Alzheimer's disease.[8] It is therefore imperative to find alternatives that are safe health – wise and also environmentally friendly.[9] A suitable alternative is *M. oleifera*, a medicinal plant that it is non – toxic.[10] *M. oleifera* seeds contain 1% active polyelectrolytes that neutralize the negatively charged colloid in the polluted water, it contains a number of benzyl glucosinolate which act as antibiotic and is an organic natural polymer.[11] The

protein powder is stable and totally soluble in water, the active ingredients are dimeric proteins and its mode of action is said to be by absorption, charge neutralization and interparticle bridging. The seeds release antimicrobial peptides which disrupt the cell membrane, inhibit the replication of bacteriophages and essential enzymes. The effectiveness of *M. oleifera* in the treatment of different kinds of waste water has been extensively reported by various authors[7, 13, 14, 15, 18] and.[20] However, there is paucity of information on the use of *M. oleifera* seeds in the treatment of abattoir waste. This mini project is therefore focused on the determination of the effectiveness of *M. oleifera* seed powder for treating abattoir waste water.

MATERIALS AND METHOD

Study area. The abattoir under study is the Iperu abattoir located in Iperu or Iperu Bale Oja, a town near the Ibu River in Ogun State in Southwestern Nigeria. It is the most populous town in Remo Region of the Ikenne Local Government Area and lies at 6°52¹N 3°43¹.

Collection, Identificationand processing of seed powder

The *M. oleifera* seeds were purchased in Shagamu Local Government Area of Ogun State, Nigeria. Theidentification was established by a botanist in the Department of Biosciences and Biotechnology, Babcock University, Illishan – Remo, Ogun State. The seeds were dried in the oven at 40°C to constant weight. The seeds were threshed, shelled, grounded using sterile mortar and pestle and sieved to produce a fine powder resembling powder milk using a 400mm sieve.

Collection of Abattoir

Waste water, Sterile sample bottles were used to aseptically collect water as it was leaving the slaughter pavements using sterile gloves. The samples were immediately transported to the laboratory and analysis of the sample was done within 6 hours of collection. The appearance and cloudiness of the water was noted by visual observation.[21] The pH of the samples were taken using a pH meter.

Media preparation

All the media used in this study were prepared according to Manufacturer's instructions. They were prepared before going for the collection of the abattoir waste water samples. Some of the media used include Nutrient agar (Lab 008), MacConkey agar (Lab M, UK), Mannitol Salt Agar (Lab 7, UK), Simmons Citrate Agar (Lab 69) and Eosin MethyleneBlue Agar.

Treatments

Total Bacterial count (TBC), Total Coliform Count (TCC), Escherichia coli Count (ECC) were taken before and after treatment. Before treatment a 1ml aliquot of the waste water sample was serially diluted, the 10^7 and 10^8 were cultured on Nutrient agar, MacConkey agar and Eosin Methylene Blue agar and the plates were incubated at 37°C for 24 hours.[22] After treatment, 1ml aliquots were taken from the abattoir waste water treated with different concentrations of the seed powder (1.0g, 2.5g, 5.0g) and serially diluted, the 10^7 and 10^8 dilution were cultured and incubated at 37°C for 24 hours along with the control (untreated sample). Plates containing colonies ranging from 30 – 150 were counted and recorded at 24 hours interval for 3 days.

RESULTS

Table 1. showed the preliminary macro and microscopic features of the colonies such as shape, colour, elevation, surface and age, the cells are also Gram

stain and checked for the cell types and arrangements followed by biochemical characterization such as oxidase, catalase, motility, oxygen used, Starch hydrolysis, glucose, lactose and mannitol utilizations.

