

PRINT ISSN 1119-8362 Electronic ISSN 1119-8362 Full-text Available Online at <u>https://www.ajol.info/index.php/jasem</u> https://www.bioline.org.br/ja

# Biochemical and Physiological Response of *Abelmoschus esculentus* (L.) Moench to Food Industry Processing Effluent

# SAM, SM

Department of Botany, Akwa Ibom State University, Ikot Akpaden, Nigeria. Email: sundaysam@aksu.edu.ng, eosamviek@yahoo.com, Tel: +2348028706841

**ABSTRACT:** This study investigated the response of *Abelmoschus esculentus* to food industry processing effluent at different concentrations of 0%, 20%, 40%, 60%, 80% and 100%. The physicochemical properties of the effluent and experimental soil were analysed. The effluent had a high BOD of 39.20mg/l compared to WHO standard of 10 and a pH of 6.12 which is within WHO allowable limit of 6.5-8.5. The conductivity was low 130.00 µs/cm compared to WHO standard of 750.5. The study also revealed that food industry effluent had a significant (p<0.05) effect on the morphological features, chlorophyll and heavy metals composition of *Abelmoschus esculentus*, The growth parameters were significantly (p<0.05) reduced with increase in the concentrations of the effluent. High concentration of the effluent at 80% induced high chlorophyll content at 24.05mg/ml and also increased the level of heavy metal bioaccumulation in *Abelmoschus esculentus*. This study suggests that high concentration of food processing industry effluent could be detrimental to the growth and productivity of *Abelmoschus esculentus*.

## DOI: https://dx.doi.org/10.4314/jasem.v26i3.21

**Open Access Article:** (https://pkp.sfu.ca/ojs/) This an open access article distributed under the Creative Commons Attribution License (CCL), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Impact factor: http://sjifactor.com/passport.php?id=21082

#### Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

### Copyright: © 2022 Sam

Dates: Received: 10 January 2022; Revised: 22 February 2022; Accepted: 15 March 2022

#### Keyword: Biochemical, Physiological, Abelmoschus esculentus, Effluent

Okra (Abelmoschus esculentus (L.) Moench, a member of the Malvacea family, is a widely cultivated vegetable crop and very important in the diet of African (Omotoso and Shittu, 2008). Okra is also known as ladies fingers, Ochro or Gumbo in many English-Speaking countries. It is valued for its edible green seed pods. The species is a perennial, often cultivated as an annual in temperate climates and often grows to around 2 metres tall. The leaves are 10-20 centimeters long and broad, palmately lobed with 5-7 lobes. The flowers are 4-8 centimeters in diameter with five white to yellow petals, often with a red or purple sport at the base of each petal. The fruit is a capsule up to 18 centimeters long with pentagonal cross section. It is a valuable crop that provides an excellent income and generates other opportunities for small scale farmers (selleck and Opeńa, 1985). It is one of the important nutritional vegetable crops cultivated in Nigeria, covering an estimated land area of 1-2 million hectares (FMAWR & RD, 1989). Okra grows in all types of soils and thrives best in a moist, friable, well-drained soil (Kochhar, 1986). The plant is

tolerant to drought stress (Majanbu et al., 1985); however, supplementary irrigation may be necessary during extended drought periods for a satisfactory production (Okunade et al., 2009). In Nigeria, the widely cultivated okra is distributed and consumed other fresh (usually boiled, sliced or fried) or in a dried form. Industrial effluents coming from different industrial and commercial establishments posing serious threats to environment, particularly in urban and semi urban areas. It becomes the source of pollution for surface and subsurface water, soil and air. Its proper management and disposal is one of the most serious challenges all over the world (Martin, 1991). Huge industrial establishment and their indiscriminate discharges pose a great threat to our environment. Industrial wastewater deteriorates not only the quality of soil, crop and environment but are also directly harmful to the human, animal and aquatic lives. Unplanned discharges of industrial wastewater degrade the quality of food crops. The total land irrigated with raw or partially diluted wastewater has been estimated to be about 20 million hectares in fifty

