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An Integrated Education Intervention Improves the Feeding Frequency of Infants and Young Children in the Upper Manya Krobo District of Ghana

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ABSTRACT

Childhood malnutrition is partly due to inappropriate complementary feeding (CF) practices. The effect of two education interventions on young child feeding practices was evaluated in the Upper Manya Krobo district of Ghana. A total of 32 child growth monitoring centers were randomly assigned to either of two intervention groups [integrated nutrition and agriculture education (IE) or nutrition education (NE)], or the control (CT) group. Over six months, mothers of children 6-24 months old in both intervention groups received monthly nutrition education delivered by community health volunteers; additionally, mothers in the IE group received agricultural education from extension agents. Information on complementary feeding practices and dietary intakes of study children were collected at baseline and at three-month intervals for nine months. Intention-to-treat analysis was complemented by a sub-sample analysis to determine the effect of attendance to education sessions on outcomes. At the end of the study, children in the IE were twice as likely to meet the minimum meal frequency compared to the CT children (aOR = 2.62; 95% CI; 1.11, 6.16), but energy, calcium, and iron intakes from complementary foods did not differ between the three groups. Additionally, children of mothers who attended at least one nutrition education session in the IE group tended to receive the minimum acceptable diet (aOR = 2.30, 95% CI; 0.98, 5.39, p = 0.055) compared to children in the CT group at the end of the study. Compliance to the intervention was low, with almost half of intervention mothers (45%) never attending an education session. A combined agriculture and nutrition education led to improved meal frequency among young children in the Upper Manya Krobo district of Ghana. Thus, this is one of the strategies that can be used to address sub-optimal complementary feeding practices in rural areas, where farming is a major source of employment.

Keywords: Complementary Feeding Practices, Nutrition Education, Community Health Volunteers, Ghana

Introduction

The prevalence of childhood malnutrition is high in Ghana, and is partly attributed to inappropriate complementary feeding (CF) practices such as feeding of low diverse meals, and inadequate nutrient intakes among young children (Ghana Statistical Service *et al.*, 2015; Nti & Lartey, 2007). The 2014 Demographic and Health survey indicated that based on a summary indicator using the consumption of breast milk, other milks or milk products, minimum feeding frequency, and minimum diet diversity, only 13% of Ghanaian children aged 6-23 months met the minimum standard of being appropriately fed (Ghana Statistical Service *et al.*, 2015). The above-mentioned report also indicated that the Eastern region recorded the least proportion of children (29.6%) who met the minimum meal frequency. Nutrition education interventions have been used to address sub-optimal practices such as inadequate feeding frequency and malnutrition in some developing countries. In environments where food availability is not a limiting factor, educational interventions have reportedly improved CF practices and young child growth (Dewey & Adu-Afarwuah, 2008; Guldan et al., 2000; Penny et al., 2005), suggesting that poor feeding practices and malnutrition may be the result of caregivers' lack of knowledge of recommended practices or beliefs about certain foods, and not the lack of food. However, in environments where food accessibility is scarce, approaches to reduce childhood malnutrition may need to include also strategies that are aimed at improving caregivers' ability to access foods at the household level. Improving household food production can lead to food availability and consumption by increasing the quantity and quality of food and/or increase income through the sale of agricultural products, thus increasing the ability of household to purchase food. A review of the effectiveness of agriculture interventions in improving nutrition outcomes concluded that interventions that were combined with other strategies such as nutrition education were likely to have positive effects on the nutritional status of young children (Berti, et al., 2004). Thus, in communities where the economy is agriculturalbased, combining strategies that aim at improving both agricultural production and caregivers' nutrition knowledge could lead to improved CF practices and infants and young children's nutrient intakes.

Health care providers have been used extensively in recent studies as channels of nutrition education (Bhandari *et al.*, 2004; Penny *et al.*, 2005; Santos *et al.*, 2001). In most of these studies, health services were easily available to caregivers of young children. Studies that have looked at the effects of educational interventions in areas where accessibility to health care was poor are few, and the researchers generally used specially trained fieldworkers to deliver the intervention (Kilaru *et al.*, 2005; Roy *et al.*, 2005). One consequence of this particular approach is that more financial input is needed to sustain the intervention after the project period. The use of existing health and agricultural services may be a sustainable way of addressing childhood malnutrition, particularly in areas where agriculture forms the basis of the economy.

Studies that explored the use of education to address the feeding of children aged 6 to 24 months are limited in Ghana. In this study, an integrated approach involving nutrition and agricultural education delivered by community health volunteers and agricultural extension agents was used to address sub-optimal CF in rural Ghana. The objective of this study was to determine the effect of the intervention on CF practices and nutrient intakes of children 6-23 months. A second intervention group was included to assess the effects of the integrated education compared to having only nutrition education.

Methods

Study design and participants

The cluster-randomized controlled trial took place in three sub-districts of the Upper Manya Krobo district of Ghana, where 85% of the communities are rural (Nubians Renewal Organization, 2010). The three sub-districts were served by the staff of two Community-based Health Planning and Services (CHPS) compounds and two subdistrict health clinics. Monthly community-based child welfare clinics (CWC) were organised by the community health nurses who were assisted by community health volunteers (CHV). Child welfare clinic centers were included in the study if there was an active CHV. Thirtytwo CWC were stratified by average monthly attendance, and then a simple random sampling method was used to assign them to two intervention groups [integrated education (IE) or nutrition education (NE)] or the control (CT) group. All infants aged 6 to 11 months old with no major birth defects, living in the study area, and attending child monthly welfare clinics were eligible for the study.

Preliminary data analysis of an earlier study in the district showed that iron was the least adequate micronutrient among infants in the area (RIING Project, unpublished results). Therefore, dietary iron intake was used in determining the sample size for this study. Assuming an effect size of 1.2 mg (Hotz & Gibson, 2005) in the mean intake of infants between the intervention and control groups and a standard deviation of 2.4 mg (Santos *et al.*, 2001), 63 mother-infant pairs were needed per group at statistical power of 80% and significance level of 0.05 (Hulley *et al.*, 2007) for two-sided hypothesis. Adjusting for cluster effect by multiplying by 1.5 (Roy *et al.*, 2005) and then accounting for a drop-out rate of 20% for follow up, the sample size needed per group was 120 mother-child pairs, making a total of 360 mother-child pairs.

Procedures

To develop the intervention, formative research was conducted in the study area to assess existing CF practices and challenges to CF education. This included interviewing community health workers and staff of the Ministry of Food and Agriculture (MOFA), focus group discussions involving mothers and grandmothers of young children, and testing potential education messages among target mothers using the trials of improved practices (TIPS) method. Based on the results of the formative research, a nutrition education training manual (Colecraft, 2008) was modified and used by community health volunteers to educate caregivers of children 6 to 24 months of age at the intervention centers. Three agricultural topics were selected in consultation with the local agricultural workers for the agricultural education component of the intervention, and delivered by two agricultural extension agents at the IE centers. Group education sessions took place monthly during CWC sessions and were delivered to all mothers who attended the clinic. Each intervention center received nutrition education for at least six months, and IE centers received additional education sessions in agriculture (six sessions). Three project education evaluators assessed the education sessions and attributed scores for recording attendance, style of teaching (interactive or not), mothers' level of interest and content of lessons. Caregivers in the control group received the standard GHS care. This involved education delivered by the community health nurses on malaria prevention, family planning, and child feeding. These topics were addressed

alternatively, so that caregivers on the average received information on child feeding at three-month intervals.

Each mother-child pair was visited by fieldworkers at baseline, then at three, six, and nine months after recruitment. The primary outcome variables that were measured during the study period were children's nutrient intakes (energy, iron, vitamin A, and calcium) and feeding practices (diet diversity and feeding frequency) using a single 24-hour recall and a food frequency questionnaire. Other forms of data collected using structured questionnaires were child morbidity (maternal recall of symptoms seven days prior to interview), and household characteristics. All questionnaires were translated into the local languages and translations were agreed upon for consistency. Seven local young women with secondary level of education were recruited and trained to administer the questionnaires.

Dietary data were converted to energy and nutrients using a database which was prepared from a combination of nutrient database (RIING Project, unpublished results). Information collected using the food frequency questionnaire was used to develop a dietary diversity score based on the seven food groups used in the WHO indicators for assessing infant and young child feeding practices (WHO, 2008; 2010).

Ethics approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the McGill University Research Ethics Board, the Noguchi Memorial Institute for Medical Research Institutional Review Board, and Ghana Health Service Review Board before the fieldwork began. Informed consent by signature or thumb-print was obtained from all mothers who gave consent also for the participation of their children.

Statistical analyses

The time points reported in the results (baseline, 6 months, 9 months) do not correspond to the age of the study children, but rather reflect the period (months) of being in the study. Analysis was by intention-totreat. Analysis of variance (ANOVA) or Krusal Wallis tests were used to compare continuous variables of the three groups, and the Pearson's chi square test was used for categorical variables (time point analysis). Generalized estimating equations (GEE) were used to investigate changes over time. To determine the effect of the intervention on CF practices and nutrient intakes, STATA software (StataCorp, 2009) was used to conduct multiple linear and logistic regressions, controlling for biological (child's sex and age, maternal age) and socio-demographic characteristics (maternal education, house ownership, type of settlement, possession of basic amenities), baseline values, and intra-cluster correlation.

Results

A total of 367 mother-child pairs, consisting of 123 IE, 122 NE, and 122 CT were recruited at baseline. Of these, 210 participants completed the nine months follow up (Figure 1). The main reasons for loss to follow up were families moving out of the study area, not locating participants at visit time points and ending the study before participants completed nine months. Study children in the three groups did not differ in age, but a greater proportion of the children in the integrated education group were males compared to the control group at baseline (Table 1). Attendance to education sessions was very low among participants, with 45% of mothers never attending an education session. Additionally, attendance to nutrition education sessions in the IE group was higher compared to the NE group (% attendance¹: IE = 70%, NE = 40%; p < 0.0001).

¹ Percentage of mothers who attended at least one nutrition education session.

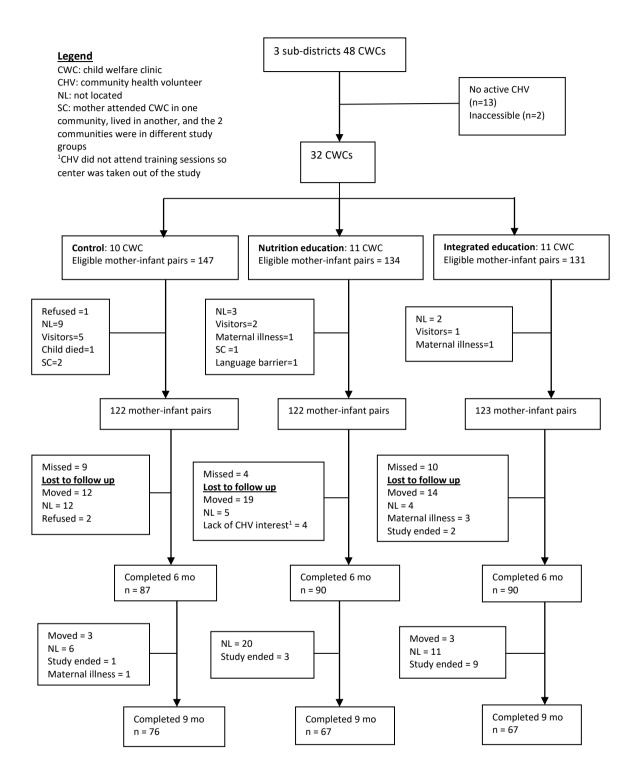


Fig. 1: Flow chart of study participants

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	Treatment ³						p-value			
	Integrated e n = 123	educat	ion	Nutrition = 122	n ed	ucation n	Control n = 122			
Child Sex (% male)	75		(61.0) ^a	64		(52.5) ^{a, b}	55		(45.1) ^b	0.045
Age (months)	7.5	±	1.5	7.5	±	1.6	7.7	±	1.5	0.303
Maternal Age (y)	26.2	±	6.6	27.3	±	7.3	27.0	±	6.4	0.463
Formal education (y)	4.6	±	3.4 ^a	4.8	±	3.6 ^{a,b}	5.7	±	4.1 ^b	0.038
Main economic activity Unemployed Farming Trading Hairdressing/seamstress Other ⁵	9 52 51 9 2		(7.3) (42.3) (41.5) (7.3) (2.4)	13 61 41 7 0		(10.7) (50.0) (33.6) (5.7) (0)	9 50 44 14 5		(7.4) (41.0) (36.1) (11.5) (4.1)	0.175
BMI (kg/m²)	22.1	±	3.6	22.2	±	2.9	22.8	±	3.7	0.308
Household Home ownership	40		(34.2) ^a	50		(41.0) ª	33		(27.1) ^b	<0.0001
Amenities score ⁶ High Low	9 114		(7.3)ª (92.7)	29 93		(23.8) ^b (76.2)	68 54		(55.7) ^c (44.3)	<0.0001

Table 1: Baseline characteristics of study children and their households in the Eastern region of Ghana^{1, 2}

¹Results presented as mean ± standard deviation or n (%). Groups were compared using analysis of variance, Kruskal-Wallis or Pearson's chi square test; ²Mean ± SD or n (%) on the same row with different superscript letters are significantly different, p < 0.017 (pair-wise Mann-Whitney or chi square test with Bonferroni correction); ³Integrated education received monthly nutrition and agricultural education; Nutrition education received monthly nutrition education (120) and integrated education (122); ⁵Includes teachers (n = 3), police (n = 1), banker (n = 1), cook (n = 1) and student (n = 1); ⁶A proxy indicator of household's possession, based on housing materials (wall and roof), source of drinking water, light and type of toilet facility. Scores 0 – 3 = low amenities, 4 – 5 = high amenities.

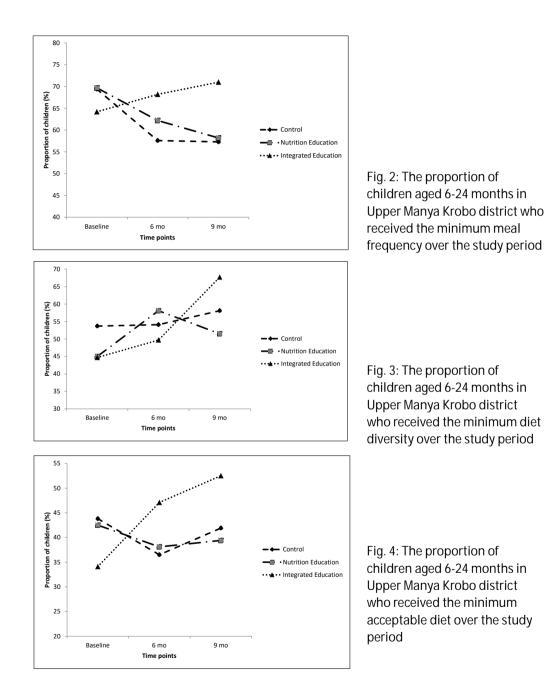
Children in the IE group were four times more likely to consume flesh foods (fish, meat, poultry, sausage) compared to the NE group (aOR = 4.14, 95% CI: 1.35, 12.75) at the end of the study, but the odds of consuming flesh foods did not differ between children of the IE and CT groups (aOR = 1.85, 95% CI: 0.42, 8.21). The complementary feeding practices of the study participants over the study period are shown in Figures 2, 3, and 4. In the bivariate analysis, CF practices did not differ between the interventions and control groups. After controlling for amenities, demographic factors, and cluster effect, IE children were twice as likely to have met the minimum meal frequency² compared to the CT children (aOR = 2.62; 95% CI; 1.11, 6.16) at the end of the study. The diversity of the children's diet increased over the study period (effect of time: p = 0.006). At nine months, IE and CT children were about six (aOR = 6.46, 95% CI: 1.71, 23.39) and three (aOR = 3.19; 95% CI; 1.05, 9.71) times respectively, more likely to have received foods from at least four food groups the previous seven days compared to children in the NE group. However, the likelihood of meeting the minimum diet diversity did not differ between the IE and CT groups (aOR = 2.02, 95% CI: 0.40, 10.16). Less than half of the children (40%) received minimum acceptable diet³ at baseline. The proportion of IE children who received minimum acceptable diet

² This refers to the proportion of children 6-23 months of age who received solids, semi-solids or soft foods the minimum number of times or more during the previous day.

³ This is the proportion of children 6-23 month of age who had at least minimum diet diversity and minimum meal frequency the previous day.

increased over the study period (time*group effect: p = 0.034) compared to NE and CT children. Additionally, children of mothers who attended at least one nutrition education session in the IE group tended to receive the

minimum acceptable diet (aOR = 2.30, 95% CI; 0.98, 5.39, p = 0.055) compared to children in the CT group at the end of the study.



	Treatment ²				p-value ³		
	Control		Nutrition	education	Integrate	d education	
Mean intakes							
Energy	679	± 39	650	± 40	691	± 50	0.794
Calcium	374	± 29	372	± 40	440	± 36	0.305
Iron	9.1	± 0.5	8.4	± 0.6	9.0	± 0.7	0.696
Proportion meeting recommendations ¹							
Energy	45	(59.2)	35	(53.8)	33	(53.2)	0.732
Calcium	57	(75.0)	41	(63.1)	48	(77.4)	0.149
Iron	59	(77.6)	40	(61.5)	43	(69.4)	0.115

Table 2: Energy and nutrient intakes of children aged 6-24 months in the Upper Manya Krobo district of Ghana at the end of study¹

¹Intakes are based on a single 24-hour dietary recall and intakes were compared to WHO recommended energy and nutrient intakes form complementary foods, assuming average breast milk intake Results are presented as mean ± standard error of mean or n (%); ²Integrated education centers received monthly nutrition and agricultural education; nutrition education centers received monthly nutrition and agricultural education; nutrition education centers received standard care by Ghana Health Service; ³Based on analysis of variance (ANOVA) test or Pearson's Chi Square test

Energy, calcium, and iron intakes from complementary foods were similar in the three groups at the end of the study period (Table 2). The proportion of children who met age-specific recommended energy intake from complementary foods decreased over the study period (time effect: p < 0.0001), while proportion of those who met iron and calcium increased over the same period.

Discussion

In this study, an educational approach that involved two existing government agencies (GHS and MOFA) was used to address complementary feeding in rural areas. Delivering the intervention through existing services was to ensure that the program could be sustained after the completion of the study at a minimum cost. The study thus increased the use of community health volunteers, who were already involved in infants and young child growth monitoring activities in the district.