The result divulged the higher population of both Gram negative and gram positive bacteria namely *P. aeruginosa*, *E. coli*, *Bacillus spp* and *Staphylococcus aureus* was all isolated. Table 2, showed 24 hours Total Bacterial Count (TBC), Total Coliform Count (TCC), *E. coli* Count (ECC) before and after treatment. Different concentrations of the powder (1.0g, 2.5g, and 50g) were used to treat 100ml of the waste water resulting in a gradual change in colour with increasing concentration and time. TBC, TCC, ECC dropped to ≈ 4.4 x 10^7, 3.2 x 10^5 and 1.6 x 10^4 respectively. Table 3 showed the numbers of colonies After 48 hours, TBC, TCC, and ECC dropped to ≈ 2.2 x 10^7, 3.1 x 10^5 and 1.1 x 10^4 respectively. Table 3. Showed the numbers of colonies After 72 hours TBC, TCC and ECC dropped to ≈ 2.1 x 10^7, 1.7 x 10^5 and 1.5 x 10^4 respectively. On treatment with 2.5g, after 24 hours the TBC, TCC and ECC reduced to ≈ 2.3 x 10^7, 3.5x 10^5 and 1.1 x 10^4 respectively.

After 48 hours TBC, TCC and ECC dropped to ≈ 2.1 x 10^7, 2.5 x 10^5 and 0.5 x 10^4 respectively. After 72 hours TBC, TCC, ECC dropped to ≈ 2.0 x 10^7, 1.5 x 10^5, and 0.3 x 10^4 respectively. After 24 hours of treatment with 5.0g, TBC, TCC and ECC dropped to ≈ 2.0 x 10^7, 2.9 x 10^5 and 0.9 x 10^4 respectively. After 48 hours, TBC, TCC and ECC dropped to ≈ 1.1 x 10^7, 2.4 x 10^5 and 0.2 x 10^4 respectively. At 72 hours TBC, TCC and ECC dropped to ≈ 1.0 x 10^7, 1.1 x 10^5 and 0.1 x 10^4 respectively.

DISCUSSION

Waste disposal in Iperu abattoir is by open dumping of solid waste while the waste water is allowed to flow into a drainage which later probably finds its way into the Ibu River, this is in agreement with the observation of,[5] that most abattoirs in Nigeria have a poor method of waste disposal. The abattoir was found in a deplorable state lacking the basic facilities needed for operation and good management, this agrees with the findings of [23] on other abattoirs in Nigeria. The condition of this abattoir exposes its workers and potential consumers to the risk of getting infected with disease causing pathogens. Based on the definition of abattoir by Alonge[1]that an abattoir is a special facility designed and licensed for receiving, holding, slaughtering and inspecting meat animals and meat products before release to the public, this abattoir does not meet the description. This research work therefore aims at finding a treatment for the abattoir effluents before they are discharged into the environment. The waste water is reddish in colour and has a very offensive odor, this agrees with the findings of[24]. Based on morphological and biochemical analysis, the probable identity of the organisms isolated are *S. aureus,E. coli*, *P. aeruginosa* and *Bacillus sp*.

This agrees with other researchers who found heavy loads of *Listeria species,Escherichia coli, Klebsiella species, Enterococcus faecalis, Pseudomonas aeruginosa* and other intestinal pathogens in untreated abattoir effluent[5, 23, 24].*M. oleifera* seed powder was used in this preliminary investigation to treat the abattoir waste. On treatment with the seed powder, the initial reddish brown colour of the abattoir waste water was slightly removed on treatment with 1g, it got lighter with 2.5g.

The change in colour was more in the sample treated with 5g. This shows that the *M. oleifera* seed has absorbent property. Before treatment with the powder, the initial bacterial count was about 7.6 x 10^7, at 24 hours the untreated sample had a higher total bacterial load and at 72 hours the bacterial

load was highest. The sample treated with 1.0g of the seed powder had a reduced Total bacterial count of about 4.4 x 10^7 at 24 hours, 2.2 x 10^7 at 48 hours and 2.1 x 10^7 at 72 hours. The sample treated with 2.5g of the Moringa oleifera powder had a reduction in total bacterial load from about 2.3 x 10^7 at 24 hours, about 2.1 x 10^7 at 48 hours to 2.0 x 10^7 at 72 hours.