countries, which is approximately 10% of total irrigated land (FAO, 2003). The heavy metal content in the soil will be increased by about 18 - 98%, compared to the present unpolluted soil (Kurnia et al., 2000). Industrial effluents are liquid wastes which are produced in the course of industrial activities. Over the years, the improper disposal of industrial effluents has been a major problem and a source of concern to both government and industrialist. In most cases the disposal or discharges of effluents, even when these are technologically and economically achievable for particular standards, do not always comply with pretreatment requirement and with applicable tonic pollutant effluent limitations or prohibitions. The consequence of these anomalies is a high degree of environmental pollution leading to serious health hazards. Among the food processing industries commonly found in the Southern region of Nigeria are noodles industries, beer breweries, soft drink bottling companies, vegetable oil industries, etc. The food processing plants produce oil sludge's and other wastewaters that are normally land applied after limited pretreatment. However, considering the types and interactions of the numerous constituents of these complex waste streams and the necessity to avoid the environmental pollution problems that can result when these wastes are land applied, it is necessary that the characteristics of the effluents from noodles and other food processing industries in a developing country like Nigeria be investigated. Effluents are wastes produced from industries and they vary depending on the human activities that produce them. Production of those wastes is an integral part of industrial activities but unfortunately our inability to anticipate or predict the types and magnitude of undesired consequences of uncontrolled release of effluents in our environment, coupled with the growth of industrialization have resulted in massive and destructive operations in our ecosystems although industrial processes are desirable, at the same time, the serious and irreversible damage done to the environment through their discharges of effluents are unquantifiable. Until now, effluents are discharged into rivers, estuaries, lagoons or the sea initiatory form of treatment. However despite the treatment being employed by some industries it is skill impossible to remove all undesirable properties from effluents. In Nigeria of today. the responsibility environmental management is for the National Environmental Standard and Regulations Enforcement Agency (NESREA) to ensure that all industries treat their effluents before they are discharged into the environment. In Rivers State, farmers especially those in sub-urban and rural areas irrigate their crops with water obtained from rivers and stream, which receive effluents from these industries. The physicochemical

Determination of Sodium, Potassium, Calcium, Zinc and Iron: One thousand parts per million stock solution of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), zinc (Zn<sup>2+</sup>) and iron (Fe<sup>2+</sup>) were prepared. Both the standard (Na, K, Ca, Zn and Fe) and sample solutions as well as the blank solutions were aspirated using a flame photometer (GallenKamp BKL – 210) with the filter of Na, K, Ca, Zn and Fe in place and the readings of elements in the sample solutions recorded as described by Salami and Egwin (1997).

*Pollution of soil samples:* The effluent was prepared in various concentrations of 20%, 40%, 60%, 80% and 100% respectively using a simple serial dilution with sterile deionized water. Sterile distilled water was used

characteristics of industrial effluents are usually

inhibitive to plants, hence; they usually produce

debilitating effects on plants when the water is used

for agricultural purposes. In recent years, increasing

awareness of water pollution and its far reaching

effects has prompted concerted efforts towards

pollution abatement (Donmez et al., 1999; Igbinosa

and Okoh, 2009). The rate at which effluents is

discharged into the environment and water bodies, has

been on the increase due to rapid growth of cities in

the world. Generally, water bodies are major sites of

heavy metal deposits due to the fact that streams and rivers flow through agricultural areas where pesticides

and fungicides may have been used, through industrial

districts where there may have been many metal waste

deposits or direct discharge of effluents into these

water bodies (Malakootian et al., 2009). Thus, this

study aimed at assessing the biochemical and

physiological response of Abelmoschus esculentus to

effluent from food processing industry which is

Sources of experimental materials: The Okra seeds

used for this study were obtained from Agricultural

Development Programme (AKADEP) under the

Ministry of Agriculture and Food Sufficiency, Akwa

Ibom State, Nigeria. The industrial effluent used was

obtained from Dufil Prima Food Plc who is the

producers of Indomie noodles in Port Harcourt, Rivers

State, Nigeria. The physical and chemical parameters

of the effluent were determined using standard

procedures for examination of water and waste water

(APHA, 1989). The effluent was analyzed for pH,

conductivity, Total Dissolved Solid, Chemical

Oxygen Demand (COD), Biological Oxygen Demand

(BOD) and oil level. The loamy soil used was obtained

from the Botanic Garden of Akwa Ibom State

University and the physicochemical properties of the

soil were also analyzed (A.O.A.C, 1995).

frequently discharged into water bodies.