The combined agriculture and nutrition education intervention led to slight improvement in some recommended feeding practices. Studies that have looked at the effect of educational interventions on WHO's recently developed infant and young child feeding indicators (WHO, 2008) are scarce. One non-randomised study in India that used locally trained counsellors to deliver monthly nutrition education to caregivers reported improved diet diversity (received at least 5 food groups: 42% vs 19%; p = 0.01) among intervention infants aged 11 months (Kilaru et al., 2005). A higher proportion of intervention infants 7-11 months old were fed at least four times daily (78% vs 51%; p < 0.001), similar to findings of the current study. Our findings are also similar to the results of the Credit with Education program developed by Freedom from Hunger intervention (MkNelly & Dunford, 1998). An evaluation of that program in the Western region of Ghana reported significant improvement of feeding frequency of the children from baseline among participants compared to controls (change 0.8 vs - 0.1, p = 0.03). The program however, required participants to be engaged in non-farming income generating activities, and thus did not include a large percentage of rural caregivers, whose only economic activity was farming. The present study collected information and examined feeding frequency and diet diversity as described by WHO (WHO, 2010), and therefore provides information on the effect of an education intervention on infant and young child feeding practices, as defined by the new international indicators. Similarly, an integrated agriculture and nutrition

and health behaviour change communication intervention marginally increased the number of children (6-12 mo) who received foods from at least four food groups (minimum diet diversity) in Burkina Faso (Olney *et al.*, 2015), as well as decrease the prevalence of anemia among younger children. Our intervention however, did not affect energy and nutrient intakes of young children. This lack of effect on nutrient intakes are inconsistent with results of other nutrition education interventions that were implemented through existing health care systems in Brazil, India, Pakistan, and Peru (Bhandari *et al.*, 2004; Penny *et al.*, 2005; Santos *et al.*, 2001; Zaman *et al.*, 2008).

The lack of effect observed in the present study may be due to various factors including the relatively short duration and issues that affected the implementation of the intervention. Mothers in the intervention groups received education monthly instead of the originally planned bi-weekly schedule. Additionally, attendance rate was low among mothers and the intervention did not occur for almost half of the participants. As such, exposure to the intervention was lower than expected. Frequent exposure of caregivers of young children to nutrition education has the potential to improve feeding practices and subsequently, the energy and nutrient status of young children. In Indonesia, caregivers of mildly wasted children who received weekly nutrition education on infant and young child feeding showed significant improvement in knowledge and practices, compared to those who received monthly education on the same topics (Inayati et al., 2012).

Exposure to the intervention in the present study was a major challenge. The low level of attendance to the education sessions was a reflection of low attendance to child welfare clinics in the district. The number of children seen within the first half of 2011 decreased to 4790 compared to 6084 during the same period in the previous year (Upper Manya Krobo District Health Directorate, 2011). There has been no study to investigate the possible causes of this decline in coverage. However, a decrease in health volunteer activities, which included home visits during which mothers were reminded of

the next CWC, could have been a contributing factor. The low level of commitment among CHV could be attributed, at least in part, to a general dissatisfaction as a result of lack of remuneration from the GHS⁴. At the time of the present study, CHV worked strictly on volunteerism basis. A nutrition education intervention in India that had the Anganwadi workers as educators, was successful in improving energy intakes (difference = 1230 kJ; p <0.001) (Bhandari et al., 2004). The Anganwadi workers are not professional health workers, but form part of the Integrated Child development Scheme of India and receive monthly remunerations (Ishrath, 2011). In addition, their activities are regularly monitored by a supervisor, who then reports to a Child Development Project officer. Having a system which provides regular remuneration to the volunteers may help enhance their willingness to participate in activities that aim at improving young child well-being and growth in their communities.

One factor that contributed to the lack of mothers' commitment to the intervention was a limited involvement of the target audience at the design phase of the intervention. Participants' commitment to change is essential in the success of nutrition education programs that aim to change behaviour (Kayman, 1989; Olson & Kelly, 1989), and this needs to be recognized during the development phase. This was partly explored during the TIPS phase, when mothers' willingness to perform potential feeding recommendations was tested. However, the formative research did not explore caregivers' willingness to receive frequent education on child feeding in their communities, including how much of their time they were ready to commit to it. The consideration of these factors during the formative phase would have led to the identification of potential problems which could have been resolved before the implementation stage.

The results indicated that the integrated nutrition and agriculture education improved feeding practices, but not nutrition education alone. This significant result was observed in both the intention-to-treat and sub-sample

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⁴Comments from volunteers to the doctoral student during the training workshops of the study.

analyses (data not shown). The lack of improvement in the nutrition education group could partly be due to the low attendance to education sessions among mothers, and reinforcement of nutrition messages by agricultural extension agents in the integrated group. Similarly, a study in China that used multiple methods such as group discussions, food demonstrations, and home visits to reinforce messages reported improved feeding frequency among intervention children compared to control children at 12 months (4.2 vs 2.9; p < 0.001) (Shi et al., 2010). Using innovative ways of bringing caregivers together which then serve as a platform for nutrition education may improve participants' interest in education interventions. In the present study, the agricultural education sessions were used to discuss topics to which the mothers could easily relate. These sessions might have provided a forum which was of interest to women in the area, serving as motivation for mothers of young children to attend CWC, where they were also exposed to nutrition education. Nutritionists and health workers need to find platforms that interest caregivers to expose caregivers to appropriate nutrition education.

Lack of information on some potential sources of bias such as geographical location of CWC, better ways of assessing compliance, and effect of educators on study outcomes are limitations acknowledged by the researchers of the present study. Information on the above-mentioned factors might have provided a clearer understanding of the results. Additionally, an in-depth qualitative study to investigate the implementation process of the present study is needed to provide better insight into the low rate of participation in the intervention.

Conclusion

The intervention resulted in improvement in feeding practices but failed to improve the nutrient intakes of young children. Implementation of the intervention was hampered and this may possibly be due to inadequate involvement of participants during the planning stage. A combined agriculture and nutrition education can be applied as one of the means of improving complementary feeding practices in the rural areas, where farming is 11

a major source of employment. However, using the community health volunteer system, as it currently operates, may not be an effective way to address infant and young child malnutrition in rural Ghana.

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Effects of different pesticide management options on the population dynamics of aphids, *Lipaphis erysimi pseudobrassicae* (Davis) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), their natural enemies and the yield of cabbage

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ABSTRACT

Cabbage is a popular vegetable grown in Ghana and it serves as an important source of livelihood for smallscale farmers. Aphids are major pests of cabbage in the field and as such, farmers have resorted to the indiscriminate use of insecticides which have had adverse environmental and health implications. The current study sought to investigate the effect of commonly used management options on the population of aphids, Lipaphis erysimi pseudobrassicae (Davis) and Myzus persicae (Sulzer) (Hemiptera: Aphididae), their natural enemies and the yield of cabbage. Cabbage seedlings were planted during the major and minor seasons of 2015 in 3 x 3 m plots and the treatments used included Chlorpyrifos, Lambda-cyhalothrin, hot pepper, Capsicum futescens fruit extract, neem, Azadirachta indica seed extract, solution of local soap (alata samina) with water as a control. Ten cabbage leaves per treated plot were randomly sampled weekly into 70% alcohol to obtain actual counts of aphids and their natural enemies. The least number of aphids was recorded in the neem treated plots, while Lambda-cyhalothrin treated plots recorded the highest number of aphids. The control and biopesticide treated plots recorded the highest numbers of the natural enemies (hoverflies, ladybirds and spiders). The highest yield and marketability was recorded in the neem treated plots for both seasons. The yield and marketability of cabbages obtained from plots sprayed with alata samina and pepper was also higher than that obtained from the control, Lambdacyhalothrin and Chlorpyrifos treated plots, with the insecticide treated plots recording the least number of marketable heads. It offered the most promising solution. The current findings suggest that the crude neem seed extract, and to some extent local soap, alata samina, and pepper, are effective and safe options for managing aphids on cabbage.

Keywords: Pesticides, Mustard aphid, Green peach aphid, Neem, Natural enemies

Introduction

Cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae) is a popular and very important vegetable grown in Ghana. It is a cold temperate crop that also thrives in other climates throughout the world (Nieuwhof, 1969; Dickson and Wallace 1986; Mochiah et al., 2011a). It has been identified by the Food and Agriculture Organization (FAO) as one of the top twenty vegetables and an important food source globally (FAO, 1998). Cultivation of this crop serves as a source of employment for most of the rural and peri-urban population in Ghana (Abbey and Manso, 2004). Cabbage is usually consumed raw or cooked in stews. This cruciferous vegetable provides important nutrients such as vitamins, dietary fibre, iron, potassium, manganese and magnesium for healthy body development (USDA, 2009). Despite these benefits, cabbage has been ravaged by several insect pest species. Among them, the aphids: Brevicoryne brassicae L., Lipaphis erysimi erysimi Kalt., Lipaphis erysimi pseudobrassicae (Davis) and Myzus persicae (Sulzer) (Hemiptera: Aphididae) cause devastating losses in the production of cabbage worldwide (Müller, 1986; Ronquist and Ahman, 1990; Mc Cullum et al., 1992; Sæthre et al., 2011; Fening et al., 2013; Vidogbéna et al., 2015). All the stages of these insects feed on the phloem, and their feeding results in stunted plants with little or no yield (Hughes, 1963; Mochiah et al., 2011b). They cause indirect damage by transmitting viral diseases to the cabbage plant during feeding (Blackman and Eastop, 1984; Flint, 1991). Also, the excretion of honeydew supports the growth of sooty mould which decreases yield and marketability of cabbage heads (Blackman and Eastop, 1984; Amoabeng et al., 2013).

Fortunately, these pests are attacked by several natural enemies, including the ladybird beetle *Cheilomenes* spp., *Coccinella* spp., (Coleoptera: Coccinellidae), the hoverflies *Paragus borbonicus* (Diptera: Syrphidae), spiders (Araneae) and the braconid parasitoid *Diaeretiella rapae* (Stary) (Hymenoptera: Braconidae) (Fening *et al.*, 2011, 2014; Liu *et al.*, 2014). These natural enemies can reduce the rate of population increase of the aphids or even wipe out infestations (Mandal and Patnaik, 2008;

Liu et al., 2014). Despite its effectiveness, this natural control is often considered inadequate, and this situation, combined with the growing craving for damage-free cabbages (vegetables), makes farmers often overuse insecticides to meet this demand (Obeng-Ofori et al., 2002; Ntow et al., 2006; Osei et al., 2013). However, several authors have attested that these chemicals often adversely affect the health of people, the environment and especially the natural enemies, which serve as a natural control measure for these pests (Obeng-Ofori et al., 2002; Ntow et al., 2006; Fernandes et al., 2010; Fening et al., 2011, 2014). It is therefore necessary to seek alternative, sustainable methods of managing cabbage pests. This study sought to assess the effect of some management options on aphids, their natural enemies and the yield of cabbage, in order to provide sustainable options for managing these insect pests.

Materials and method

Study site

The study was carried out at the University of Ghana Soil and Irrigation Research Centre, Kpong (00 04' E, 60 09' N), located within the lower Volta basin of the Coastal Savannah agro-ecological zone of Ghana. The study hsite has an annual rainfall of between 700 and 1100 mm, an average annual temperature of 28 °C and relative humidity of between 59%-93%. The main soil type is the vertisols (black clay soil). The experiments were conducted from May to August and September to December, 2015 in the major and minor seasons, respectively.

Land preparation, transplanting and application of treatments

The land used was cleared of weeds, ploughed, harrowed and ridged. Seeds of certified healthy hybrid white cabbage (*B. oleracea* var. *capitata*) (*cv. oxylus*) obtained from AGRIMAT Ltd in Accra were sown on raised beds in the field. The young seedlings were protected from pest attack with mosquito-proof netting (1.2 mm \times 1. 2 mm of mesh size). Appropriate agronomic and cultural practices such as weed control and watering were employed regularly throughout the growing period. The experimental design was a randomized complete block with six treatments and three replicates. Cabbage seedlings were transplanted 30 days after germination. Spacing was $0.5 \ge 0.75$ m and each plot measured 3 = 3 = 3 m, giving a total of 30 plants per plot. Inter-plot and inter-block distance was 2 = 10 to prevent drift between adjacent plots and blocks. NPK 15-15-15 (5g/plant) and Sulphate of Ammonia (3g/plant) were applied in a ring form around each plant 10 and 42 days respectively after transplanting.

Treatments used were neem Azadirachta indica seed extract (50g/L of water), hot pepper Capsicum frutescens fruit extract (20g/L of water), local soap (alata samina) solution (7g/L of water), two commonly used synthetic insecticides, Conpyrifos* (Chlorpyrifos 480g/L, applied at 2ml/L of water), Lambda M^{*} (Lambda-cyhalothrin, 25g/L, applied at 2ml/L of water) and tap water as control. For the hot pepper, ripe fruits were obtained from a local market and the required weight obtained using an electronic balance. The weighed fruits were homogenised using an electric blender. The required amounts of dry neem seeds were also weighed and pounded in a wooden mortar using a wooden pestle. The homogenate was mixed separately with 1L tap water, and a few drops of liquid soap (Madar Renzo^{*}) and vegetable oil were added to enhance its delivery and stickiness onto the leaf surfaces of the cabbage plant. The resultant mixture was left overnight, and later sieved through a fine linen material. The mixtures were further diluted with the required volume of water for spraying. The required amount of alata samina was also weighed and dissolved in the required volume of water. Treatments were applied using a 15L capacity knapsack sprayer on the top and the underside of leaves. Applications commenced 14 days after transplanting the seedlings for both the major and minor seasons and continued weekly thereafter until cabbage heads were fully mature, about 14 days before harvesting. There were seven applications for each season.

Sampling for aphids was modified from the method according to Hughes (1963). Ten leaves per plot were randomly sampled into 70% alcohol to obtain actual counts of aphids. Natural enemies were also separated and counted. In addition, weekly field observations were made to determine the number of natural enemies per plot. For yield assessment, a total of 12 cabbage heads in the three inner rows (4 cabbages per row) was harvested and weighed, and the mean for each treatment calculated. Mean yields were extrapolated into tonnes/ hectare. The cabbage head damage was assessed by using a standard scoring scale of 0-5 (Aboagye, 1996): zero (0) = no head damage, 1= 1-15% head damage, 2= 15-30% head damage, 3=30- 45% head damage, 4= 45-60% head damage, 5= 60-100% head damage, and categorised into marketable and unmarketable heads.

Identification of pests and their natural enemies

The aphids and their natural enemies collected in this study were identified using reference specimens at the Insect Museum of the Department of Animal Biology and Conservation Science, University of Ghana, Accra. Samples of the immature stages of coccinellids and syrphids were cultured in the laboratory to the adult life stage to allow identification by comparison with labelled specimens in the insect museum. Voucher specimens of all the insect species collected were also deposited in the insect museum. The aphid species were identified using taxonomic keys by Blackman and Eastop (1984).

Data analysis

Count data for aphids and natural enemies were square root transformed and subjected to repeated measures of ANOVA. Mean cabbage head weight was analysed using ANOVA. Significant differences in means were separated using SNK test ($P \le 0.05$). All analyses were carried out in GENSTAT V12.

Results

Generally, the neem was very effective in controlling the aphids *Lipaphis e. pseudobrassicae* and *M. persicae* for the two seasons, even more than the synthetic insecticides (Figs. 1-4). *Lipaphis e. pseudobrassicae* infestation started in the first sampling week for the two seasons and increased progressively throughout the sampling period. The population of *Lipaphis e. pseudobrassicae* was at its peak in the third and fourth week for the major season and the fifth week for the minor season of 2015 (Figs. 1 and 2). Lambda-cyhalothrin treated plots recorded the highest number of *Lipaphis e. pseudobrassicae* for both the major and minor seasons. Infestations in the botanical treated and Chlorpyrifos treated plots were minimal. Neem however recorded the least number of *Lipaphis e.*

pseudobrassicae for both seasons. There was a significant difference in the effect of the different treatments on *Lipaphis e. pseudobrassicae* for both seasons ($F_{5,125} = 3.69$; P = 0.0380, and $F_{5,125} = 5.58$; P = 0.010, respectively). There was also a significant difference in the effect of each treatment on the population of *Lipaphis e. pseudobrassicae* among the weeks of sampling for both seasons ($F_{6,125} = 21.54$; P < 0.001 and $F_{6,125} = 9.82$; P = < 0.0010). However, the interaction between the spray formulations and weeks of sampling of *Lipaphis e. pseudobrassicae* was not significant for the major season ($F_{30,125} = 1.75$; P = 0.1340) but significant for the minor season ($F_{30,125} = 3.03$; P = 0.008).

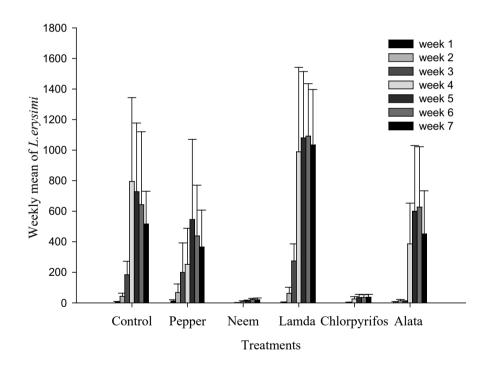


Fig. 1: Effects of treatments on mean (± SE) weekly count of Lipaphis e. pseudobrassicae per cabbage plant during the major season, 2015 in Kpong, Ghana.

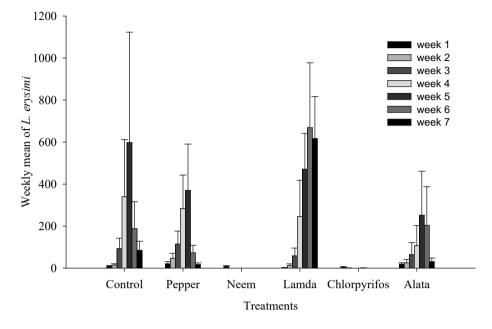


Fig. 2: Effects of treatments on mean (± SE) weekly count of Lipaphis e. pseudobrassicae per cabbage plant during the minor season, 2015 in Kpong, Ghana.

Myzus persicae infestation started in the first week of sampling, depending on the treatment for the major season, and in the fourth week of the minor season, and increased progressively throughout the sampling period of 2015 (Figs. 3 and 4). Infestation by this pest was generally low throughout the sampling period for both seasons. Plots sprayed with botanical insecticides and water had very minimal infestation for the major season compared to the synthetic insecticides. In the minor season, infestation was minimal in the botanical treated and Chlorpyrifos treated plots. Highest *M. persicae* numbers were recorded in the Lambda-cyhalothrin treated plots for the major season and the control plots

for the minor season. However, neem treated plots recorded no *M. persicae* for both seasons. There was no significant difference in the *M. persicae* population among the treatments for both seasons ($F_{5,125} = 1.67$; P = 0.2290 and $F_{5,125} = 0.71$; P = 0.6310). The effect of treatments on the *M. persicae* population among the sampling weeks was significant for the major season ($F_{6,125} = 12.55$; P = 0.0030) but not for the minor season ($F_{6,125} = 2.10$; P = 0.170). However, the interaction between the treatments and the sampling time for the *M. persicae* population was not significant for both seasons ($F_{30,125} = 1.54$; P = 0.2400 and $F_{30,125} = 0.79$; P = 0.5850).