Treatment with 5g of the powder showed a marked decrease in the total bacterial load from 7.6 x 10^7 before treatment to 2.0 x 10^7 at 24 hour, 1.1 x 10^7 at 48 hours and 1.0 x 10^7 at 72 hours. This shows a significant reduction in the total bacterial load on application of the *M. oleifera* seed powder. The Escherichia coli count also dropped from 2.1 x 10^4 before treatment to 0.1 x 10^4 on treatment with 5g of the powder.

The result shows decreasing microbial load with increasing concentrations of the *M. oleifera* seed powder. The disinfectant property of *M. oleifera* has been reported by [17], in a similar study using dried pulverized *M. oleifera* seeds and observed a 95% reduction in bacterial load of turbid water at 15 minutes residence time.

However, in contrast to the 15minutes recorded by,[17] the effect took a longer time, perhaps due to the turbidity of the water and also the use of methanolic extracts of the seed powder. In the developed nations of the world, there are government agencies with relevant laws and standards guiding the treatment and disposal of abattoir effluents in order to protect the health of the people. These laws make the treatment of abattoir effluents before discharge mandatory for operators of the abattoir.[26] In Nigeria, there are similar agencies with relevant laws and standards guiding the treatment and discharge of effluent.[26, 27] However, while these laws and standard are enforced and adhered to in the developed nations like United States of America and United Kingdom, there is no such enforcement and adherence in Nigeria. Abattoir effluents are discharged untreated into the drainage system, with the consequent health hazard to populace.

In addition to identifying the pathogenic organisms that are present in abattoir effluents, this research work also focused on attempting to treat the abattoir waste water using *M. oleifera* seed powder.

RECOMMENDATION

From the result of this research work it is recommended that Government and private individuals should stop the indiscrimate disposal of untreated abattoir effluent into the environment since this poses a threat to the public health and aesthetically unsightly. Facilities for better operations and good management should be put in place in the abattoir. Since the use of chemicals and waste treatment facilities are prohibitively expensive, the use of *M. oleifera* seed powder should be adopted in abattoir waste water treatment since they are relatively cheap and non - toxic. It is also an environmentally friendly method of cleaning the ecosystem.

CONCLUSION

In conclusion, *M. oleifera* seed should be adopted as a cheap source of phytocoagulant and phytodisinfectant to clean ecosystem. Since using *M. oleifera* in place of alum will save foreign exchange and generate farm and employment income, the use of this seed in the sterilization of domestic water, industrial and agricultural waste water should be encouraged.

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Conflict of interest statement

We hereby declare that we have no conflict of interest.