MATERIAL AND METHODS

as 0%. Five kilograms (5kg) of loamy soil was weighed into a perforated planting bucket and labeled appropriately. Each soil thoroughly mixed with concentrations of 20%, 40%, 60%, 80% and 100% of the effluent and incubates for three days (Udo and Oputa, 1984). The unpolluted soil served as control for each treatment (Amadi *et al.*, 1992; Esenowo and Umoh, 1996). The soil sample was mixed with hand trowel to achieve uniformity for three days after and four of each seeds was placed into the perforated planting buckets. Each of the treatment was replicated 4 times, which were kept under a conducive environmental condition.

Assessment of Biochemical and Physiological parameters: The parameters assessed were shoot height, leaf length, leaf width, leaf area, root length, fresh and dry weight, and moisture content. Other parameters were chlorophyll content and the presence of heavy metals in the leaf.

*Viability and Germination Test:* Viability of 3 replicates of 26 seeds was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). Seeds were placed in 1% tetrazolium chloride solution at 30°C and darkness for 24 hours. Seeds were then cut in half and examined. Only uniformly stained red/dark pink embryos were considered 'viable'.

*Statistical analysis:* The growth parameters were subjected to one-way analysis of variance (ANOVA) to determine the level of significance using standard method of (Sokal and Rohlf, 1995).

## **RESULTS AND DISCUSSION**

The results of the physicochemical properties of the experimental soil shows that the soil was rich in particle sizes, sand, silt, clay, available phosphorus and calcium while exchange magnesium, organic carbons and total nitrogen were relatively low in their values (Table I). Table 2 shows the physical and chemical properties of the food industry processing effluent. The pH was 6.12 which is within the admissible limits of 6.5-8.8 for pH value in effluent water as stated by WHO (2004). The conductivity was 130.00 µs/cm which was low compared to WHO (2004) standard of 750.5. The Total Dissolved Solid was 22.00mg/l. The Biological Oxygen Demand (BOD) was 39.20mg/l which was high as compared to WHO (2004) standard of 10. The Chemical Oxygen Demand (COD) was 130.00mg/l which was higher than BOD. For the heavy metals analysis, the values for the elements 100% conc. were Sodium 279.49mg/kg, Potassium 950.82mg/kg, Calcium 3809.22mg/kg, Zinc 7.65mg/kg and Iron 12.355mg/kg (Table 3).

The effect of different concentrations of the effluent on the growth parameters are shown in figures 1 to 8. The results shows that at 20% concentration the effluent significantly (P<0.05) enhanced shoot length, leaf length, leaf width, leaf area, fresh weight and dry weight of *Abelmoschus esculentus* at 12WAP (Figures 1-5) while at 60% concentration the effluent significantly (P<0.05) enhanced the moisture content (figure 6), at 80% concentration the root length and chlorophyll content were significantly (P<0.05) enhanced (figure 7 and 8).

Table 1:	Physicochemical	properties of ex	perimental soil
		P	

Parameters	Values Of Soil Sample			
pH (1:1) H <sub>2</sub> O	5.54			
pH (1:1) KCl	4.78			
Oc (%)	1.474			
N (%)	0.153			
P (mg/g)	36.945			
Ca (Cmol/kg)	37.051			
Mg (Cmol/kg)	2.516			
K (Cmol/kg)	0.159			
Na (Cmol/kg)	0.609			
Acidity (Cmol/kg)	0.06			
Al (Cmol/kg)	0.00			
ECEC (Cmol/kg)	40.395			
Mn (mg/g)	30.02			
Fe (mg/g)	59.96			
Cu (mg/g)	157			
Zn (mg/g)	18.47			
Sand (%)	75.8			
Silt (%)	13.8			
Clay (%)	10.40			
Ec (m/s)	0.24			

The physical and chemical of properties of the food industry processing wastewater showed that the pH is 6.12 which falls within the admissible limits of 6.5-8.5 for pH value in effluent water as stated by WHO (2004). The electrical conductivity of the effluent was low indicating low presence of conducting ions.