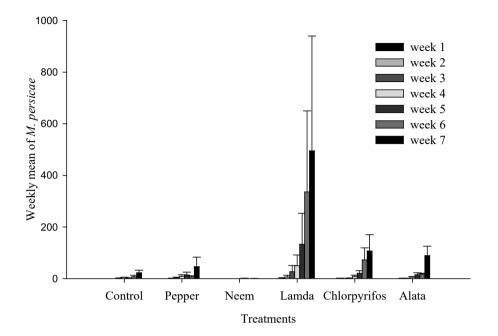


Fig. 3: Effects of treatments on mean (±SE) weekly count of *M. persicae* per cabbage plant during the major season, 2015 in Kpong, Ghana

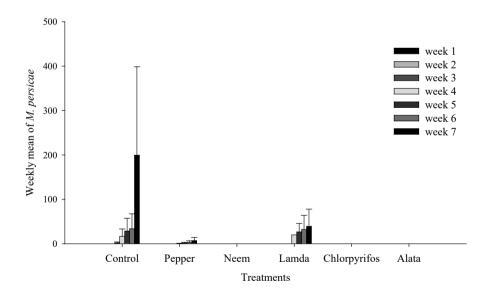


Fig. 4: Effects of treatments on mean (±SE) weekly count of *M. persicae* per cabbage plant during the minor season, 2015 in Kpong, Ghana.

The incidence of hoverfly, *P. borbonicus* was recorded in the first week of sampling and increased progressively until it reached its peak population in the fifth week of sampling for the control plots for both seasons during 2015 (Figs. 5 and 6). Plots sprayed with neem, Lambdacyhalothrin and Chlorpyrifos had the least number of hoverflies for both seasons. Control plots recorded the highest number of hoverflies, followed by the pepper and *alata samina* treated plots. The number of hoverflies was significant among the treatments for both seasons ($F_{5,125}$ = 5.23; *P* = 0.0130 and *F*_{5,125} = 4.17; *P* = 0.0260). There was also a significant difference in the effect of treatments on the hoverfly population in the different weeks of sampling for the major season ($F_{6,125} = 13.00; P < 0.0010$) but no significant difference during the minor season ($F_{6,125} = 2.63; P = 0.0920$). The interaction between the treatments and sampling time was significant for the major season ($F_{30,125} = 2.30; P = 0.0260$) but not for the minor season ($F_{30,125} = 1.82; P = 0.1090$).

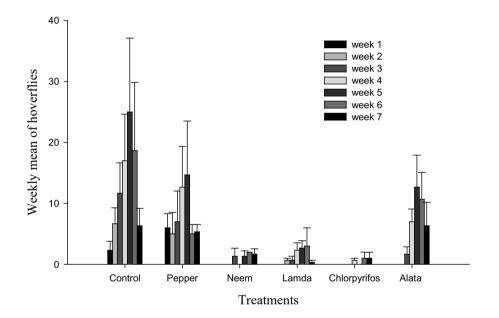


Fig. 5: Effects of treatments on mean (± SE) weekly count of the hoverfly, P. borbonicus, per cabbage plant during the major season, 2015 in Kpong, Ghana.

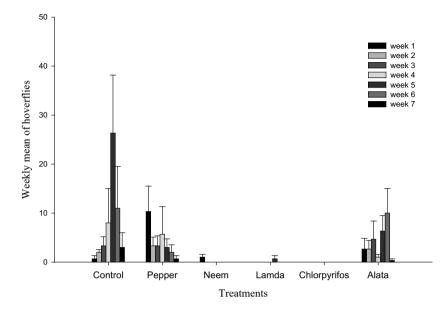


Fig. 6: Effects of treatments on mean (± SE) weekly count of the hoverfly, P. borbonicus, per cabbage plant during the minor season, 2015 in Kpong, Ghana.

The occurrence of ladybird, *Cheilomenes* spp. started in the first week of sampling and reached its peak population in the fifth and fourth week of sampling for the major and minor seasons of 2015 respectively (Figs. 7 and 8). Control plots, pepper-treated plots and *alata samina* treated plots recorded the highest number of ladybirds for both seasons. The neem, Lamda-cyhalothrin and Chlorpyrifos treated plots recorded the least number of ladybirds for both seasons. There were significant differences in ladybird abundance among the treatments for the major season ($F_{5,125} = 3.92$; P = 0.0310), but no

significant difference in the minor season ($F_{5,125} = 1.82$; P = 0.1960). There was also a significant difference in the effect of each treatment on the ladybird population among the weeks of sampling for the major season ($F_{6,125} = 12.87$; P < 0.0010) but no significant difference in the minor season ($F_{6,125} = 1.47$; P = 0.2520). The interaction between the treatments and sampling time was not significant for both seasons ($F_{30,125} = 1.69$; P = 0.1530 and $F_{30,125} = 0.69$; P = 0.7060).

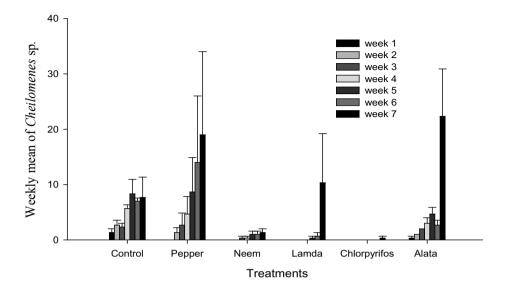


Fig. 7: Effects of treatments on mean (± *SE*) weekly count of the ladybird beetle, *Cheilomenes* spp., per cabbage plant during the major season, 2015 in Kpong, Ghana.

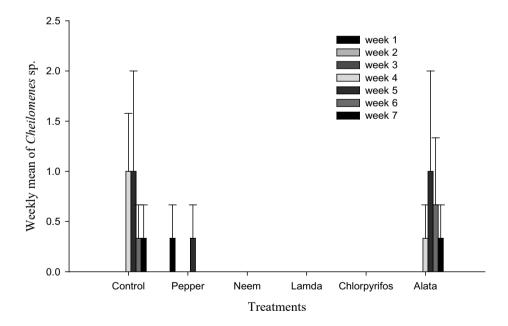


Fig. 8: Effects of treatments on mean (± *SE*) weekly count of the ladybird beetle, *Cheilomenes* spp. per cabbage plant during the minor season, 2015 in Kpong, Ghana.

The occurrence of spiders started in the first week of sampling and continued increasing until its peak in the fifth week of sampling for both seasons (Figs. 9 and 10). Control plots recorded the highest number of spiders, followed by the botanical treated plots for both seasons. Plots sprayed with synthetic insecticides recorded the lowest number of spiders for both seasons. There was a significant difference among the different treatments in controlling the spiders for both seasons ($F_{5,125} = 21.27$; P < 0.0010 and $F_{5,125} = 61.95$; P < 0.0010). The effect of each treatment on the spider population among the weeks of sampling for both seasons was also significantly different ($F_{6,125} = 12.30$; P < 0.0010 and $F_{6,125} = 55.91$; P < 0.0010). However, the interaction between the sampling weeks and treatment was not significant for the

major season ($F_{30,125}$ =1.68; P = 0.0670), but significant for the minor season ($F_{30,125}$ = 4.40; P = 0.0010).

Populations of the cabbage aphid parasitoid, *D. rapae* were very low throughout the sampling period for the major season. Most treatments recorded no *Diaeretiella rapae* for the entire season. Only control plots and pepper treated plots recorded *D. rapae*. The different treatments showed no significant difference for the *D. rapae* populations ($F_{5,125} = 1.82$, P = 0.1960). The effect of the treatments on the weekly sampling of *D. rapae* was not significant for the major season ($F_{6,125} = 1.70$, P = 0.2070). The interaction between the sampling weeks and treatments on *D. rapae* numbers was also not significant ($F_{30,125} = 1.26$, P = 0.3140). *D. rapae* was absent in the minor season.

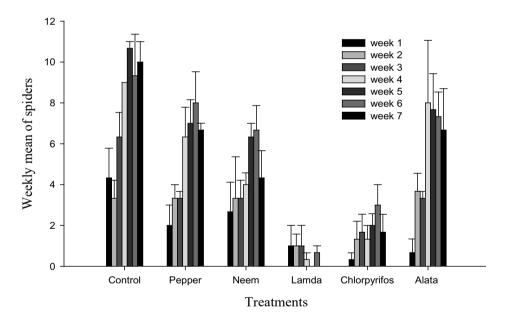


Fig. 9: Effects of treatments on mean (± SE) weekly count of the spiders (Araneae) per cabbage plant during the minor season, 2015 in Kpong, Ghana.

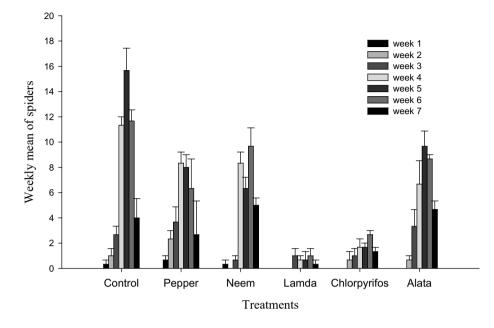


Fig. 10: Effects of treatments on mean (± SE) weekly count of the spiders (Araneae) per cabbage plant during the minor season, 2015 in Kpong, Ghana.

The mean yield among the treatments was significantly different for the major and minor seasons in 2015. The neem treated plots had significantly higher yields than the Lambda-cyhalothrin treated plots, but it was not significantly different from the rest of the treatments during the major season (Table 1). However, during the minor season, the highest yield was obtained in the neem sprayed plots, followed by the *alata samina* treated plots. Damage on cabbage heads was highest in the synthetic insecticide treated plots (damage 5 category). Cabbage heads from botanically treated plots were lower (damage

0-2 categories). Within the different treatments, the neem recorded the highest number of marketable heads for both seasons (Figs. 11 and 12). Lambda-cyhalothrin, however, did not record any marketable head for the major season. For the minor season, Chlorpyrifos, control and Lambda-cyhalothrin did not record any marketable head. The yield of cabbage during the major season was significantly higher than that of the minor one for the control and pepper treated plots. However, the yield of the neem treated plot was significantly higher during the minor than the major season.

Treatment	Mean yie	eld (tonnes/hectare)	t-value	Р	
	Major season	Minor season			
Water (Control)	12.37 ±1.27ab	0.59 ± 0.07c	8.94	0.0120*	
Pepper	12.37 ± 1.30ab	6.50 ± 0.74c	3.93	0.0170*	
Neem	17.80 ± 2.61a	28.36 ± 0.97a	4.08	0.0150*	
_ambda-cyhalothrin	6.03 ± 2.08b	5.07 ± 2.81c	0.27	0.7970	
Alata samina	14.57 ± 2.75ab	15.05 ± 3.05b	0.12	0.9120	
Chlorpyrifos	13.40 ± 1.99ab	10.02 ± 3.70bc	0.80	0.4660	
F	3.47	22.14			
Þ	0.0359*	< 0.0010*			

Table 1: Mean (± *SE*) yield of cabbage under different treatments during the major and minor seasons of 2015, Kpong, Ghana.

Means with the same letter(s) are not significantly different within (P < 0.05, SNK test) within columns. Means between the same row, for both seasons, were compared using *t* test (P < 0.05).

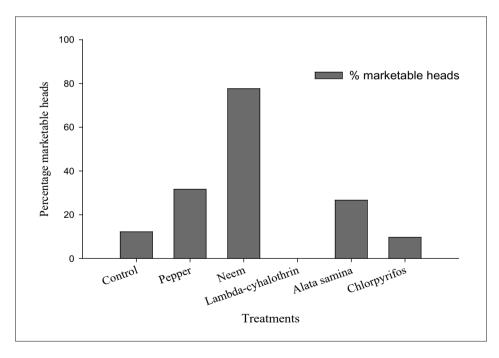


Fig. 11: Mean percentage marketable heads for different treatments for the major season of 2015, Kpong, Ghana.

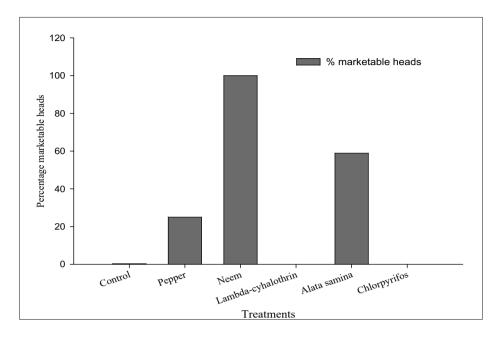


Fig. 12: Mean percentage marketable heads for different treatments for the minor season of 2015, Kpong, Ghana.

Discussion

The main species of aphids identified during the study were Lipaphis erysimi pseudobrassicae and Myzus persicae. The two species and have been observed to be the main aphids occurring on crucifers in the Hangzhou suburbs in China (Liu et al., 1997). Lipaphis erysimi is known to be the specialist aphid on cabbage in Benin and Mali (James et al., 2010; Sæthre et al., 2011), but this is the first report of its presence on cabbage in Ghana. Myzus persicae, however, is a polyphagous aphid and has already been reported in Ghana (CIE, 1979). This study has shown that neem is very effective in managing the population of the aphids, L. e. pseudobrassicae and M. persicae on cabbage, while conserving the natural enemies, especially spiders. However, the abundance of the main aphid predators (hoverflies and ladybird) was very low or absent throughout the sampling period on the neem treated plots. This could be attributed to the fact that the predators' main prey, the aphids, were absent due to their effective control, leading to the low numbers or absence of their predators. However, the spider, being a generalist predator, was found in the neem plot throughout the

sampling period, because of the availability of other sources of prey. This implies the neem treatment did not really have any detrimental effect on the natural enemies, including the ladybirds and hoverflies.

The efficacy of the neem is in line with previous work done by other authors. The neem oil formulation was proven effective against *L. erysimi* and did not have any detrimental effect on its hoverfly predator, *Ischiodon scutellaris* (F.) (Diptera: Syrphidae) (Boopathi and Pathak, 2011). The application of 3% neem seed kernel extract also was reported to be effective in reducing the aphid population on cabbage (Patel *et al.*, 1996). According to the current study, the highest yield was recorded in the neem treatment, which supports earlier work done by Baidoo and Adam (2012) that reported improved cabbage yield when the neem was used against insect pests.

This contrasts with the Lambda-cyhalothrin plot which had a continuous build-up of aphids and a detrimental effect on the natural enemies. A similar observation was made in a field experiment in Ghana in which Lambda-

cyhalothrin failed to control aphids on cabbage, leading to low yields compared to plots sprayed with garlic, chili pepper and Attack (Fening et al., 2013; 2014). Amoabeng et al. (2013) also observed poor control of aphids on cabbage by Lambda-cyhalothrin in a field cage experiment. The lack of control by these insecticides was attributed to the reduction of natural enemies: hoverflies, Cheilomenes spp. and spider, and also the resistance of this pest to Lambda-cyhalothrin. This is also likely to have been the case in the present study. Lambdacyhalothrin is a pyrethroid with a broad spectrum action which could be harmful to non-target organisms such as natural enemies of aphids (Devotto et al., 2007). Though Chlorpyrifos (organophosphate) controlled the aphids better than Lambda-cyhalothrin, it also had a negative effect on the natural enemies. Organophosphates are broad spectrum insecticides and are known to be highly toxic to predators (Fernandez et al., 2010). For example, Chlorpyrifos 20% EC was found to be highly toxic to the maggots of *I. scutellaris* (Boopathi and Pathak, 2011).

Pepper and *alata samina* also reduced pest populations more than Lambda-cyhalothrin, with no effect on the natural enemies. This observation is confirmed by earlier findings of Zehnder et al. (1997) and Fening et al. (2013) that pepper controlled aphids on cabbage and conserved its natural enemies better than the insecticides Lambda-cyhalothrin. The ability of pepper to kill or repel pests is speculated to be associated with the presence of capsaicinoid elements in the extract, although this has not been proven (Antonious et al., 2006; Fening et al., 2013). Alata samina has insecticidal properties and is thus considered as an insecticidal soap. The soap is composed of potassium salts of several fatty acids whose mode of action is not well understood, but it is believed to disrupt the cellular membrane of pests, especially soft bodied insects, resulting in the loss of cellular contents, and hence, death (Osborne and Henley, 1982). A lower dose of Alata samina is recommended for pest control, as a high dose could have a phytotoxic effect on the plant (Organic Resource Guide, 2010).

Control plots (plots sprayed with only water) had higher yields than Lambda-cyhalothrin in the major season. This

was likely to be a result of high natural enemy abundance (spiders, ladybirds and hoverflies) which offered natural control as opposed to Lambda-cyhalothrin. Despite the high numbers of natural enemies in the control plot, the attack by the aphids was very severe due to their large numbers, leading to very low yields in the minor season. This shows that the control of pests solely by natural enemies was inadequate. The addition of botanicals such as neem and pepper will therefore offer a synergy between the two strategies to reduce the population of aphids in the field and improve yields.

Conclusion

The current study has revealed that the crude neem seed extract is an effective option for managing aphids on cabbage. The method of preparation is not very difficult and can easily be adopted by resource-poor farmers, especially for small scale production of cabbage. Cabbage is often eaten raw and using the neem as a management option for aphids will contribute to food safety and further prevent contamination of produce by insecticide residues.

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Estimating Exceedance Probability of Extreme Water Levels of the Akosombo dam

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ABSTRACT

The Akosombo dam is a major source of electric energy in Ghana. Considering the current increase in the demand for electricity in the country, where such an increase in demand implies more pressure on the dam, it is of key interest to study the tail behaviour of the water levels of the dam. Such a study is important because the level of water in the dam determines the amount of electricity generated. The study employed the Univariate Extreme Value Theory to model the monthly maximum and minimum water levels of the dam. The Generalized Extreme Value Distribution was fitted to the data and the Maximum likelihood estimation method was employed to estimate the model parameters. The study indicated that, the water levels cannot fall below 226.00ft which is the critical water level of the Akosombo dam. It further showed that, the lowest ever level of water the dam can attain is 226.69ft and the highest 279.07ft. The study also found that, though the water cannot fall below the critical level, there was evidence of its falling below the minimum operation head.

Keywords: Extreme value theory; Generalized extreme value distribution; Maximum likelihood estimation; Exceedance probabilities; Tail behaviour

Introduction

Since the late 1980s, Ghana has been facing an energy crisis. A major source of energy in the country is electrical energy and this has been insufficient due to generation problems. According to the Ghana Energy Commission's 2016 energy supply and demand outlook, currently the country's sources of electrical energy are hydro power, thermal power and renewables (solar). Hydro power alone contributes 49.80% (1,580MW) of the country's electrical energy. Of this quantity, the Akosombo hydroelectric plant contributes 32.14% (1,020MW) (Ghana Energy Commission, 2016). The water levels of the dam are a key determinant of the amount of power generated. On the one hand, water levels below the required level for operation result in low power output since not all turbines can function at such a minimal level. On the other hand, very high water levels above the maximum operation level can result in the collapsing of

the dam, leading to flooding. In the event of low or very high water levels, lives, businesses, social activities and the economy of the country can be affected. For these reasons, the tail behaviour of the Akosombo dam, which is the largest dam and a major contributor to Ghana's energy, needs to be investigated.

Extreme Value Theory (EVT) is a field of statistics which deals with the statistical techniques for modeling and the estimation of rare (infrequent) events. By definition, extreme values are infrequent, meaning that estimates are often required for levels of a process that are much greater (or lower) than those which have already been observed, which implies extrapolation from observed events to unobserved events (Coles, 2001). Extreme value theory also provides a class of models to enable such extrapolation. EVT is an exclusive statistical discipline, since it develops techniques and models for describing the unusual rather than the usual, and because it offers a framework in which an estimate of expected forces could be made using past data (Coles, 2001).