REFERENCES

1. Alonge, D. O. (1991). Textbook of Meat Hygiene in the Tropics. Farmcoe Press, Ibadan, Nigeria, University press.Pp58.
2. Ezeoha, S.L., and Ugwuishiwu, B.O. (2011). Status of Abattoir wastes Research in Nigeria. *NigerianJournal of Technology*. 30 (4) Pp 34-41.
3. Narfanda, W.D and Icubkomawa, H.I. (2005). Impact of abattoir wastes on aquatic life. *Global journal of pure and applied sciences*. 21: Pp31-33.
4. Sobsey, M.D; Khatib, V.R; Hill, V.R; Alocija, E; and Pillai, S.(2002). Pathogens in Animal Wastes and the Impacts of Waste Management Practices on their Survival, Transport and Fate, White Paper for the NCMAWM. Pp7-9
5. Adeyemo, O.K. (2002). Unhygienic Operation of a city abattoir in Southwestern Nigeria: Environmental Implication. *African Journal of Environmental Assessment and Management*. 1: pp23 – 28.
6. Alhassan, O. (2008) Federal Government of Nigeria Endorses Local Water Treatment Purification Initiative. *The Nation Newspaper*. 5: Pp22.
7. Yongabi, K.A. (2004). Studies on the Potential use of Medicinal Plants and Macrofungi (Lower Plants) in Water and Waste water Purification. Proceedings of an E-seminar organized by the International Organization for Biotechnology Bioengineering. Pp7-9.
8. Zhang,Fang Zhang, Yuhong Luo, Hong Yang (2006). A Preliminary Study on Cactus a Coagulant in Water Treatment. *Process Biochem*, Elsever; 41(3); Pp730 – 733.
9. Yerima, M.B. Maccido, D.A; Farouq, A.A. Jodi-Sa'adatu M. Ibrahim, A.D. Umar, A.F., Ali-Mustapha, N.M. (2008). Evaluation of seed Extracts from Moringa oleifera in water disinfection. *Nigerian Journal of Biotech*. Pp19.
10. Grabow Wok, Slabert, J. Morgan, M.S.G., Jahn, S.A.A., Watt, S.A. (1985). A medicinal plant that it is non – toxic, 11: Pp 9 – 14.
11. Eilert U., Wolters B. and Nahrstedt. (1981). The antibiotic principle of Moringa oleifera and Moringa stenopetalla. *Planta medical*. 42: 42; Pp55-61.
12. Yongabi, K.A. (2009). The Role of Phytobiotechnology in Public health: In Biotechnology Ed. Horst. W. Doelle Edgar. J. Dasilva in Encyclopedian of Life Support Systems (EOLSS) Developed under the auspices of the UNESCO EOLSS Publishers, Oxford. Pp76.
13. Oliver, B. (1995). Medicinal Plants in Nigeria, college of Arts, Science and Technology. Ibadan. Pp27.
14. Jahn, S.A.A. (1981). Studies on natural Water Coagulants in the Sudan with special reference to *Moringa oleifera* seeds. Waterlines. 2: 27-28.
15. Muyibi Suleyman A. and Evison Lilian M. (1994). The effectiveness of *M. oleifera* in the treatment of different kinds of waste water; 4:1099-1105.
16. Akinwore, A.O and Jok, I.I. (2006). An investigation of the Potentials of *Moringa oleifera* Seed as a Coagulation and Suspended Solids in Fish Culture Waste water. *The Nigerian Journal of Forestry*, 31st Annual Conference. Pp 26 – 30.
17. Yongabi1, K. A. Lewis D. M and Harris P. L. (2011). Application of Phytodisinfectants in water Purification in Cameroon. *African journal of microbiological Research*, 5(6) Pp628 – 635.
18. Chebemicheal, K., Gunaratna, K.R., Henrikisson, H., Bruner, H., and Dalhammer, G. (2005). A Simple Purification and Activity of Coagulation Protein from *Moringa oleifera* Seed. Water Research, 39 (11) Pp. 2338 – 2340.
19. Oluwalana, S.A., Bankole, W., Bolaji, G.D., Martu, D and Alegbeleye, O. (2004). Domestic Water Purification Using *Moringa oleifera*. *The Nigerian Journal of Forestry,* 29 (1): 28 – 32.

20. Global Health and Education Foundation (GHEF), (2007). Conventional Coagulation Flocculation Sedimentation. Safe Drinking Water is Essential. National Academy of Scence, Retrieval.
21. Burns, N.M, Van Otterloo, H.R (1974). Standard Method for Examination of Water and Waste Water in American Public Health Association.
22. Jeanine I. Boulter, Jack T. Trevors, Greg J. Bo land (2002). Microbial Studies of Compost, bacterial identification and their potential for turtgrass pathogen suppression. *World Journal of Biotechnol*. 18: pp661 – 671.
23. Akano SO, Moro DD, Deji-Agboola AM and Oluwadun A (2013**).** Public health implication of *Listeria species* and other bacteria isolates of abattoir effluent in Lagos, Nigeria. *International Research Journal of Microbiology*. 2013; 4:162 – 167.
24. Abogenin, T.P. (2014). Some Aspects of Physicochemical and Microbiological Characteristics of Effluent from two Abattoirs in Ogbomosho, *International Journal of Biological and Physical Sciences*. pp14: 4.
25. Amagloh and Benang (2009). Effectiveness of *Moringa oleifera* seeds as a coagulant for water purification, University for Development Studies, Faculty of Applied Sciences, Department of Applied Chemistry, pp6.
26. Federal Environmental Protection Agency (FEPA) (1988). National Interim Guidelines and Standards for Industrial Effluents, Gaseous Emissions and Hazardous Waters Management in Nigeria's FEPA Decree. pp33 – 46.
27. Lagos State Environmental Protection Agency (LASEPA) (1996). Functions and Power of the Agency LASEPA Edict *International Journal of Advanced Biological Research*. 3:117 – 20.