The increase of conductivity and decrease of total dissolved salts are due to the excessive mineralization of organic matter (Arrignon, 1998). According to Kalra and Maynard (1992), the conductivity of a solution helps to estimate the total dissolved salt contents. COD, which is an overall as sessment of organic pollution, shows a higher value at the output of the plant. The pollutant indicators as shown on table 2 can be reduced in their units when the effluents are treated with suitable active carbons as documented by Okafor and Egwin (2004) in which it was reported that treatment of cassava effluent with locally developed adsorbents reduced BOD and COD by up to 50 and 75% respectively. There were significant (p<0.05)reductions in the percentage germination and growth parameters of Abelmoschus esculentus with increased levels of wastewater tested. The low rate in germination and growth performance at high

concentrations of the effluent was probably due to toxicity resulting from effluent contamination around the seeds.

Table 2: Physicochemical analysis of effluent						
Sample identity	рН	Conductivity µs/cm)	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	Oil and grease (mg/l)
Dufil-prima food limited effluent	6.12	130.00	39.20	130.00	22.00	3.36

Table 3: Analysis of heavy metals						
Concentrations	Na	K	Ca	Zn	Fe	
20%	90.72	801.40	1,636.15	40.06	37.79	
40%	120.98	1,736.03	3,284.09	9.76	97.29	
60%	52.81	299.63	1,420.67	1.68	11.34	
80%	197.63	812.06	8538.59	27.89	83.31	
100%	279.49	950.82	3,809.22	7.65	12.355	
0%	191.36	1,683.09	3512.05	11.59	60.20	

This agrees with the findings of Dutta and Biossyna (1997) that worked on the effect of paper mill effluent on germination of rice seed and growth behavior of its seedlings and discovered that effluents particularly at higher concentration inhibit germination. Sam *et al.*, (2017) reported the Biochemical Characterization of Cassava processing waste water and its effect on the growth of maize seedlings.





Their results showed that higher concentrations of the wastewater were inhibitory to the germination and early seedling growth of maize. Rajni and Chauchan (1996) discovered in their investigation that the effect of tannery effluent on seed values of *Hordeum vulgaris* L. showed that the effluents caused a significant reduction in germination percentage. Ogunwenmo *et al.* (2010) also found out that paints effluents generally inhibited the germination of Leafy vegetables-*Amaranthus hybridus* and *C. argentea*. The reduction in growth parameters may also be due to heavy metal poisoning, as a result of high accumulation of the heavy metals by the plant.







Fig 3: Effect of different concentrations of food processing industry effluent on the Leaf width of *Abelmoschus esculentus* 

The fresh and dry weights significantly (p<0.05) decreased with increase in food processing wastewater. This reduction may be due to a reduction in the proportionate amount of nutrients and biomass at the elongation phase of seedlings (Mayer *et al*, 1973), or due to low water potential of the food

processing effluents which weakened nutrient absorption in combination with the acidic nature of the food processing waste water.



Fig 4: Effect of different concentrations of food processing industry effluent on the Leaf area of *Abelmoschus esculentus* 



Fig 5: Effect of different concentrations of food processing industry effluent on the Fresh and dry weight of *Abelmoschus esculentus* 



Fig 6: Effect of different concentrations of food processing industry

effluent on the Moisture content of Abelmoschus esculentus



#### Treatment

Fig 7: Effect of different concentrations of food processing industry effluent on the Root length of *Abelmoschus esculentus* 



Fig 8: Effect of different concentrations of food processing industry effluent on the Chlorophyll content of *Abelmoschus* esculentus

This result is in line with the study of Pandey et al (1994), who showed that there was significant reduction in the fresh weight of seedlings. There were high accumulations of heavy metals Na, K, Ca, Zn and Fe in the leaf of Abelmoschus esculentus. Heavy metals disrupt the metabolic processes of living organism by inducing anatomical changes in primary leaves (Chaudhry and Qurat-ul-Ain, 2003). It could have been that the high rate of pollution enhanced the production of trace elements. Similar observations were made by Lowei (1968), who showed that increased levels of trace elements were indicative of extensive pollution. Therefore, these results should be a source of major concern not only to food processing industries but to all others who discharge effluents indiscriminately without adequate prior treatment.