Extreme value distributions arises as limiting distributions of maximum or minimum values in random samples, as the samples become sufficiently large (Katz et al., 2002). There are two main families of extreme value distributions, namely the Generalized Extreme Value (GEV) distribution introduced by Jenkinson (1955) and the Generalized Pareto (GP) distribution introduced by Pickands (1975). This paper investigates the tail behaviour of the Akosombo dam using the GEV distribution. Minkah (2016) studied the tail distribution of the Akosombo dam using the GP distribution. The objective of this paper is to complement Minkah (2016) by studying the tail distribution of the Akosombo dam using the GEV distribution to estimate the lower and upper bounds of the water levels of the dam and to determine the exceedance probabilities for very low water levels and very high water levels of the dam which have not yet been observed.

The rest of the paper is organized as follows: section 2 details the GEV methodology and parameter estimation of some extreme events. Section 3 provides a brief background of the Akosombo dam and a brief description of the data employed. Section 4 examines the fitness of the model and an estimation of some extreme events, that is, the bounds and the exceedance probabilities. Section 5 concludes the discussions of the results.

Method

Extreme value theory enables us to study the tail behaviour of stochastic processes. A finite-sample approximation to asymptotic results is the basis of extreme value analyses (Watts *et al.*, 2006).

This paper employs the definitions of Coles (2001). Let $X_{n,n} = \max(X_1, X_2, ..., X_n)$ denote the sample maximum, where $X_1, X_2, ..., X_n$ is a sequence of independent and identically distributed random variables with distribution function . Suppose there exists a suitable sequence $\{a_n > 0, n \ge 1\}$ and $\{b_n, n \ge 1\}$ such that for a sufficiently large sample and at all continuity points x of F, with x taken from the set of real numbers:

$$\lim_{n \to \infty} P\left(\frac{X_{n,n} - b_n}{a_n} \le x\right) = \lim_{n \to \infty} \left\{ F\left(a_n x + b_n\right)^n \right\} \to H\left(x\right)$$
(1)

where F belongs to the domain of attraction of H and H is a non-degenerate distribution function, then H(x) is the Generalized Extreme Value distribution given by:

$$H(x) = \begin{cases} \exp\left\{-\left[1+\gamma\left(\frac{x-\mu}{\sigma}\right)\right]^{-\frac{1}{\gamma}}\right\}, \gamma \neq 0\\ \exp\left\{-\exp\left[-\left(\frac{x-\mu}{\sigma}\right)\right]\right\}, \gamma = 0 \end{cases}$$
(2)

 $-\infty < \mu < \infty, \sigma > 0$ and $-\infty < \gamma < \infty$ with $-\infty < \mu < \infty, \sigma > 0$ and $-\infty < \gamma < \infty$. This distribution is a three parameter distribution: a location parameter μ , a scale parameter σ and a shape parameter γ . The GEV distribution is a combination of three families of extreme value distributions, namely the Gumbel, the Fréchet and the

Weibull distributions. If $\gamma = 0$, then the GEV corresponds to the Gumbel distribution; $\gamma > 0$ corresponds to the Fréchet distribution with $\alpha = \frac{1}{\gamma}$; and $\gamma < 0$ corresponds to the Weibull distribution with $\alpha = -\frac{1}{\gamma}$.

The GEV distribution provides a model for the distribution of block maxima (or minima). The block maxima (or minima) method consists of partitioning the sample into r disjoint and independent blocks. The blocks are usually of equal length and the block sizes are usually selected naturally, for example, yearly. The maximum (or minimum) of each block is chosen as rindependent sample maxima (or minima) from the observed data and then fitted to the GEV distribution. The choice of block size is a sensitive issue since it is a trade-off between bias and variance (Coles, 2001). Blocks of small size are likely to yield poor approximation by the GEV distribution and blocks of larger size produce few block maxima (or minima), which might lead to large variance. In this paper, the block size will be monthly (see Katz et al., 2002; Reiss and Thomas, 2007; Mendez and Menendez, 2006). The monthly maxima and minima water levels of the Akosombo dam will be used, instead of the ussal annual extremes. The block sizes in this case may differ since the months do not have the same number of days. We however do not expect this to affect the result significantly. We used monthly extremes in order to consider more than one datum per year. Also, according to Mendez and Menendez (2006), monthly random extreme variables are expected to be more homogeneous

than yearly ones and therefore expected to improve the asymptotic approximation..

The GEV distribution is for modelling only block maxima; hence to be able to model the block minima, we employ the duality between the distribution for maximum and minimum. Thus, given that $x_1, x_2, ..., x_n$ are realizations from the GEV distribution for minima with parameter (μ_*, σ, γ) , then fitting the GEV distribution for maxima to the data $-x_1, -x_2, ..., -x_n$ yields the estimates for the minima with just a correction of $\mu_* = -\mu$ (Coles, 2001).

The parameters of the GEV distribution can be estimated using the Maximum Likelihood (ML), the Probability Weighted Moment (PWM), the Generalized Probability Weighted Moment (GPWM) or the L-moment method (Ribereau *et al.*, 2008; Katz *et al.*, 2002; Bezak *et al.*, 2014; Hawks *et al.*, 2008). However, this paper employed the ML estimation method due to its "off-the-shelf" large sample inference properties (Coles, 2001).

Considering the assumption that the $X_1, X_2, ..., X_n$ are independent and identically distributed, the loglikelihood function of the GEV is given as:

1/

If $\gamma \neq 0$, we have:

$$l(\mu,\sigma,\gamma) = -n\log\sigma - (1+1/\gamma)\sum_{i=1}^{n}\log\left[1+\gamma\left(\frac{x_{i}-\mu}{\sigma}\right)\right] - \sum_{i=1}^{n}\left[1+\gamma\left(\frac{x_{i}-\mu}{\sigma}\right)\right]^{-/\gamma}$$
(3a)
Provided that $.1+\gamma\left(\frac{x_{i}-\mu}{\sigma}\right) > 0$ for $i = 1, 2, ..., n$

If $\gamma = 0$, the log-likelihood reduces to:

$$l(\mu,\sigma,\gamma) = -n\log\sigma - \sum_{i=1}^{n} \left(\frac{x_i - \mu}{\sigma}\right) - \sum_{i=1}^{n} \exp\left\{-\left(\frac{x_i - \mu}{\sigma}\right)\right\}$$
(3b)

The derivatives of (3a) and (3b) with respect to the parameters $\theta = (\gamma, \mu, \sigma)$ yield the likelihood system of equations which has no explicit solution; therefore the system of equations is solved iteratively. For computational details see Prescott and Walden (1980).

When the estimates μ , σ and γ of the GEV distribution have been obtained, the extreme quantile, the exceedance probabilities and the endpoints (bounds) of the GEV can be estimated.

By inverting the GEV distribution function, we obtain the quantiles for the original X data as:

$$q_{X,p} = \begin{cases} \hat{\mu} - \frac{\hat{\sigma}}{\hat{\gamma}} \Big[1 - \{ -\log(1-p) \}^{-\hat{\gamma}} \Big], \hat{\gamma} \neq 0 \\ \hat{\mu} - \hat{\sigma} \log \Big[-\log(1-p) \Big], \quad \hat{\gamma} = 0 \end{cases}$$
(4)

Where $H(q_{X,p}) = 1 - p$ and $0 \le p \le 1$, p is the exceedance probability. Hence $P(X > q_{X,p}) = 1 - H(q_{X,p})$

Equation (4) is known as the extreme quantile. The parameters $\hat{\mu}, \hat{\sigma}$ and $\hat{\gamma}$ are corresponding ML estimates of μ, σ and γ respectively.

For $\gamma < 0$, an inference can be made on the upper endpoint of the distribution and the ML estimate of the upper endpoint is $q_{\chi,0} = \hat{\mu} - \hat{\sigma}/\hat{\gamma}$, with probability zero (Coles, 2001; Beirlant *et al.*, 2004).

Data and brief background of the Akosombo dam

The data used for the study is the daily water level readings of the Akosombo dam obtained from the office of the Volta River Authority (VRA) at the Akuse station. The data consists of 17,525 data point, covering the period January 1, 1966 to December 31, 2013.

The Akosombo dam was commissioned into operation in January 1965. The dam is located on the Volta river in South-eastern Ghana in the Akosombo gorge. The construction of the Akosombo dam resulted in the creation of the Lake Volta, the world's largest man-made lake, covering 3.6% of Ghana's land area. The Akosombo hydroelectric plant has six 170MW Francis turbines. Each turbine is supplied with water via a penstock which is 112-116 meters long and 7.2 meters in diameter. The dam operates, under normal conditions between a minimum water level of 240ft and a maximum water level of 278ft. Increased power demand, coupled with unanticipated environmental conditions, has resulted in rolling blackouts and major power outages over the years. The dam has over the years experienced some trends of water levels below the minimum requirement for operation. The critical minimum level of the dam is 226ft and at this level all the turbines must be shut down completely. The dam has also had a fair share of very high water levels, very close to the maximum requirement for the dam's operation. For instance, in November 2010, the water peaked at 277.54ft, and to safeguard the integrity of the dam, the spill gate was opened. This resulted in the flooding of some houses and farmlands around the dam (VRA, 2010).

Results and discussion

This paper applied EVT to the water levels of the Akosombo dam. All the extreme value analyses in this paper are limited to the water levels of the dam recorded over the period 1966 to 2013. The methodology described in the previous section is applied to the data and discussed in this section.

Table 1 presents some descriptive statistics of the Akosombo water levels. The lowest ever water level for the period under study is 234.00 ft., and this was recorded on June 28, 1966. The electricity demand during that time was at a minimal level, so the electricity generated by such low water levels was enough to go round. However, with the nation's current increasing demand for electricity, any water level below 240ft may pose a problem which may lead to nationwide load shedding. The highest ever water level recorded is 277.54ft, and this occurred on 8th and 9th November 2010. The Skewness value indicates the distribution is asymmetric and therefore has a tail much lighter than that of the normal distribution.

Table 1: Descriptive Statistics of the Water levels

Minimum	1 st Quartile	3 rd Quartile	Mean	Skewness	Maximum
234.00	248.06	265.73	256.67	-0.124	277.54

The partitoning of the data into monthly blocks resulted in 576 data points in each case (i.e., for maxima blocks and minima blocks). In modelling the minimum, we employed the duality between maximum and minimum, that is, we converted the minimum into a maximum problem by using the negated forms of the minimum values. According to Coles (2001), the ML estimates of the parameters of this distribution correspond exactly to those of the required GEV distribution for minima except the sign correction $\mu_* = -\mu$. Figure 1 shows the time series plots of the sample maxima and minima water levels for the monthly blocks. From the figure, it can be seen that the maximum and minimum plots are similar in terms of variations and trends. The Extreme Value Index (EVI), that is, the shape parameter, is the primary problem in extreme value analyses.

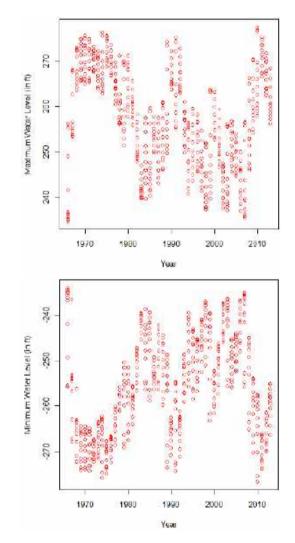


Fig. 1: Monthly Maximum Water Levels, top panel; Monthly Minimum Water levels, bottom panel

Table 2: Parameter estimates of the GEV distribution

Estimates	Right tail	Left til
Location ($\hat{\mu}$)	254.90 (0.5287)	259.10 (0.52)
Scale ($\hat{\sigma}$)	11.42 (0.4198)	10.69 (0.40)
Shape ($\hat{\gamma}$)	-0.47 (0.0338)	-0.33 (0.041)
95% conf. Interval ($\hat{\gamma}$)	(-0.54, -0.41)	(-0.4, -0.25)

* Standard errors are in parentheses

Table 2 presents the parameter estimates for the GEV distribution for the right and left tails of the Akosombo water levels. The EVI for the right tail of the Akosombo water levels is $\hat{\gamma} = -0.47$ with an associated standard error of 0.034, and that of the left tail is $\hat{\gamma} = -0.33$ with associated standard error of 0.04. In both cases, $\gamma > -0.5$

indicates that, regularity conditions are satisfied and the ML estimators have the usual asymptotic properties (Kotz *et al.*, 2000; Coles, 2001). The negativity of EVI's in both cases indicates that, the Akosombo dam water levels belong to the Weibull family of distribution.

We further used diagnostic plots to assess the fitted GEV models for the monthly maximum and minimum water levels (figure 2a and 2b). The plots are the QQ-plot (a), PP-plot (b) and a histogram overlay with the density curves of the fitted GEV (c). For the QQ-plot and the PP-plot, a good fit should yield a straight one-to-one line of points. Generally, the QQ-plot is preferred to the probability plot. The approximate linearity of the QQ-plot and the PP-plot indicates that the model is valid. From the histogram, the density also appears to be consistent with the data points. We therefore conclude that the diagnostic plots are in favour of the fitted model and that the GEV model is a good fit for the Akosombo data.

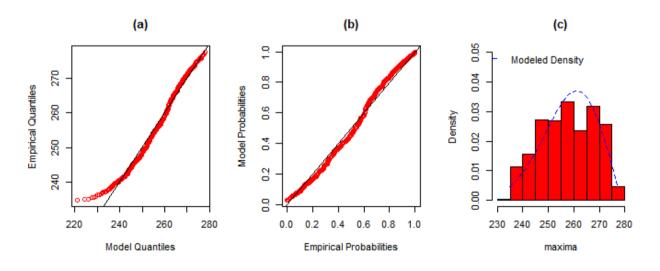


Fig. 2a: Diagnostic plots for the fitted GEV model for the monthly maximum water levels

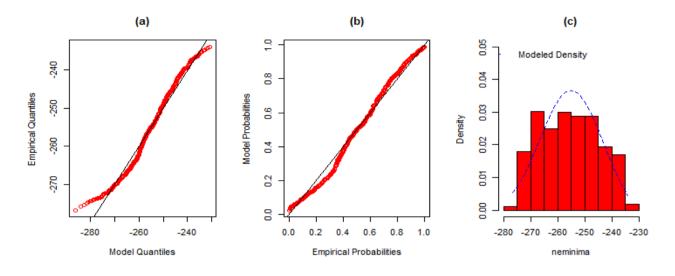


Fig. 2b: Diagnostic plots for the fitted GEV model for the monthly minimum water levels

Now that there is no doubt that the GEV model is valid for modeling the Akosombo dam data, we can estimate the upper endpoint of the distribution. The upper bound for the left tail is 226.69ft, which is much below the lowest water level ever recorded for the period under study but very close to the critical level of the dam. The upper bound for the right tail is 279.07ft, which is higher than the maximum water capacity (278ft) of the dam. Deducing from the endpoint estimates of the left tail and right tail, the water levels of the dam are bounded below by 226.69ft and above by 279.07ft. This implies that, the water level can neither fall below 226.67ft nor go above 279.07ft.

Table 3: Exceedance Probabilities of water levels of the dam

Tail	Water level (ft.)	Probability
Right	277.6	$1.872*10^{-4}$
Right	277.90	0.0000
	234.00	$1.09*10^{-2}$
Left	232.00	$4.154 * 10^{-3}$
20.0	228.00	$6.04*10^{-5}$
	226.00	0.0000

Table 3 presents the probability of exceedances for some selected water levels of the dam. We observed that, very high water levels and very low water levels are associated with small probabilities. The exceedance probability for the right tail is interpreted as, the probability that the water level of the dam will exceed the specified level. Additionally, any level greater than the maximum capacity of the dam has an exceedance probability of zero, which implies that it is impossible to observe a level higher than what the dam can actually contain. Since there is a ceiling (278ft.) on the amount of water in the dam, any level close to it leads to spillage of water from the dam to save it from collapsing; therefore it is impossible for the dam to attain a level higher than 278ft. The exceedance probabilities for the left tail are interpreted as, the probability that the water level of the dam will fall below the specified level. Very low probabilities implies that the chances of observing such water levels are rare, but not impossible, and the zero probabilities imply that it is impossible to observe such water levels.

Conclusion

This study set out to investigate the tail behaviour of the water levels of the Akosombo dam using EVT, particularly the GEV distribution. The paper had two objectives: to estimate the bounds of the water levels of the dam and to estimate the exceedance probabilities of some selected (very high and low) water levels.

The ML estimation method was employed to estimate the EVIs and the other parameters. The EVI determines the tail behaviour and the domain of attraction of the underlying distribution (Coles 2001; Kotz and Nadarajah, 2000; Beirlant *et al.*, 2004). The tail distribution of the Akosombo water levels follows the Weibull distribution; this confirms Minkah (2016).

The diagnostic plots indicate that the block maxima approach, that is, the GEV distribution, is very good for the description of the Akosombo water levels.

Considering only the water levels of the dam and all other things being equal, a complete shutdown of the dam is impossible, but a shutdown of a couple of turbines is inevitable. Therefore, the dependence on the Akosombo hydroelectric power plant for electricity supply should be reduced so that even when some turbines are shut down, "communities" depending on the dam for electricity supply would have enough power to carry out their activities without any power interruptions.

It can be observed from the study that water levels very close to the maximum operation head which would necessitate water spillage from dam are possible. Therefore, the dam's reservoir should be extended to retain the excess water to prevent flooding downstream and reduce the rate at which the water levels fall below the minimum operation head.

We conclude by stating that multivariate extreme value analysis which would include other factors likely to influence the water levels of the dam should be employed to investigate the problem further.

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Gross and Histo-Pathologic Findings in Goats with Plastic bags in the Rumen

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ABSTRACT

The objective of this study was to investigate the gross and histo-pathological changes caused by the presence of plastic bags in the rumen of goats. Sixteen castrated one-year old small East African goats were used for the study. The animals were divided into 4 groups, of 4 goats (n=4). Three groups were implanted with 129g, 258g and 387g of plastic bags, respectively, introduced into the rumen through rumenotomy, while the fourth group without implants served as control. All goats with or without plastic bags were observed daily for 6 weeks following implantation. Dead and surviving goats euthanized after the 6-week period were subjected to postmortem and histopathological examination. Gross pathological lesions observed were atrophy of the muscle and body fat, and atrophy and fibrosis of the spleen, liver, kidneys and hydropericardium. Lesions on the rumen epithelium included stunting, atrophy, thinning and loss of ruminal papillae, erosion, ulcerations and nodular formations on the ruminal mucosa. The histopathological examination revealed atrophy, erosion, ulceration, and disruptions of the stratified epithelial layer of the papillae. Other changes included parakeratosis, hyperkeratosis, prominent rete pegs, oedema and severe hydrophic degeneration of different parts of the mucosal layer. There were areas of increased mononuclear cell infiltration, increase in the number of lymphatic vessels and lymphangiectasis in the submucosa, and oedema in the muscularis and serosal layers. The pathological changes observed may interfere with the digestion and absorption of nutrients, resulting in poor animal condition, overall productivity and production.