Table 1: Microscopic and biochemical characterization of bacterial isolates

Physicochemical tests	1	2	3	4
Gram staining	+ve	+ve	-ve	-ve
Elevation	Flat	Convex	Flat	Flat
Shape	Circular	Circular	Circular	Circular
Surface and age	Dry and rough	Smooth and entire	Smooth and irregular	Smooth and entire
Colour	Cream	White	Green	Cream
Cell type and arrangement	Cocci in chains	Cocci in clumps	Rods in chain	Road in chain
Oxidase	+ve	-ve	+ve	-ve
Motility	+ve	-ve	+ve	+ve
Oxygen used	Aerobe	Facultative	Facultative	Facultative
Catalase	+ve	+ve	+ve	+ve
Citrate	+ve	+ve	-ve	-ve
Starch hydrolysis	+ve	+ve	+ve	+ve
Glucose	-ve	+ve	-ve	-ve
Lactose	-ve	+ve	-ve	+ve
Mannitol	-ve	+ve	-ve	-ve
Probable identity	*Bacillus sp.*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*

Key: -+ve = Positive; -ve = Negative

Table 2: Bacterial count after 24 hours of treatment ≈ (cfu/ml)

Treatment	TBC	TCC	ECC
Control (untreated)	7.6×10^7	3.6×10^5	2.1×10^4
1.0g	4.4×10^7	3.2×10^5	1.6×10^4
2.5g	2.3×10^7	3.0×10^5	1.1×10^4
5.0g	2.0×10^7	2.9×10^5	0.9×10^4

Table 3: Bacterial count after 48 hours of treatment ≈ (cfu/ml)

Treatment	TBC	TCC	ECC
Control (untreated)	7.8×10^7	3.8×10^5	2.1×10^4
1.0g	2.2×10^7	3.1×10^5	1.1×10^4
2.5g	2.1×10^7	2.5×10^5	0.5×10^4
5.0g	1.1×10^7	2.4×10^5	0.2×10^4

Table 4: Bacterial count after 72 hours of treatment ≈ (cfu/ml)

Treatment	TBC	TCC	ECC
Control (untreated)	8.5×10^7	4.0×10^5	2.1×10^4
1.0g	2.1×10^7	1.7×10^5	1.5×10^4
2.5g	2.0×10^7	1.5×10^5	0.3×10^4
5.0g	1.0×10^7	1.1×10^5	0.1×10^4

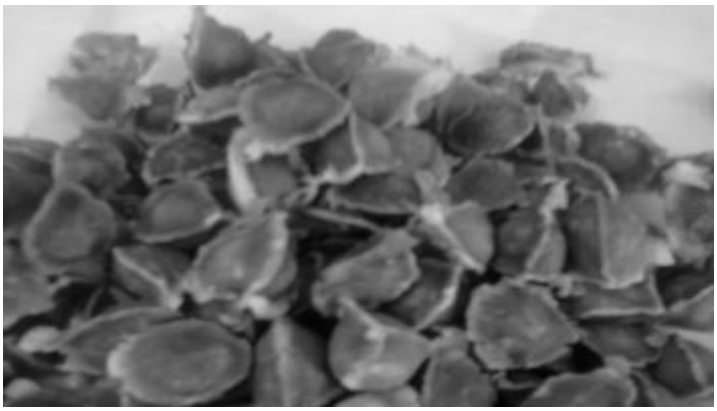

Figure 1: Unshelled Moringa seeds.

Figure 2: Moringa powder.

Figure 3: Shows the control, treatment with 1g, treatment with 2.5g, and treatment with 5g respectively, gradual colour change with increase in concentration.