*Conclusion:* Industrial effluents can be considered as a potential source of irrigation water and nutrients for the growth of agricultural crops in areas where industrial effluents are being discharged. However,

proper treatment and removal of toxic substances are necessary before using industrial effluent for irrigation purpose. In this study, effluent from food processing industry is found to be less toxic at low concentration on the germination and growth performance of *Abelmoschus esculentus* but at high concentrations of the effluent, germination and growth performance were inhibited.

# REFERENCES

- AOAC. (1995). Association of Official Analytical Chemist. *Method of Analysis*, 10<sup>th</sup> and 17<sup>th</sup> ed. USA: Washington DC press, pp.201-205
- Amadi, A; Dickson, AA. and Maaire, GO. (1992) Remediation of oil polluted soil, air and water. J. soil Sci., 66: 59-76
- APHA (1989). Standard methods for the examination of water and wastewater. American Public Health Association, 17th edition pp. 110 -200.
- Arrignon, J. (1998). Aménagement piscicolede seauxdouces (5eédition). Lavoisier. Tec. Doc, Paris.
- Avasan, MY; Ramakrishna, S. (2001). Effect of sugar mill effluent on organic resources of fish, *Pollut. Res.* 20(2) 167–171.
- Chaudhry, NY; Qurat-ul-Ain, 2003. Effect of growth hormones i.e., IAA, Kinetin and heavy metal i.e., lead nitrate on the internal morphology of leaf of *Phaseolus vulgaris* L. *Pak. J. Bio. Sci.* 6: 157-163.
- Dönmez, G.; Aksu, Z.; Özturk A.; Kutsal, T., (1999). A comparative study on heavy metal biosorption characteristic of some algae. Process. *Biochem.*, 34 (9), 885-892.
- Dutta, SK and Boissya, CL. (1997). Effect of paper mill effluent on germination of rice (*Oryza sativa* L.) and growth behaviour of its seedlings. *Journal* of Industrial Pollution Control, 13:41-47.
- Esenowo, GJ and Umoh, NS. (1996). Effect of used engine oil pollution on growth and yield of *Arachis hypogea* and *Zea mays. Trans. Nig. Soc. Bio. Conser.* 5: 700-711
- FAO (2003). Food and Agriculture Organization of the United Nation, World Water Development Report, Natural Resources and Environment Department, Water Development and Management Unit. Web address: <u>http://www.fao.</u> org/nr/water/dated 21 July 2010.

- Federal Ministry of Agriculture, water resources and rural development (FMAWR & RD). (1989). Fertilizer use and management: practices for crops in Nigeria. Series No2. Edited by Enwezor, W.D., Udo, E. J., Usoroh, N.J., Ayotade, K. A., Adepet, J.A., Chude, V.O., and Udegbe, C.I. Bobma Pulishers, U.I.P.O Pp 9555.
- Henry, G; Howeler, R. (1996). Cassava in China in an era of change. A CBN case study with farmers and processors. Working Document No. 155. CIAT, Cali, Columbia p. 18.
- Igbinosa, EO; Okoh, AI, (2009). Impact of discharge wastewater effluents on the physicochemical qualities of a receiving watershed in a typical rural community. *Int. J.Environ. Sci. Tech.*, 6 (2), 175-182
- International Seed Testing Association (ISTA) (2003). Working Sheetss on Tetrazolium Testing. Vols I and II. ISTA, Bassersdorf, Switerland. pp. 8-10. International Tree Journal. 6(1):59-66.
- Kalra, YP and Maynard, DG.(1992). Méthodesd'analyse dessols forestier setdestissus végétaux. rapportd'information NOR-X-319FCanada,129p.
- Kochhar, SL. (1986). Tropical crops: A textbook of Economic botany. 1<sup>st</sup> Edition, Macmillan Publishers Lordon, UK. P.256.
- Kurnia, U; Sutono, S; Markus Anda, Sulaeman, Kurniawansyah, AM. Tala'ohu SH. (2000). Pengkajian baku mutu tanah padalahan pertaniah. Laporan Akhir Kerjasama Penelitian Bapedal-Puslitbangtanak, Indonesia. Web address: http:// www.agnet.org/library/eb/521/ dated 09 Feb. 2011.
- Lowei, B. (1986). Effect of Bowl Sludge on growth of two pasture legumes, *Brachia mutica* and *Axonopus compressus* Malaysia. J. Agri. 25:19-31.
- Majanbu, 1S; Oginlela, VB; Abmed, MR; Olarawaju, JD. (1985). Response of two okra (*Abelmoschus esculentus* L.. Moecnh) verities to fertilizers: yield and yield components as influenced by Nitrogen and phosphorus application. *Fertilizer Res.* 6(3):257-267.
- Malakootian, M.; Nouri, J.; Hossaini, H., (2009). Removal of heavy metals from paint industries wastewater using Leca as an available adsorbent. *Int. J. Environ. Sci. Tech.*, 6 (2), 183-190.