Keywords: Goats, Pathology, Plastic Bags, Rumen

Introduction

Goats reared under the traditional extensive husbandry systems mainly roam and scavenge for food, and may ingest various indigestible foreign bodies, especially in urban and peri-urban areas (Remi-Adewunmi *et al.*, 2004). These materials, particularly plastic bags, pollute the grazing environment due to indiscriminate disposal of waste in many parts of the developing world (RemiAdewunmi *et al.*, 2004; Ghurashi *et al.*, 2009). The risk factors that compel goats to ingest these non-food materials include deficiency of minerals such as calcium, phosphorus and other micronutrients (Radostitis *et al.*, 2009); feed scarcity (Igbokwe *et al.*, 2003); depraved appetite (Reddy and Sasikala, 2012), poor management of animals leading to scavenging at dumping sites;

poverty among animal owners which makes them unable to provide feed; and increased number of animals per given land space (FAO, 1999).

Accumulation of indigestible foreign bodies in the rumen and other parts of the digestive tract may cause complications in the rumen and other organs of the body (Otsyina et al., 2016). The complications caused by ingested foreign bodies may vary with the type of material, the duration of its presence in the body, its location and the degree of obstruction caused by the offending foreign body (Tesgaye and Chanie, 2012). The effect of indigestible materials, particularly plastic bags, in the rumen of goats includes interference with the flow of ingesta, ruminal tympany, anorexia, hypoglycaemia, depression, absence of defaecation, reduced milk production, reduced fattening of the animal, loss of weight and death of the affected animal (Igbokwe et al., 2003; Reddy et al., 2004; Remi-Adewunmi et al., 2004; Debaris and Mousumi, 2010; Vanitha et al., 2010; Otsyina et al., 2014).

Gross and histopathological lesions observed in the rumen and tissues of goats with indigestible foreign bodies in abattoir studies and at postmortem include sloughed and hyperplastic epithelia; atrophy and loss of ruminal papillae; rumenitis; erosion, ulceration and scarred ruminal pillars, and epithelia (Robbins et al., 1984; Hailat et al., 1998; Bakheit, 2008; Pitroda et al., 2010). However, the types of foreign bodies, the quantities and the degree of obstruction, severity of damage caused as well as the duration of their presence in the rumen that produced the reported effects are not known. Gross and histo-pathological changes in goats with indigestible materials experimentally implanted into the rumen have not been previously studied. Since goats have occasionally been found to have plastic bags in their rumen at slaughter and at necropsy (Otsyina et al., 2015), the current study reports the gross and histopathological lesions in goats with specific quantities of plastic bags experimentally implanted into the rumen over a period of 6 weeks.

Material and methods

Experimental animals

Sixteen (16) castrated small East African goats with a mean body weight of 24.5kg and a body condition score of 3.0 ± 0.5 (on a scale of 1-5) were used for the study. The animals were housed in groups of four (4) for the whole period of the experiment and allowed 6 weeks to acclimatize to the environment and the feed. They were fed on chopped Rhodes grass hay supplemented with commercially produced small stock concentrate meal (UNGA AFYA Meal, UNGA Farm Care Ltd, Nairobi, Kenya). Feed and drinking water were provided ad libitum. They were treated against endoparasites with 2.5% Albendazole (Alfabas[®] Norbrook, Kenya) administered at a dose rate of 4ml/kg of body weight. They were also treated against ectoparasites with Ivermectin at a dose rate of 1ml/50kg of body weight. All the animals were administered 20% injectable Oxytetracycline HCl (Alamycin LA 20°, Norbrook, Ireland) at a dose rate of 20mg/10kg of body weight as a prophylactic measure against infection often associated with transportation stress. The animals were subjected to routine physical examination over the acclimatization period.

They were then assigned to 4 experimental groups (4 groups with 4 goats each) using stratified random sampling based on the weight of the animals, such that the mean weight of animals in each of the experimental groups was not statistically different. Three of the groups (GE1, GE2 and GE3) had 129 g, 285 g and 387 g of plastic bags respectively implanted into the rumen through rumenotomy, as previously described by (Hendrickson, 2007). The plastic bags implanted were non-perforated small soft polythene bags (KEBS Industries Ltd, Nairobi, Kenya). Each poly bag measured 167 mm x 290 mm in size and 30 micrometers thick, and a pack of 100 pieces weighed 129 g. These were the most common type of plastic bags found in the rumen of sheep and goats during an abattoir study carried out prior to the experimental study. The fourth group (GC4) served as control on which rumenotomy was done but no plastic bags were implanted. Both test and control animals were monitored daily for a period of 6 weeks (42 days). The duration of implantation of the plastic bags was informed by a pilot study and an abattoir study conducted at two abattoirs in Nairobi, Kenya. All vital parameters as well as clinical manifestations were noted and recorded.

Euthanasia of experimental animals

After 6 weeks of implantation of plastic bags into the rumen, all were euthanized for postmortem and histopathological examination. Euthanasia was carried out humanely by sedation using Xylazine hydrochloride at a dosage of 0.2mg/kg body weight, followed by stunning with a captive bolt pistol, after which the animals were exsanguinated.

Postmortem examination

Postmortem examination of the euthanized goats was done through inspection of individual carcasses of both test and control groups. The carcasses were weighed, then flayed to examine the state of the musculature and to note any abnormalities. They were opened and the gastrointestinal tract, liver, kidneys, spleen, pancreas, lungs, heart, lymph nodes, adrenal glands, gall bladder and urinary bladder were examined for gross lesions. The rumen was examined while intact with the contents, and then incised to inspect the contents and the nature of the implanted plastic bags. The contents were removed and the rumen thoroughly washed for better inspection of the wall, mucosa, the papillae and the pillars for any abnormalities. The organs of goats implanted with plastic bags were weighed and the weights compared with those of the control. All findings in all tissues and organs were recorded. Photographs of carcasses and body organs were taken using an iPAD 4 with retina display application (Apple Computers Inc, USA).

Histopathological examination

Rumen tissues were collected from areas with and without gross pathological lesions. They were immediately preserved in 10% buffered formalin and allowed to fix for a minimum of one week before processing.

The formalin fixed tissue specimens were processed for histological examination as previously described by Smith and Bruton (1977). They were cut into a thickness of 3-5µm. Four sections were made from every paraffin wax block, stained with haematoxylin and eosin (H&E) and mounted on microscope slides using Destrene 80, dibutyl Phthalate and xylene (DPX). The slides were examined under the light microscope using x4, x10, x40 and x100 objective lenses. Results were recorded and photomicrographs taken using the photomicroscope (Olympus CXSF1, Olympus Corporation, Tokyo. Japan).

Animal use ethical approval

Animal use ethical clearance was approved by the Biosafety, Animal use and Ethics Committee (BAEC) of the Faculty of Veterinary Medicine, University of Nairobi, Kenya, according to international standards of animal use in research; clearance certificate number: 11250313.

Results

Pathological changes in carcasses and gastrointestinal tract

The gross lesions observed in carcasses and gastrointestinal tract of goats with plastic bags implanted in the rumen are presented in Table 1. The carcasses of the goats in the control group and those with 129g of plastic bags implanted in them appeared normal. However, goats with 258g and 387g of plastic bags implanted in them had atrophy of the muscles and body fat, atrophy and degeneration of the omental fat, hyperemia and oedema of the prescapular lymph nodes.

			Gross Pathological Changes	
Carcass/Organ	Control (GC4)	Group GE1	Group GE2	Group GE3
	No plastic bags	129 g	258 g	387 g
Carcass	Good body condition well covered with fat	Fairly good body condition; reduced body fat; lymphadenitis of prescapular lymph nodes	Poor body condition, atrophy of the muscles, body fat and omental fat, lymphadenitis of prescapular lymph nodes	Poor body condition, atrophy of the muscles, body fat, hyperemia and oedema of prescapular lymph nodes, atrophy of omental and mesenteric fat
Rumen	No significant findings	Slightly reduced in size	Eroded patches devoid of papillae, short and stunted papillae, oedema and haemorrhages of the ruminal folds and rumen mucosa	Hyperemia and congestion of the rumen mucosa; eroded patches devoid of papillae; short, stunted and fungoid papillae; oedema and haemorrhages of the ruminal folds and rumen mucosa, ulcers on ruminal folds
Reticulum	No significant findings	No significant findings	Atrophied and empty	Atrophied and empty, no papillae in the combs
Omasum	No significant findings	Slightly reduced in size	Atrophied and empty	Atrophied and empty, hyperemia and oedema of the folds, ulcerations at the reticulo-omasal orifice
Abomasum	No significant findings	Slightly reduced in size.	Empty, atrophied, congested and haemorrhagic. Ulcers at the pyloric region	Atrophied and empty, congested and haemorrhagic, ulcers at the pyloric region
Small intestines	No significant findings	No significant findings	Atrophied, congested and haemorrhagic and filled with fluid	Atrophied, congested, haemorrhagic and filled with blood tinged fluid
Large intestines	No significant findings	No significant findings	Atrophied, congested and haemorrhagic and filled with fluid	Atrophied, congested, haemorrhagic and filled with blood tinged fluid

Table 1: Gross pathological changes observed on carcasses and gastrointestinal tracts of goats with plastic bags implanted in their rumen

Key: SE1 - Goats with 129 g of plastic bags implanted in them; **SE2** - Goats with 258 g of plastic bags implanted in them; **SE3** - Goats with 387 g of plastic bags implanted into the rumen; **GC4** (control) – Goats with no plastic bags in the rumen; **n**- number of goats; **g** – grams.

The rumen contents looked normal and the rumen did not show significant pathologic changes in the control goats and those with 129 g of plastic bags implanted in them. However, goats with 258 g and 387 g of plastic implanted in them showed congestion, hyperemia, haemorrhages, erosions, excoriations, scarification and loss of papillae on the walls of the rumen. The rumen papillae were stunted, atrophied and thin. Scars, ulcerations and nodular lesions were found on the ruminal pillars in all the goats with 258 g and 387 g of plastic bags implanted in them. In the same groups of goats, the walls of the rumen were very thin. Furthermore, in these two groups, the reticulum, omasum and abomasum were atrophied, congested and haemorrhagic. The gastrointestinal tract distal to the rumen was completely devoid of ingesta and the intestines were found to have a blood tinged fluid.

Pathological changes in other organs and tissues

Gross pathological changes in various organs and tissues in goats with various quantities of plastic bags implanted in them are shown in Table 2. Atrophy and fibrosis of the liver, spleen, kidneys, heart and lungs were observed in all the goats with 258 g and 387g of plastic bags implanted in them. Distension of the gallbladder with thickening of the bile ducts and viscous bile were observed in 3 of the 4 goats in each of the two groups. In all the goats with 258 g and 387 g of plastic bags implanted in them, the heart muscles were thin; the coronary fat was atrophied and there was hydropericardium with straw coloured fluid. The severity of gross pathological changes in the goats increased with the weight of the implanted plastic bags. Three of the goats with 258 g and all goats with 387 g of plastic bags implanted died before the end of the 6 weeks. The animals that died showed similar lesions as observed in those that were euthanized. The goats with 129 g of plastic bags implanted in them had very minimal changes while those without plastic bags (control) did not have any observable gross pathology upon examination at 6 weeks.

Table 2: Gross patholo	ogical changes observed in o	raans of goats with	plastic baas im	planted in the rumen

		Gross Path	ological Changes	
Organs	Control (GC4) (No plastic bags)	Group GE1 129 g	Group GE2 258 g	Group GE3 387 g
Liver	No significant findings, normal consistency	Slight reduction in size, normal consistency	Firm and fibrotic liver,distended gall bladder, viscous bile	Firm and fibrotic of liver, distended gall bladder with viscous bile
Spleen	No significant findings	Slightly reduced in size, normal consistency	Atrophy of spleen with fibrotic capsule	Atrophy of spleen with fibrotic capsule
Heart	No significant findings	Slightly atrophic with slight degeneration of coronary fat	Hydropericardium (straw coloured fluid), gelatinous coronary fat	Very thin cardiac muscles, no coronary fat, hydropericardi- um with straw coloured fluid
Kidneys	No significant findings	Slightly atrophied, renal fat atrophy	Atrophy and congestion of the renal fat, fibrotic capsule	Atrophy of the renal fat,fibrotic capsule and congestion of medullary area
Lungs	No significant findings	No gross changes	Presence of froth	Oedematous and congested

Key: GE1 - Goats with 129 g of plastic bags implanted in the rumen; **GE2** - Goats with 285 g of plastic bags implanted in the rumen; **GE3** - Goats with 387 g of plastic bags implanted in the rumen; **GC4** (control) – Goats with no plastic bags in the rumen; **n** - Number of goats; **g** – grams.

Histopathological findings of the rumen

Histopathological findings of rumen tissues of goats in control and experimental animals are presented in Figures 1-4. The control group showed normal papillae with a thin and continuous stratified epithelium without disruptions or erosions. The long, intermediate and short papillae were all present. The long papillae were about 2-3 microscope fields (x40 Magnification) long from their base to the tip. The mucosa epithelium was 3-5 cells thick with cornified layers and no rete pegs. The submucosa had good connective tissues with few lymphatic ducts and without infiltration of mononuclear or polymorphonuclear cells. The muscular layer and the serosa had no observable gross lesion (Fig 1).

With goats with 129 g of plastic bags in them, the stratified epithelium of the rumen was disrupted with degeneration, necrosis and patchy hyperplasia in many areas (Fig 2). The papillae were shortened, stunted, sometimes broadened, compressed or flattened, atrophied and shredded in some areas. The length of the papillae was about half (1/2) of the field of the microscope at x40 Magnification (Fig 2). There were clefts at the tips of some of the atrophied papillae. Degeneration, necrosis and hyperplasia of the mucosa with prominent rete pegs of variable length projecting into the submucosa were observed. The mucosa had up to 28-32 cells in thickness, with both extracellular and intracellular epithelial cell oedema characterized by hydrophic degeneration, cellular vacuolation and spongiosis (Fig 2). The submucosa appeared widened and oedematous, with dilated lymphatics between the rete pegs. There was degeneration, necrosis and fibrosis of the connective tissue with increased mononuclear cell

infiltration in the submucosa (Fig 2). The muscular layers were atrophied, compressed and shredded in some areas. Muscle fibers were separated by oedema. The serosa was shredded, widened and oedematous (Fig 2).

Goats with 258 g and 387 g of plastic bags implanted showed similar but more severe changes than those with 129 g of plastic bags implanted in them. The severity of the lesions increased with the quantity of plastic bags in the rumen. These changes included degeneration, erosion, necrosis, disruption, sloughing, and segmental hyperplasia of the keratinized epithelia in many areas of the rumen (Figs 3 and 4). In some areas of the rumen, the rumen papillae were atrophied, shortened, compressed, flattened and disrupted.

In goats with 258 g of plastic bags implanted, the papillae were broadened but shortened in length to less than a quarter (1/4) of the microscope field (Magnification 40x) (Fig 3). While in those with 387 g of plastic bags implanted, the length was less than an eighth (1/8) of the microscope field (Magnification 40x) (Fig 4). The mucosa epithelium was 35-37 cells in thickness in those with 258 g and 40-45 cells thick in those with 387 g, with both extracellular and intracellular epithelial cell oedema being characteristic of hydrophic degeneration, cellular vacuolation and spongiosis of the cells (Figs 3 and 4). The submucosa in the two groups was oedematous with dilated lymphatics, increased mononuclear cell infiltration and vascularization (Fig 3). The muscular layers were also atrophic and shredded in some areas while the muscle fibers were separated by oedema. The serosa was also shredded, widened and oedematous (Figs 3 and 4).

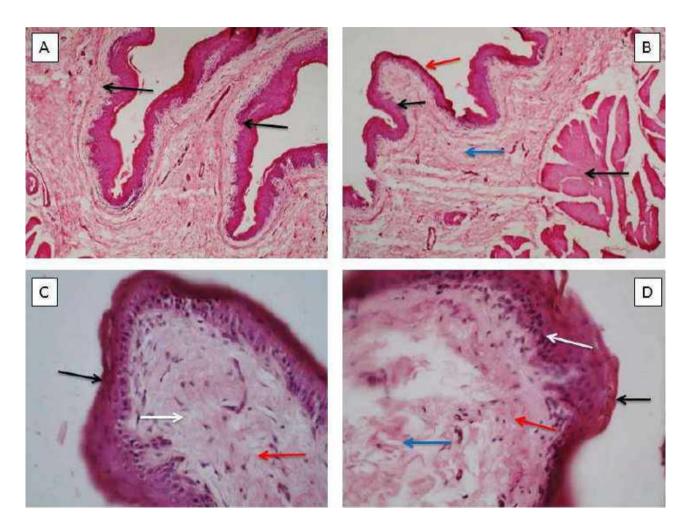


Fig. 1: Rumen of goats without plastic bags (control): (A) showing normal ruminal papillae (*arrows*) with normal keratinized epithelium, mucosa and submucosa (Magnification 40x); (B) showing normal papilla (*red arrow*), normal mucosa (*white arrow*), submucosa (*blue arrow*), muscular (*black arrow*) (Magnification 40x); (C) higher magnification of the tip of ruminal papilla showing its 3-4 cell epithelium with a thick keratinized layer (*black arrow*), submucosa (*white arrow*), and muscularis parts (*red arrow*)(Magnification 100x); (D) higher magnification of the tip of the papilla showing epithelium with keratinized layer (*black arrow*), submucosa (*red arrow*) (Magnification 100x); (D) higher magnification of the tip of the papilla showing epithelium with keratinized layer (*black arrow*), submucosa (*red arrow*) and muscularis layer (*blue arrow*) (Magnification 400x) (H &E stain).

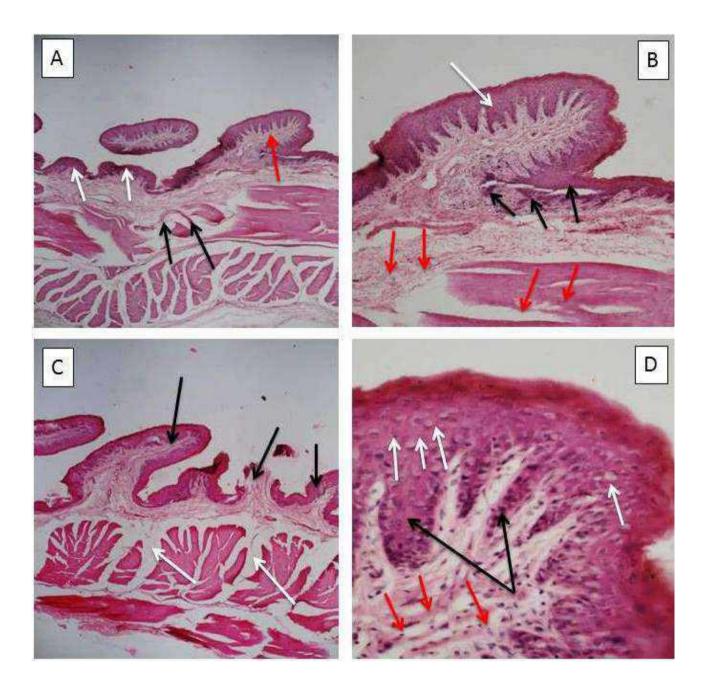


Fig. 2: Rumen of goats with 129 g of plastic bags implanted in them: (A) showing atrophied (*white arrows*), flattened (*red arrow*) ruminal papillae and shredded submucosa and muscle layers (*black arrows*) (Magnification 40x); (B) showing atrophied and flattened papilla (*white arrow*) with regenerated epithelium (*black arrows*), and compacted muscle layers (*red arrows*) (Magnification 100x); (C) showing atrophied and destroyed papillae (*black arrows*) and separated bundles of the muscle layer (*white arrows*) (Magnification 40x); (D) showing tip of the papilla with hyperplasia of the mucosa and prominent rete pegs (*black arrows*), oedema and spongiosis of the cells (*white arrows*) and oedema of the submucosa (*red arrows*) (Magnification 400x) (H & E stain).

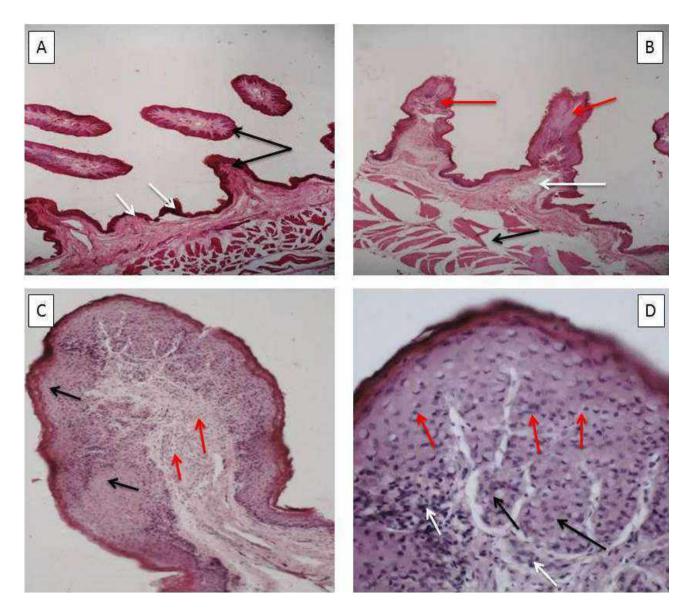


Fig. 3: Rumen of goats with 258 g of plastic bags implanted in the rumen: (A) showing disrupted (*black arrows*) and atrophied (*white arrow*) papillae (Magnification 40x); (B) showing destroyed ruminal papillae (*red arrows*), submucosa (*white arrow*) and atrophic separated muscle layer (*black arrow*) (Magnification 40x); (C) showing atrophied and fungoid papilla with hyperplastic mucosa (*black arrows*), compaction and vascularization of the submucosa (*red arrows*) (Magnification 100x); (D) showing tip of an atrophic papilla with hyperplastic epithelium with prominent rete pegs (*black arrows*), vacuolation and spongiosis of the cells (*red arrows*) and massive infiltration of mononuclear and polymorphonucear cells (*white arrows*) (Magnification 400x) (H & E stain).

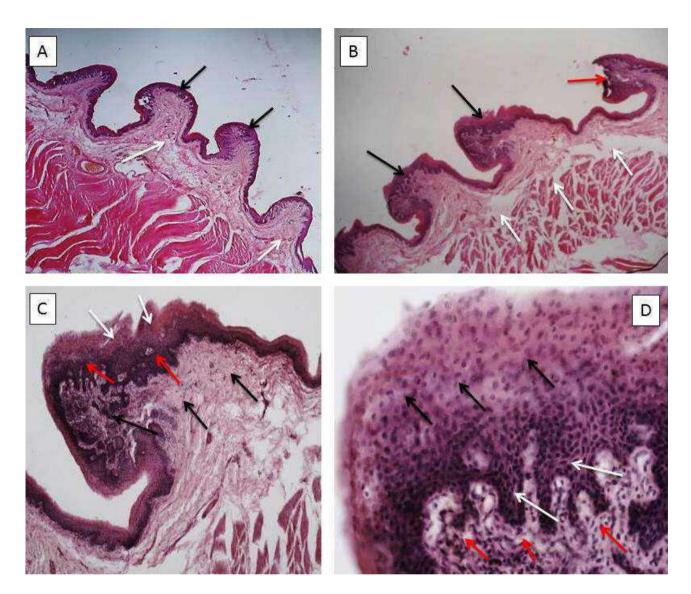


Fig. 4: Rumen of goats with 387 g of plastic bags implanted in the rumen: (A) showing stunted, clubbed atrophied and broadened (*black arrows*) ruminal papillae, widened and disrupted submucosa (*white arrows*) (Magnification 40x); (B) showing atrophied and bent papillae (*black arrows*), ruptured papilla (*red arrow*), disrupted, degenerated submucosa, and atrophic degenerated muscularis (*white arrows*) (Magnification 40x); (C) showing atrophied papilla with disrupted stratified epithelium (*white arrows*), degenerated and hyperplastic epithelium (*red arrows*) and degenerated submucosa (*black arrows*) (Magnification 100x); (D) showing tip of the atrophied papilla with hyperplastic epithelium (*black arrows*) and interlocking rete pegs (white arrows), compressed and degenerated submucosa with massive infiltration of mononuclear and polymorphonucear cells (*red arrows*) (Magnification 400x) (H & E stain).

Discussion

This study described the gross and histopathological changes associated with accumulation of plastic bags in the rumen of goats. The severity of the gross and histopathological changes varied with the quantity of plastic bags present in the rumen. This confirms what was previously suggested, that the severity of pathological changes depends on the type of foreign body, the duration in the rumen and the degree of obstruction (Calfee and Manning, 2002; Tesgaye and Chanie, 2012). However, quantitative damage by the same type of foreign body as evaluated in this study has not been previously reported.

Generalized muscle and organ atrophy; degeneration of body; omental, mesenteric and pericardial fat observed in the goats may be due to anorexia and physiological nutrient imbalances. The extent of stretching of the ruminal wall and reduced ruminal motility due to the presence of plastic bags in the rumen may have stimulated the hypothalamus and satiety centre, leading to anorexia, emaciation and dehydration (Ghurashi et al., 2009; Mozaffari et al., 2009; Otsyina et al., 2016). In addition, the plastic bags may have produced toxicants, while the damage to the ruminal mucosa may allow these toxic substances from the rumen into the body to cause toxaemia and anorexia. Chronic wasting diseases, malnutrition and cachexia result in mobilization of fat deposits (Jubb et al., 1985), hence the degeneration and atrophy (Abdalla et al., 2010). The selected organs in the body showed a reduction in size and weight due to atrophy. Radostitis et al. (2009) reported that atrophy of the liver is related to chronic distension of adjacent structures of the alimentary canal such as the rumen or colon, which exerts pressure on the organ, and this could explain the observation in the current study.

Shortening, stunting and atrophy of the papillae; erosion, excoriation of the papillae as well as oedema of the ruminal pillars, ulcerations, scars, nodules, hyperemia and haemorrhages of the rumen mucosa and thinning of the ruminal wall, were consistent findings in previous reports in clinical and abattoir studies involving different types of foreign bodies (Bakhiet, 2008; Ghurashi *et al.*, 2009). Similarly, Raoofi et al. (2012) found a significant reduction in the thickness of the rumen in goats with a nylon rope implanted in them. However, these changes varied with the quantity of plastic bags present in the rumen. The degree of papillae and pillar atrophy was also dependent on the quantity of plastic bags. The degree of shortness of the papillae has not been evaluated and graded as in this study. These pathological changes may be attributed to the pressure exerted against the ruminal walls by the plastic bags (Bakhiet, 2008) and perhaps toxicants from the plastic bags and the ruminal contents. Furthermore, constant irritation of the wall of the rumen by continuous movement of the plastic bags may have led to erosion and excoriations of the rumen papillae, ruminal pillars and mucosa, resulting in inflammation and hyperplasia of the epithelial mucosa (Bakhiet, 2008). It is also possible that some of the lesions may have resulted from poisonous substances released from the plastic bags and toxicants absorbed from the rumen. Hyperemia, haemorrhages and oedema observed in the reticulum, omasum, abomasum and intestines could be due to irritation, circulatory disturbances and inflammation as a result of toxic substances released from the plastic bags and absorbed from the gastrointestinal tract.

Fibrosis and atrophy of the liver observed in the current study could be due to hepatocyte damage from absorbed toxic substances from the rumen after severe ruminal mucosa damage by the plastic bags. It has been reported previously that fibrosis and necrosis of the liver could occur as a result of toxic injury to the liver parenchyma, and the degree and pattern of the damage is determined by the duration of injury and the metabolic reactions triggered by the intoxication (Jubb et al., 1985). This could mean that the animals with 387 g of plastic bags implanted in them had more injury to the liver, hence the variegated discolourations of the liver associated with the fibrosis. This could have contributed to the early deaths observed in the goats in this study. The distended gall bladder, viscous bile and thick bile ducts observed in goats implanted with plastic bags could be due to anorexia and starvation (Radostitis et al., 2009).

The changes in the spleen and kidneys including atrophy and capsular fibrosis observed in the goats with plastic bags implanted in them may be associated with the anorexia, malnutrition and cachexia in these animals as a compensatory body mechanism during stressful conditions such as chronic wasting diseases (Jubb et al., 1985). Similar compensatory body mechanisms may also be responsible for the gross pathological changes on the heart including atrophy and thin atrial and ventricular musculature, the absence of pericardial fat and hydropericardium observed in most of the goats with 285 g and 387 g of plastic bags implanted in them. Oedema and congestion of lungs could be due to starvation resulting in hypoproteinaemia and difficulty in breathing due to pressure exerted on the diaphragm by the distended rumen. Impaired function of the heart could be due to myocardial atrophy, degeneration and hydropericardium. Pulmonary congestion due to cardiac insufficiency resulting in interstitial pulmonary oedema has been reported in some heart conditions (Jubb et al., 1985). Such findings have not been previously associated with foreign bodies in the rumen.

Histopathological changes observed in the rumen involving the papillae, pillars and the epithelia can be attributed to excessive pressure exerted on the ruminal walls by the plastic bags. Similar findings have been reported in abattoir studies by other authors (Bakhiet, 2008; Hailat, 1998), although the time span and quantities of foreign bodies were not evaluated as in this study. The degree of atrophy and estimation of cell numbers, and thickness in hyperplastic tissue have not been previously reported in goat rumen with indigestible foreign bodies. These changes may have been due to mechanical irritation induced by the plastic bags or chemical substances released by these bags as suggested previously (Bakhiet, 2008).

The hyperplasia, degeneration, oedema, cellular vacuolation and karyolysis observed in the mucosa of the rumen could be the effects of plastic bags (Raoofi *et al*, 2012; Bakhiet, 2008). According to Robbins *et al*. (1984), pathologic hyperplasia constitutes precursors from which cancerous proliferation may eventually arise.

Hence the papilloma-like hyperplasia of the mucosa of the rumen with prominent interlocking rete pegs projecting into the underlying submucosa and nodules observed grossly in this study could be an indication of an early process. Further investigations are however needed to elucidate this assertion. The prominent mucosal and submucosal oedema, cellular oedema and spongiosis observed in this study may be due to fluid seeping through the ruminal walls into the tissues because of the physical damage caused by the plastic bags or generalized oedema resulting from the severe starvation and cachexia (Jubb *et al.*, 1985).

The degeneration, necrosis and fibrosis of the connective tissue with increased mononuclear and polymorphonuclear cell infiltrations into the submucosa could be due to progressive cellular change attracting phagocytic cells to remove destroyed ruminal tissue and provide stimuli for tissue repair after irritation and cellular damage by the plastic bags. Dowan *et al.* (1995) previously reported similar inflammatory response after intra-arterial injection of plastic particles into the brain parenchyma of a sheep. The exostosis of the polymorphonuclear cells and increased vascularization with congestion of the blood capillaries in the submucosa can occur in the early stages of regeneration of the epithelia in response to chronic irritation by the plastic bags. Hyperplasia, oedema and separation of the muscular layer and serosa observed could be due to the pressure exerted on the ruminal wall by the plastic bags as previously suggested (Bakhiet, 2008).

Conclusion

The presence of plastic bags in the rumen of goats, depending on the quantity and duration, caused significant damage to the ruminal wall and the ruminal papillae as well as major body organs. This could adversely affect digestion leading to impaired nutrient absorption, ill-health and death of the animal. Goats should therefore not be left to roam freely if they are to be prevented from ingesting plastic bags and suffering the effects on their overall well-being, productivity and production.

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Leaching of trace metals from mixed electronic waste using four extraction methods

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ABSTRACT

Rapid developments in technology in recent times, coupled with increasing demands for electronic products, have led to a rapid increase in electronic waste (e-waste) in the environment. The leaching of metals from e-wastes into the environment is a potential health and environmental hazard. The present study investigates the leaching potential of fourteen metals (Ag, Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Na, Ni, Pb and Zn) in mixed waste printed circuit boards (PCBs) and plastic housing (PH) from recycling/dump sites. The assessment was carried out using rain water, deionized water, synthetic precipitation leaching procedure (SPLP) solution and seawater over a period of 192 hours at regular intervals. The data obtained was compared to TCLP and WET limits. The toxicity characteristic potential of the elements extracted by these solvents were in the order deionized water>rainwater>SPLP>seawater. The levels of the 14 elements studied were generally low and in varying concentrations, with Ca, K, Mg and Na being relatively higher in all the leaching fluids. The results further proved that leaching from PCBs and PH was a significant source of trace metals from recycling/dump sites. Therefore, proper handling and disposal of these waste materials should be encouraged to lessen their impact on the environment.

Keywords: E-waste, Plastics, Printed circuit boards, Metals, Leaching fluids, Toxicity, Dump site

Introduction

Rapid technological advancement, coupled with the proliferation of electrical and electronic equipment (EEE), has made life easier for humankind. Nonetheless, the increasing demand for EEE has resulted in the generation of unwanted, discarded or malfunctioning electrical and electronic appliances (Michael and Sugumar, 2013). Newer EEE are brought into the market, rendering older versions obsolete. This pattern contributes to the soaring quantities of e-waste globally.

Currently e-wastes are known to present potential threats to the environment and human health as a result of growing volumes of the waste stream and the presence of toxic elements. It is estimated that about 20 to 50 million tons of e-waste are generated worldwide each year; less than 5% of all municipal solid waste (Keith *et al.*, 2008). The number of mobile phone subscribers worldwide is reckoned to reach a global population mark of 6.8 billion which will translate into more e-waste components in the years to come. This phenomenal growth is primarily driven by developing countries, which accounted for over 80% of all new subscriptions as at 2011 (Yadav and Yadav, 2014). This suggests that developing countries, especially Sub-Saharan African countries (including Ghana), will become the hub of e-wastes as EEE approach their end of life (EoL), besides the already existing volumes of e-waste. Other electronic devices also contribute significantly to the waste disposal problem; for instance, about 100 million obsolete computers and televisions are disposed of annually in the United States alone (Hileman, 2006).

Great concerns have been raised over the management practices for waste electrical and electronic equipment (WEEE) in developing countries, where potentially toxic materials are discarded with municipal solid waste at open dump sites and even in surface waters, the sea (Osibanjo and Nnorom, 2007) and on tracts of land (Michael and Sugumar, 2013). A large portion of the e-waste is also recycled. Most e-waste contains toxic inorganic [lead (Pb), cadmium (Cd), mercury (Hg)] and organic [polybrominated diphenyl ethers (PBDEs) and dioxins] substances. The improper disposal of WEEE can pose environmental problems like leaching of heavy metals if not suitably managed (Zhou et al., 2013). Subsequently, the e-waste components corrode and the heavy metals become mobile and travel with the leachate, eventually entering the environment. Thus leaching studies of toxic substances from e-waste in landfill scenarios is very necessary for an assessment of their potential environmental impact in the long run.

Accordingly, several nations have carried out comprehensive studies on environmental contamination resulting from leachability of heavy metals in e-waste (Keith *et al.*, 2008; Kiddee *et al.*, 2013; Oliverira *et al.*, 2012; Yadav *et al.*, 2014) and e-waste recycling activities (Osibanjo and Nnorom, 2007). Findings from these works have enabled policy makers in developed nations to establish legal directives to effectively manage e-waste. However, this is not the case with most third world nations. In Ghana, there is no report on heavy metal leachability from e-waste materials. This is in addition to the paucity of data on e-waste composition and management. However, the few studies so far conducted have dwelt on e-waste contamination of soils in sites and health hazards among the workers in this industry within the Agbogbloshie vicinity (e.g. Amankwaa, 2014; Nukpezah et al., 2014; Amfu-Otu, 2013; Asante et al., 2012; Atiemo et al., 2012). In the present work, the toxicity potential of metal leaching from mixed e-waste (e.g. PCBs and PH) of WEEE from e-waste recycling/dump sites has been assessed using rain water, deionized water, SPLP and sea water over a period of 192 hours. The data are compared with the prescribed toxicity characteristic leaching procedure (TCLP) limit for classifying the e-waste as hazardous (or not). This work also provides a simulation of the metal toxicity potential of the e-waste discarded at open dump or landfill sites and the effectiveness of the four leaching fluids from WEEE. The study additionally gives information on the metal leaching from mixed e-waste in natural leaching solution (e.g. rainwater, sea water) without using any chemical or buffering agent.

Methods

Sample Collection and Preparation

E-waste materials litter the environment in heaps in many locations across the country, particularly the regional cities. In this study the e-waste materials were randomly collected from two large e-waste recycling centres/dump sites at Agbogloshie and Ashaiman motorway (Fig 1). The components consisted mainly of discarded plastic housing and printed circuit boards from different types of e-waste products (e.g. mobile phones, printers, computers, televisions, etc.). The components were reduced in size (about 1cm) by crushing them with hammer before subjecting them to the leaching test.

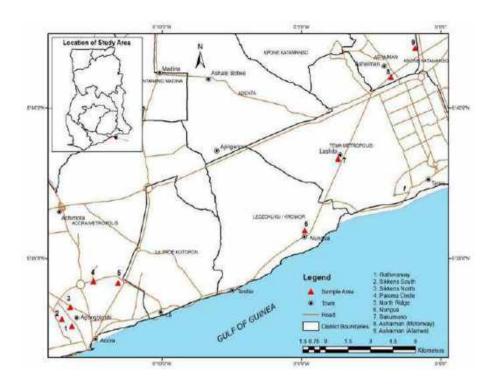


Fig. 1: Map of study area

Leaching Solution

Synthetic precipitation leaching procedure (SPLP) extraction fluid, a mixture of sulphuric (H_2SO_4) and nitric (HNO_3) acids (3:2), was added to water until pH reached 4.2±0.1. Sea water samples were collected from the Nungua beach in Accra Metropolis along with rain water in the month of June 2014 using acid cleaned polypropylene bottles. De-ionized water used was obtained from the Department of Chemistry, University of Ghana, Legon, Ghana.