- Martin, AM. (1991). Biological degradation of wastes, Great Yarmouth, GB, Elsevier Science publishers Ltd.
- Mayer, BS; Anderson, DB; Bohning, RH and Friatiana DG. (1973). *Introduction to Plant Physiology* 2<sup>nd</sup> edition, D. Van Nostrand Company New York. pp. 431–463.
- Neha, S; Sreemoyee, C; Pradeep, B. (2013). An evaluation of physicochemical properties to assess quality of treated effluents from Jaipurdairy, *Int. J. Chem. Environ. Pharm. Res.* 4(2–3) 54–58.
- Ogunwenmo, KO; Oyelana, OA; Ibidunmoye, O; Anyaso, G and Ogunnowo, AA. 2010. Effects of brewery, textile and paint effluent on seed germination of leafy vegetables-Amaranthus hybridus and Celosia argentea (Amaranthaceae). J. Bio. Sci. 10:151-156.
- Okafor, JO. and Egwim E. (2004). Effect of Chemically and Physically treated Adsorbents on wastewater from Garri Processing Industry, 27th Annual International Conference of Chemical Society of Nigeria, Benin City, Nigeria. 21st to 22nd August.
- Okunade, DA; Olanusi, OA; Adekalu, KO. (2009). Growth, yield and economics of okra and amaranth production under irrigation. *Inter. J. Veg. Sci.* 15: 29-44.
- Omotosho, SO; shihu, OS. (2008). Soil properties, leaf nutrient composition and yield of okra (*Abelmoschus esculentus* (L.) moench J as affected by broiler litter and NPK 15: 15: 15 fertilizers in Ekiti state, Nigeria. *Inter. J. Agri.* 3: 140-147.
- Pandey, DK; Soni, P. and Dhiman RC. (1994). Utilization Potential of Distillery effluent *VOSANA* 38:12 – 13.
- Rajni, A. and Chauchan, SVS. (1996). Effect of tannery effluent on seed germination and total biomass in some varieties of *Hordeum vulgare* L. *Acta Ecology*, 18: 112-115.

- Salami, SJ. and Egwin, IN. (1997). Impact of Tannery Effluents on the Quality of Receiving Stream, *Afr. J. Nat. Sci.* 2(1) pp 17-20..
- Sam, SM.; Esenowo, GJ. Udosen, IR. (2017). Biochemical characterization of cassava processing waste water and its effect on the growth of maize seedlings. *Nig. J. Basic. Appl. Sci.* 25(2): 12-20
- Selleck, GW; Opena, RT. (1985) National programs: the need for increased emphasis on the development of vegetables and legumes. In: National agricultural programs and future needs. Petersen, J.B., and Macgregor, P.W. (eds). FFTC books series No. 30. Food and fertilizer technology centre, Taipei, Taiwan, p.184.
- Sokal, RR and Rohlf, FJ. (1995) *Biometry*. New York: Colt Freeman and Company, p. 887
- Vijayarengan, P; Lakshmanachary, AS. (1993). Effect of textile Mill Effluent on Growth and Development of Green gram Seedlings. Adv. Plant Sci. 6(2): 359- 365.
- World Health Organization (WH0). (2003). Guidelines for safe recreational water environments, Volume1: Coastal and Freshwaters, vol.1, Geneva, Swit-zerland.
- World Health Organization (WHO). (2004). Guidelines for drinking water quality. Health criteria and other supporting information. 3<sup>rd</sup> world health organization, Geneva.
- World Health Organization (WH0). (2006). Guidelines for the safe use of wastewater, excreta and greywa-ter, in: Excreta and Greywater Use in Agriculture, second edition, Geneva, Switzerland.