Leaching Test

This laboratory experiment was designed to mimic the attitude of many Ghanaians in disposing of wastewater or sachet water on heaped piles of wastes (e.g. garbage, e-waste, etc.) besides other environmental conditions (rainfall and sea sprays). Based on this, four (4) different solvents (de-ionized water, rain water, sea water and SPLP) were chosen for the leaching test. Fifty grams (50

boards) was used for each solvent in a liquid to solid ratio of 4:1 in eight batches corresponding to 24, 48, 72, 96, 120, 144, 168 and 192 hours. The leaching experiment was carried out in a column, with gentle stirring. The leachates were drained according to the defined time periods for each extractant. The modified synthetic precipitation leaching procedure (SPLP) was also adopted to evaluate the leachability of contaminants from soils and waste samples under acid precipitation conditions (Li et al., 2009). The leachates were acid digested, cooled, diluted and finally filtered through a filter paper (0.45µm pore size) using the pressure filtration pump, then analyzed with ICP-OES for 14 selected trace metals (silver (Ag), aluminum (Al), arsenic (As), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn)).

g) of sample (a mixture of plastic housing and circuit

Quality Control and Assurance

For quality control and assurance purposes, the leaching tests were performed in triplicates to ensure accuracy and reproducibility. A procedural blank and solvent blank were run for each batch of samples. Recoveries for surrogate standard used were in the range of 86-105 %. All glass and plastic wares used were cleaned with 5% nitric acid (HNO₃) overnight and rinsed thoroughly with deionized water before being dried and stored. Analytical grade reagents were used.

Data Analysis

The experimental data obtained were evaluated by descriptive statistics using Microsoft Excel.

Results and discussion

The concentrations of 14 trace metals detected in four leaching fluids (rain water, deionized water synthetic precipitated leaching procedure-SPLP solution and sea water) over 24, 48, 72, 96, 120, 144, 168 and 192 hours are presented in Tables 1-5. The studied elements were present in varying concentrations in the 4 different extraction fluids. Averagely, the macro elements (Na, Ca, K, Mg), and Fe and Zn concentrations in general were extremely high, particularly in de-ionized and rain water, except the SPLP solution. However, the mean concentrations of As, Cd, Cr, and Pb were the lowest for all the solvents. Overall, the toxicity characteristic potential of the elements extracted by these solvents were in the order deionized water>rainwater>SPLP>seawater.

Table 1: Average concentrations (mg/L) of trace metals in the four leaching solutions

Sample	Rain water	Sea water	SPLP solution	De-ionized water
Ag	0.031	0.027	0.099	0.065
AI	12.31	3.86	9.72	11.59
As	0.024	0.014	0.021	0.068
Са	1083	157	7.39	1267
Cd	0.02	0.005	0.021	0.690
Cr	0.002	0.213	0.035	0.159
Cu	14.15	0.145	0.282	15.23
e	72.83	2.95	5.33	48.20
<	238	38.94	49.98	295
Иg	105	27.75	0.645	123
Na	494	10.88	24.29	614
Ni	3.68	1.03	1.56	30.20
Ър	23.79	0.001	0.004	0.002
Zn	42.85	0.813	1.43	68.47

Rain water

Table 2: Mean concentrations (mg/L) of extractable heavy metals in e-waste using rain water.

Sample	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs	Average	Std	Min	Мах	TCLP Limit
Ag	0.002	0.019	0.011	0.019	0.025	0.045	0.049	0.079	0.031	0.025	0.002	0.079	5
Al	2.12	1.58	2.20	3.30	3.41	9.66	10.25	66.00	12.31	21.96	1.58	66.00	-
As	nd	nd	nd	0.019	0.029	nd	nd	nd	0.024	0.007	0.019	0.029	5
Ca	617	866	861	940	1001	946	1185	2249	1083	497	617	2249	-
Cd	0.011	0.012	0.012	0.013	0.014	0.016	0.022	0.057	0.02	0.016	0.011	0.057	1
Cr	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.001	0.001	0.003	5
Cu	2.86	7.95	4.76	9.64	9.70	14.46	14.97	48.83	14.15	14.63	2.86	48.83	-
Fe	11.67	62.50	53.00	31.69	80.50	97.25	113	133	72.83	41.10	11.67	133	-
К	167	190	196	216.87	229	255	228	418	238	77.94	167	418	-
Mg	69.24	80.95	85.42	90.98	96.96	96.39	215	102	105	45.84	69.24	215	-
Na	369	398	424	560	392	655	539	613	494	111	369	655	-
Ni	1.34	2.04	2.61	3.80	4.85	4.30	4.21	6.32	3.68	1.62	1.34	6.32	-
Pb	19.63	30.62	56.54	63.18	1.53	3.26	7.31	8.24	23.79	24.26	1.53	63.18	5
Zn	22.54	29.42	34.78	34.23	34.53	41.45	52.02	93.86	42.85	22.33	22.54	93.86	-

hrs: hours, std: standard deviation, min: minimum value, max: maximum value, nd: not detected

Table 2 shows the distribution and levels of the leachable metals in rain water for the entire test period of 192 hours. The 14 different metals were detected in varying concentrations, with many of them having low concentrations except the elements Na, K, Fe, Mg, Ca, and Zn which were relatively higher, with mean values 494 (Na), 238 (K), 72.83 (Fe), 105 (Mg), 1083 (Ca), and 42.85 (Zn) mg/L respectively. This depicts the different solubility properties of the metals; thus Ca had the highest concentration among all elements in the rain water. The levels of Zn in the leachate were quite high and its presence is attributed to its use as a coating for ferrous metals. This may be likened to the report by other researchers (e.g. Yadav and Yadav, 2014; Li et al., 2009) who demonstrated that Zn as an external layer dissolves first; hence being high with almost uniform concentrations. Fe also had a relatively high overall concentration, with the observed pattern indicating an increase in concentration with time. Furthermore, the presence of Al and Cu is explained by their use as small connecting wires embedded in the plastics and as a major component of most EEEs, especially the hard disc drives of computers, respectively (Li et al., 2009; Yadav et al., 2014). The presence of Ni with an average concentration

of 3.68mg/L is due to its application as alloy or the metal parts on the printed wire board (PWB) (Vuorinen *et al.,* 2006; Yadav *et al.,* 2014).

By contrast, the concentrations of well-known toxic heavy metals in e-waste, such as As, Cr and Cd, were very low. The behavior of As could not be explained. It was only detected in the 4th and 5th days averaging 0.24mg/L whereas Cr and Cd leached in minimal quantities with an average accumulation of 0.02 and 0.002mg/L respectively. Their presence is attributed to their addition as plastic polymers (pigments), UV stabilizers, etc. (Piorek, 2004; Nnorom and Osibanjo, 2009).

Pb concentration ranged from 1.53 to 63.18 mg/L with an average of 23.79 mg/L which exceeded the TCLP limit of 5 mg/L (Table 2). The concentration of Pb leached in rainwater was much higher than the other three leaching fluids (Fig 2). However, these values are lower compared to those from previous studies. Lincoln *et al.* (2007) reported 87.4 mg/L Pb and Keith *et al.* (2008) reported 83.3 mg/L Pb in PWBs. The major source of Pb is Pbcontaining solders on motherboards. Overall, rainwater is slightly acidic and very low in dissolved minerals; as such, it is relatively aggressive during leaching.

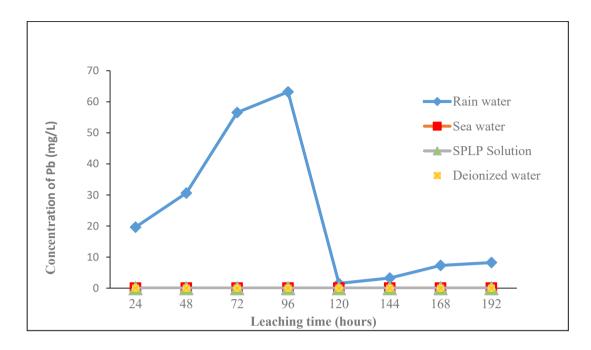


Fig 2: Leaching pattern of Lead (Pb) in four leaching fluids

Sea water

Leaching results from 14 elements using sea water are presented in Table 3. Among the leaching solutions used, sea water had the lowest concentration of metals. However, similar to rainwater, K, Ca, Na, and Mg were relatively higher compared to other elements present in the sea water leachate. According to Keith *et al.* (2008), the amount of elements leached is usually dependent on the type of extraction fluid. Therefore, the low levels of metals in this extraction fluid might be due to the saturated nature of the sea water. Among the eight heavy metals on the US-EPA D list of toxic metals, Ag, As, Cd, Cr and Pb did not exceed their TCLP threshold limits with average concentrations of 0.027, 0.014, 0.005, 0.213 and 0.001 mg/L respectively (Table 3). Pb had concentrations of 0.001 mg/L throughout the leaching period except the first 24 hours. The toxicity characteristic potential of the elements in sea water was almost non-existent as elements detected were very low.

Sample	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs	Average	Std	Min	Мах	TCLP Limit
Ag	0.018	0.022	0.024	0.023	0.028	0.035	0.026	0.038	0.027	0.007	0.018	0.038	5
Al	1.12	1.57	2.19	3.83	3.20	7.15	5.42	6.40	3.86	2.26	1.12	7.15	-
As	nd	nd	nd	0.01	0.012	0.015	nd	0.017	0.014	0.003	0.01	0.017	5
Са	139	147	151	157	165	166	167	166	157	10.62	139	167	-
Cd	0.001	0.003	0.004	0.005	0.006	0.01	0.007	0.005	0.005	0.003	0.001	0.01	1
Cr	0.053	0.105	0.174	0.225	0.312	0.438	0.367	0.032	0.213	0.149	0.032	0.438	5
Cu	0.263	0.131	0.119	nd	0.006	nd	nd	0.206	0.145	0.097	0.006	0.263	-
Fe	0.725	1.49	4.01	2.94	4.08	5.42	2.98	1.96	2.95	1.54	0.725	5.42	-
К	33.52	35.09	35.00	37.40	37.75	40.94	49.13	42.66	38.94	5.15	33.52	49.13	-
Mg	27.66	27.66	27.69	27.71	27.73	27.78	27.90	27.88	27.75	0.095	27.66	27.90	-
Na	6.58	8.50	9.63	11.21	11.32	12.48	14.41	12.93	10.88	2.54	6.58	14.41	-
Ni	0.603	0.660	0.840	0.965	1.10	1.23	1.52	1.34	1.03	0.325	0.603	1.52	-
Pb	nd	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0	0.001	0.001	5
Zn	0.650	1.28	0.856	0.831	0.769	0.500	0.944	0.675	0.813	0.232	0.500	1.28	-

Table 3: Mean concentrations (mg/L) of extractable heavy metals in e-waste using sea water.

hrs: hours, std: standard deviation, min: minimum value, max: maximum value, nd: not detected

SPLP

Table 4 shows the concentrations of trace metals from the SPLP solution. Generally, metals leached minimally over the entire period. Except for K and Na, the remaining metals recorded mean levels below 10 mg/L in the entire leaching cycle. Concentrations of K and Na ranged from 40.93 to 62.20 mg/L and from 14.05 to 36.15 mg/L respectively. The leaching pattern for the SPLP extraction followed the same order as the other extraction fluids. The pattern was a little more consistent for concentrations of leached metals throughout the cycles at regular intervals. Compared to previous studies (e.g. Li *et al.*, 2009; Zhou *et al.*, 2013), SPLP leached minimally in this work, which could be a result of the differences in environmental conditions as well as the technique and method employed.

Sample	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs	Average	Std	Min	Мах	TCLP Limit
Ag	0.021	0.034	0.044	0.055	0.066	0.085	0.299	0.184	0.099	0.095	0.021	0.299	5
Al	2.99	6.30	7.73	9.67	13.61	12.47	13.13	11.85	9.72	3.78	2.99	13.61	-
As	0.017	0.018	Nd	0.019	0.021	0.023	0.024	0.022	0.021	0.003	0.017	0.024	5
Ca	6.76	6.03	7.74	6.23	6.76	6.91	10.52	8.14	7.39	1.45	6.03	10.52	-
Cd	0.008	0.023	0.037	0.045	0.006	0.007	0.023	0.016	0.021	0.014	0.006	0.045	1
Cr	0.006	0.013	0.028	0.042	0.053	0.085	0.03	0.021	0.035	0.025	0.006	0.085	5
Cu	0.069	0.119	0.238	0.263	0.331	0.356	0.438	0.438	0.282	0.137	0.069	0.438	-
Fe	1.84	3.37	4.53	5.21	5.73	6.47	7.85	7.68	5.33	2.07	1.84	7.85	-
К	40.93	43.66	45.97	44.52	49.34	53.83	62.20	59.38	49.98	7.75	40.93	62.20	-
Mg	0.463	0.506	0.588	0.613	0.638	0.675	0.981	0.694	0.645	0.157	0.463	0.981	-
Na	14.06	16.51	16.87	22.17	23.12	32.17	36.15	33.29	24.29	8.54	14.06	36.15	-
Ni	1.13	1.25	1.34	1.50	1.59	1.75	2.09	1.87	1.56	0.33	1.13	2.09	-
Pb	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.003	0.004	5
Zn	0.075	0.619	0.850	0.888	1.29	1.42	3.83	2.47	1.43	1.19	0.075	3.83	-

Table 4: Mean concentrations (mg/L) of extractable heavy metals in e-waste using SPLP solution.

hrs: hours, std: standard deviation, min: minimum value, max: maximum

De-ionized water

Table 5 displays the concentrations of elements from the e-waste pile in de-ionized water. In all, the amount of metals leached from deionized water was higher than the other three leaching solutions but followed a similar trend to that of the rainwater. Comparing this to sea water which had the least leaching, it is clear that deionized water is devoid of ions and species that did not enhance the leaching of elements. Ca had the highest concentration of the leached metals ranging from 2695 to 2694 mg/L. Additionally, Na, K and Mg had high concentrations with the respective averages of 614, 295, and 123 mg/L. Fe, Zn, and Ni, considered as having potential toxicity characteristics, were relatively high and leached in a gradual manner to the end (Fig. 3). Another pattern typified by Cr, Cd, and Ag is shown in Fig 4. Here, the metal levels increased gradually and then sharply towards the end of the leaching cycle. On the other hand, Ni concentrations exceeded the waste extraction test (WET) regulatory limit of 20 mg/L (CDTSC, 2006).

Of the toxic elements, As, Pb and Cd, Cd was the highest with an accumulated concentration of 3.28 mg/L over the entire period and an average value of 0.69 mg/L. This concentration of Cd exceeded the TCLP and WET regulatory limits of 1.0 mg/L. Pb was almost constant in concentration in the hours found, averaging 0.002 mg/L while As averaged 0.068 mg/L. Thus As, Cd and Pb concentrations were within TCLP limits.

Sample	24	48	72	96	120	144	168	192	Average	Std	Min	Мах	TCLP Limit
	hrs												
Ag	0.148	0.105	0.018	0.018	0.057	0.061	0.037	0.079	0.065	0.045	0.018	0.079	5
Al	27.25	15.50	32.65	2.45	3.37	3.42	2.55	5.53	11.59	12.19	2.45	5.53	-
As	0.069	0.016	0.223	nd	0.018	0.016	nd	nd	0.068	0.089	0.016	0.233	5
Са	738	858	1081	1177	1169	1177	1245	2695	1267	603	738	2695	-
Cd	0.093	0.148	0.184	0.243	0.277	0.308	0.993	3.28	0.690	1.08	0.093	3.28	1
Cr	0.090	0.108	0.129	0.141	0.141	0.183	0.201	0.276	0.159	0.06	0.090	0.276	5
Cu	6.33	6.93	7.73	8.07	9.65	11.20	17.74	54.17	15.23	16.15	6.33	54.17	-
Fe	9.60	39.16	41.02	41.68	46.20	54.70	63.65	89.59	48.20	22.88	9.60	89.59	-
К	183	193	232	290	291	321	313	539	295	112	183	539	-
Mg	79.80	82.10	101	107	111	128	134	244	123	52.18	79.80	244	-
Na	398	485	549	588	652	678	719	842	614	140	398	842	-
Ni	13.89	18.73	29.82	29.22	29.24	33.26	36.32	51.13	30.20	11.24	13.89	51.13	-
Pb	nd	nd	0.001	0.001	0.001	0.001	0.003	0.003	0.002	0.001	0.001	0.003	5
Zn	17.59	26.40	58.57	59.75	82.74	82.59	95.33	125	68.47	35.51	17.59	125	-

Table 5: Mean concentrations (mg/L) of extractable heavy metals in piled e-waste material using de-ionized water.

hrs: hours, std: standard deviation, min: minimum value, max: maximum value

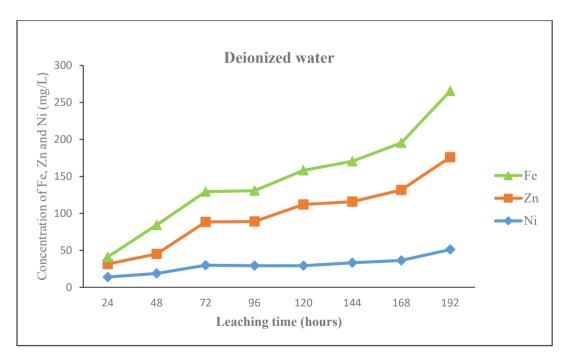


Fig. 3:. Leaching pattern of Fe, Zn and Ni in deionized water

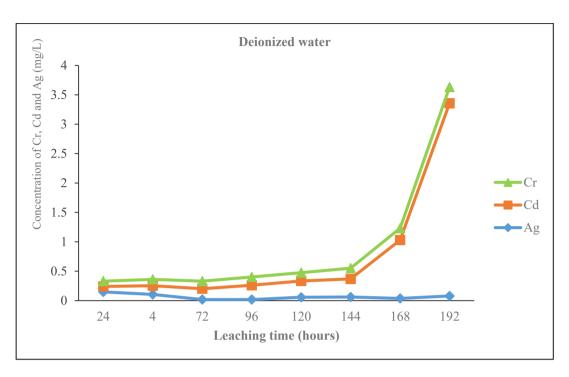


Fig. 4: Leaching pattern of Cr, Cd and Ag in deionized water

Conclusion

The leachability of 14 elements from four extraction fluids, notably rain water, sea water, deionized water and SPLP solution was studied. De-ionized water was the most predominant extraction fluid from which significant elements could leach in relatively higher concentrations while sea water extraction fluid showed the least leaching. Ca, K, Mg and Na were relatively higher in all the leaching fluids.

Comparing results obtained in this study for rainwater with the TCLP limit, concentrations of Pb were about 4-12 times higher than the TCLP limit of Smg/L.

The results further confirmed that these trace elements do leach out into the environment, which could pose a significant danger to human health and the environment due to the poor management practices of these waste materials. Therefore, proper handling and disposal of these waste materials can be embarked on to mitigate the environmental impact. Further studies could be carried out, investigating several types of e-waste components such as steel housing, batteries, and CRTs.

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Mechano synthesis of co-crystals of Sulfamethoxazole and 8-Hydroxy-7-lodoquinoline-5-Sulfonic acid based on Green Chemistry principles

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Abstract

Co-crystals have been used to produce new drugs and to improve the physical properties of drugs. Some disease causing germs have developed resistance to newer drugs, hence there is a general trend to take 'old' but efficacious drugs, improve their properties and use them to treat the conditions. This study used mechano-synthesis to produce a co-crystal of two active pharmaceutical ingredients (API), Sulfamethoxazole and 8-hdroxy-7-iodoquinoline-5-sulfonic acid (Ferron), based on Green Chemistry principles. The two APIs were combined by mixing, grinding and kneading. The resulting co-crystals were characterized using analytical methods such as spectroscopy, thermal analysis and diffractometry. In-Silico techniques based on computer modelling using Gaussian 09 and VEDA 4 software were used to interpret the vibrational spectrum of the co-crystals. The study found that kneading gave the highest yield compared to mixing and grinding. The study also confirmed that a combination of the analytical methods was necessary to characterize a co-crystal. Co-crystals of Sulfamethoxazole and 8-Hydroxy-7-lodoguinoline-5-Sulfonic acid can therefore be formed by kneading, implying that the production of the co-crystals can be done using a process that reduces pollution by the minimal use of solvents. Another implication is that Sulfamethoxazole, whose use has been minimised because of problems such as allergic reactions from patients, can be looked at again and used to produce 'polydrugs' which can be used to manage conditions at relatively cheaper costs.

Keywords: Mechano-synthesis, co-crystallization, DSC, VEDA 4, Green Chemistry, Sulfamethoxazole, 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid

Introduction

Co-crystals have been defined differently by several authors, but in all these definitions, a common theme is that a co-crystal consists of two or more non-ionic compounds in a single crystalline phase in a given stoichiometric ratio held together by hydrogen bonds or other non-covalent bonds (Dunitz, 2003 ; Rodríguez-Hornedo, *et al.*, 2007).The components in a co-crystal maintain their chemical properties even after the process since no covalent bonds are broken or formed. Crystalline salts on the other hand interact electrostatically and the components are ionized (Stahly, 2007). Cocrystals are useful especially in the pharmaceutical industry in the design and preparation of drug forms in which one or more of active pharmaceutical ingredients or components are non-ionic.

This usefulness stems from the possibility of improving the properties of drugs by forming co-crystals of the APIs. Some of the documented properties of drugs which have been improved are bioavailability and/or dissolution of drugs which have poor solubility; chemical stability and melting point (Blagden *et al.*, 2007; Kotak *et al.*, 2015).

Old drugs whose use has been discontinued either due to the discovery of newer drugs or because of some unfavourable properties discovered in their use have recently attracted renewed attention. This is because of the emergence of drug-resistant strains of the disease causing agents. Antibiotics and antibacterials fall in this category (Falagas *et al.*, 2008; Maviglia *et al.*, 2009; Falagas & Kasiakou, 2005; Giamarellou & Poulakou, 2009).

An additional advantage that drugs formed using cocrystals have over crystalline salts is that many more numerous neutral compounds (mainly GRAS¹) can be used in drug formulations, rather than the relatively fewer counterions which are used in crystalline salts (Halden, 2014).

The use of co-crystallization in the design and production of drugs is one of the ways that scientists can protect people and the environment at the same time since it promotes the use of Green Chemistry principles. Green Chemistry uses methods and conducts reactions at low or ambient temperatures; reduces the use of chemicals, which in turn reduces the generation of hazardous wastes or substances that must be safely disposed of (Anastas & Warner, 1998).

Though the production of co-crystals by the slow evaporation of a solvent in which the components are dissolved is very common, solid grinding of the components has also been used and is still being used to produce co-crystals. In some instances, cocrystals produced by solid-grinding exhibit different characteristics from those produced using solvent evaporation (Bruni *et al.*, 1999).

Mechano-synthesis is defined as co-crystal formation in the solid state based on mechanical activation of materials by processes such as grinding or milling (RodríguezHornedo *et al.*, 2007). Here the components are ground together with a mortar and pestle or in a mixer mill to induce co-crystal formation. This method uses at least two of the Green Chemistry principles: low temperature and very little or no crystallization solvent (Alatas, *et al.*, 2013).

A complex set of reactions take place when two solid substances are ground together, from the breaking of the crystalline structure to the production of cracks and the development of new surfaces (Fernández-Bertran, 1999). This method of synthesis is already in use in the local pharmaceutical industry since it does not require sophisticated machinery and/or solvents which may be needed when using solvent evaporation methods.

This study is, therefore, aimed at co-crystallizing Sulfamethoxazole, a well-known Active Pharmaceutical Ingredient (API), used as an antibiotic, with a lesser known but old API, 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid, which has anti-protozoan properties, using Green Chemistry principles.

Experimentals

Sulfamethoxazole (SMX), 98% purity Analar grade, was purchased from Central Drug House (CDH), New Delhi, India, and was used without further purification. 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid (8H7QS), Spot-Test reagent grade, was purchased from BDH Chemicals Ltd., Poole, England, and was used without further purification. Ethanol, 99.7 – 100%, Analar grade, was purchased from Pharmacos Banbury, United Kingdom, and was used without further purification. Distilled water was prepared using an Accumax (India) stainless steel water distillation unit.

A solvent system consisting of Ethanol and distilled Water in a 1:1 volume ratio was prepared and used in the study.

¹ Generally Recognised as Safe

Mechano-Synthesis

A Fritsch 'Pulverisette 2' Mortar Grinder was used for both grinding and kneading. The ground sample was prepared by weighing 0.5020 g of SMX and 0.6978 g of 8H7QS into the mortar bowl and grinding for 30 minutes. The kneaded sample was prepared by weighing 0.5020 g of SMX and 0.6978 g of 8H7QS into the mortar bowl, adding 5 drops of the solvent system and kneading for 30 minutes. The 'mixed' sample was prepared by weighing 0.5020 g of SMX and 0.6978 g of 8H7QS into the mortar bowl and mixing with a spatula for 10 minutes. The powdered products were then analysed.

Infra-Red Spectroscopy

The infra-red spectra of a suitable quantity of crystalline Sulfamethoxazole (SMX), crystalline 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid (8H7QS) and co-crystals (of 8H7QS and SMX) were measured in the 4000 – 400 cm⁻¹ region at 4 cm⁻¹ resolution and an average of 23 scans on a Perkin Elmer Spectrum Two FT-IR Spectrophotometer. The spectra were elaborated using the Spekwin 32 (Menges, 2015) software to convert the transmittance to absorbance.

Ultraviolet Spectroscopy

A 0.0128M solution of each of the pure compounds and the co-crystals was prepared by dissolving the compound/powdered co-crystal in a mixed solvent system of Ethanol:Water (1:1 by vol.). The solution was used subsequently for the Ultraviolet spectroscopy. The UV spectra of Sulfamethoxazole, 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid and the co-crystal were determined using a Spectroquant Pharo 300 UV-Vis Spectrophotometer in the region 190 nm – 1100 nm. The spectra were elaborated using the Spekwin 32(Menges, 2015) software.

Powder X-ray Diffraction:

Suitable quantities of Sulfamethoxazole (SMX), 8-Hydroxy-7-Iodoquinoline-5-Sulfonicacid (8H7QS) and the powdered co-crystals of 8H7QS and SMX were introduced into a PANalytical Empyrean X-ray Diffractometer with a copper anode. The K –alpha1 wavelength was 1.54060Å and the K-alpha2 wavelength was 1.39225Å.

Each sample was scanned continuously in steps of $.08^{\circ}$ in 3 seconds with 2θ from 5.04° to 80° while spinning to acquire the Powder X-ray Diffractograms. Spekwin 32 (Menges, 2015) software was used to sketch the diffractograms.

Thermal Analysis

An appropriate quantity of crystalline Sulfamethoxazole (SMX), 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid (8H7QS), and the co-crystals of 8H7QS and SMX were analysed using the thermal analytical methods of Thermo Gravimetry, TGA, and Thermal Calorimetry, DSC. The equipment used was TA Instruments SDT-Q600 V20.9 Build 20 (Bernstein, 2002).

The DSC results were collected on a Mettler DSC 822e under an inert atmosphere (nitrogen at 20 ml/Min) at a heating rate of 10 deg C per minute (Lu *et al.*, 2008).

Crystal Structure

The crystal structure for Sulfamethoxazole was acquired from Crystallography Open Database (Gražulis *et al.*, 2012; Grazulis *et al.*, 2009; Downs & Hall-Wallace, 2003) as determined and published by Perlovich G. L. *et al.*, 2013), was visualized using OLEX 2 software (Dolomanov, Bourhis, Gildea, Howard, & Puschmann, 2009) and plotted using ORTEP-plot in PLATON version 271014.

X-ray crystal structure data on 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid was obtained from Crystal Open Database (COD) (Information card for 2005313) as published by Balasubramanian and Muthiah (1996), was visualized using OLEX 2 software (Dolomanov *et al.*, 2009) and plotted using ORTEP-plot in PLATON version 271014.

Results and Discussions

Infra-Red Spectroscopy

The experimentally determined infra-red spectrum of 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid showed no distinct peaks above the 3000 cm⁻¹ wavenumber. This confirms the IR spectrum as reported in the data base of NIST (*8-Hydroxy-7-iodo-5-quinoline sulfonic acid*, 2011)

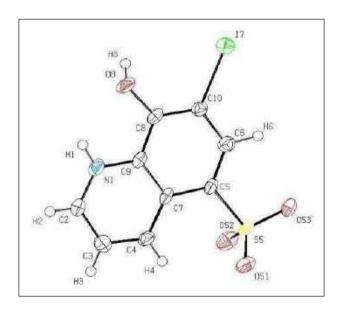
Using IR spectroscopy, the structural consequences of combining the two compounds using mechano-synthesis were shown using the vibrational modes of the bonds maintained or newly formed in the process (Thanigaimani *et al.*, 2015).

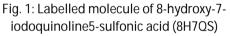
The number of atoms in 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid, 8H7QS, is 22 and that of Sulfamethoxazole, SMX is 28. The degrees of freedom which constitute vibrational motion for each of the compounds are therefore 60 and 78 respectively (Banks *et al.*, 2010).

The Total Energy Distribution, TED data generated from the Potential Energy Distribution, PED, was used in discussing the IR spectrum of the two starting compounds. The PED analysis is more accurate than the visualization of an atom movement to interpret a theoretical vibrational spectrum of a molecule. It also quantitatively describes the contribution to movement of a given group of atoms in a normal mode (Jamróz, 2013). The PED analysis is indispensible tool in serious analysis of the vibrational spectra. To perform the PED analysis it is necessary to define 3N-6 linearly independent local mode coordinates. Already for 20-atomic molecules it is a difficult task. The VEDA program reads the input data automatically from the Gaussian program output files. Then, VEDA automatically proposes an introductory set of local mode coordinates. Next, the more adequate coordinates are proposed by the program and optimized to obtain maximal elements of each column (internal coordinate.

Figure 1 is a labelled molecule of 8H7QS based on its crystal structure. The highest peak from the experimental data for 8H7QS was at 598 cm⁻¹ and this was shown in the calculated data at 600.82 cm⁻¹. This was attributed to

the stretching of the bond between C6 and H6. There was also a contribution from the torsion involving H2, C2, C3 and C4.





A C - C pure mode was determined at 1401 cm⁻¹ to be due to stretching between C4 and C7. This was shown experimentally at 1383 cm⁻¹. Most of the C – C bonds gave peaks theoretically varying from 850 cm⁻¹ to 1500 cm⁻¹, but these were mixed with other modes.

C – H had a pure stretching mode at a calculated wavelength of 1037.18 cm⁻¹; experimentally it was shown at 1042 cm⁻¹. At a wavelength of 707.52 cm⁻¹ (experimentally at 726 cm⁻¹), the stretching of both C-H and N-H were mixed, though the N-H contributed more to the absorbance.

The stretching of the O8-H8 bond was assigned the wavelengths 3254, 1710, 237 and 209 cm⁻¹, but these were not shown clearly experimentally. This may be due to the location of the lone pair of electrons in the antibonding orbital of H8 as determined with the NBO analysis.

The stretching of the S -O bond gave a pure mode at 397.3 cm⁻¹ (experimentally at 403 cm⁻¹).

Figure 2 below is a labelled molecule of SMX based on its crystal structure. The highest peak in the calculated spectrum of Sulphamethoxazole is at a frequency of 507.75 cm⁻¹ corresponds to the peak at 561.84 cm⁻¹ in the experimental spectrum. This is attributed to the torsion of the planes which pass through H2-C2-C3-C4 and H3-C3-C4-S1. The peaks around 3000 cm-1 frequency are due mainly to torsion of some planes in the molecule. For example, the peaks at 3377 cm⁻¹ and 3298 cm⁻¹, calculated to be at 3354.13 cm⁻¹ and 3262.24 cm⁻¹ respectively, are due in part to torsion in the plane through H1B-N1-C1-C2. However, the peaks at calculated frequencies of 3171.04 cm⁻¹ and 3088.09 cm⁻¹ are due to pure bending modes between O3-N3-C7 and H2A-N2-O2 respectively.

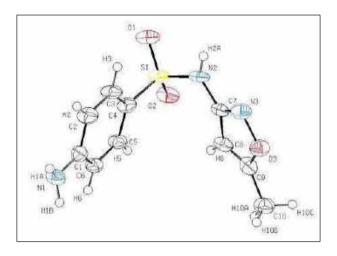


Fig. 2: Labelled molecule of Sulfamethoxazole (SMX)

The peak at 1509.14 cm⁻¹ in the calculated spectrum, corresponding to the peak at 1501 cm⁻¹ in the experimental spectrum, is also due to a pure mode of torsion in the plane C1-C6-C5-C8. The stretching modes between atoms in the group frequency region are mainly mixed with other modes except for the calculated modes at 1053.32 cm⁻¹ and 1038.59 cm⁻¹ which are single modes attributed to the stretching of the C3-H3 bond and the O2-N2 bonds respectively. These bonds are seen around the 1000 cm⁻¹ in the experimental spectrum. The pure bending mode between atoms H10A-C10-C9 gives a peak at a calculated frequency of 1300.24 cm⁻¹ which corresponds the peak at 1303 cm⁻¹ determined experimentally. The is a pure bending modes between the atoms H6-C6-C1 and N1-C1-C6 give peaks at calculated frequencies of 1361.9 cm⁻¹ and 1358.44 cm⁻¹ respectively. These peaks correspond to the peak at the frequency of 1363 cm⁻¹ determined experimentally.

Vibrational modes from bonds in 8H7QS, especially in the 'fingerprint' zone, which were not available when the co-crystal was formed by Grinding, Kneading and 'Mixing' have been given in Tables 1 to 3 respectively below. Also shown are the assignments as determined from PED calculations using the VEDA 4 software (Jamróz, 2015).

Calculated Experimental No. Assignment **IR Absorbance** Wavenumber **IR Absorbance** Wavenumber (cm⁻¹) (cm⁻¹) 1 53.1 1449.47 0.816 1459 13δΗΝC 2 18.56 1342.05 0.8246 1348 11τCCCS+11τCNCC 0.8529 3 1298 141.36 1291.64 4 55.53 1181.96 0.9561 1174 12vCC+11vCC+11δHCC 5 8.69 950.05 0.8817 956 78δOOS 6 40.33 867.54 0.857 936 11δHCC+22δHCC 7 3.87 856.15 0.8839 917 11νCC+10δHCC+16δICC 8 11.25 801.96 0.8565 847 11δHCC+16τHOCC+18τHCCC+18τHCCN

Table 1: Vibrational modes of absorptions of 8H7QS remaining after Grinding

	Calcu	llated	Expe	rimental	
No.	IR Absorbance	Wavenumber (cm ⁻¹)	IR Absorbance	Wavenumber (cm⁻¹)	Assignment
1	53.1	1449.47	0.816	1459	13δHNC
2	18.56	1342.05	0.8246	1348	11τCCCS+11τCNCC
3	141.36	1291.64	0.8529	1298	
4	55.53	1181.96	0.9561	1174	12vCC+11vCC+11δHCC
5	40.33	867.54	0.857	936	11δΗCC+22δΗCC
6	3.87	856.15	0.8839	917	11νCC+10δHCC+16δICC
7	11.25	801.96	0.8565	847	11δΗCC+16τΗΟCC+18τΗCCC+18τΗCCN

Table 2: Vibrational modes of absorptions of 8H7QS remaining after Kneading

Table 3: Vibrational modes of absorptions of 8H7QS remaining after 'Mixing'

	Ca	lculated	Ex	perimental	
No.	IR Absorbance Wavenumber (cm ⁻¹)		IR Absorbance	Wavenumber (cm ⁻¹)	Assignment
1	127.36	1616.22	0.8461	1621	12δHOC
2	53.1	1449.47	0.816	1459	13δHNC
3	18.56	1342.05	0.8246	1348	11τCCCS+11τCNCC
4	40.33	867.54	0.857	936	11δΗCC+22δΗCC
5	3.87	856.15	0.8839	917	11νCC+10δHCC+16δICC
6	11.25	801.96	0.8565	847	11δΗCC+16τΗΟCC+18τΗCCC+18τΗCCN
7	29.99	707.52	0.8658	726	10vCH+18vNH
8	23.56	620.03	0.8954	624	16τHNCC
9	182.25	600.82	1	598	10vCH+13τHCCC

Co-crystals from all mechano-synthesis methods used have similar IR spectra, especially in the 'finger print' region.

The wavenumbers at which the co-crystals absorbed which are not in the starting compounds are highest for the kneading method; these were at 3105 cm⁻¹, 1502 cm⁻¹, and 571 cm⁻¹. In the case of grinding, there was an absorption at only one new wavenumber, 3070 cm⁻¹. Similarly, when the compounds were mixed, there was only one new wavenumber with absorption at 3104 cm⁻¹.

This implies that new bonds are formed by kneading whose vibrational modes are at those wavenumbers.

Under all three syntheses, the 8H7QS bonds which were de-emphasised in the co-crystallization process were the bending of HNC, HCC, ICC, the twisting of CCCS, CNCC, HOCC, HCCC, and HCCN; and the stretching of CC.

Similar vibrational modes of both 8H7QS and SMX were not available in the co-crystals formed through either grinding or kneading. For example, the vibrational modes which absorbed between wavenumbers 1830 cm⁻¹ and 2170 cm⁻¹ in the spectrum of 8H7QS were all absent in the co-crystals, no matter the method of production. This is shown in Figure 3.

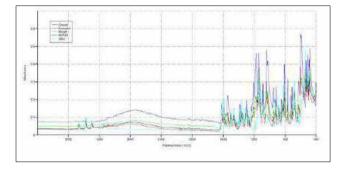


Fig. 3: IR spectra of both compounds and Co-crystals

At wavenumbers common to all products of the mechano synthesis, the lowest absorption were from the kneaded products as shown in Figure 4. This supports the conclusion that the kneading process produces the most co-crystals since the formation of the co-crystals has the effect of reducing the amount of starting compounds available which could have absorbed at those wavenumbers.

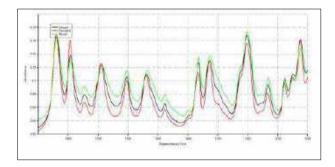


Fig. 4: IR spectra of both compounds and Co-crystals

UV Spectra

The 8H7QS had a peak at 297 nm and a shoulder between 325 nm and 345 nm while the SMX had a single peak at 300 nm.

When the two compounds were ground together for 30 minutes, the peak was at 292 nm and a shoulder between 320 nm and 350 nm.

When the compounds were kneaded together, the peak was at 297 nm and a shoulder between 315 nm and 345 nm.

When the compounds were 'mixed' together, the peak was at 296 nm and the shoulder between 315 nm and 350 nm. The UV spectra is given in Figure 5.

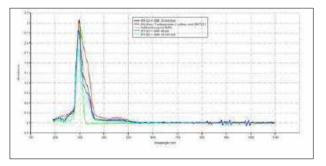


Fig. 5: UV spectra of Compounds and Co-crystals

Differential Scanning Calorimetry

The melting point of 8H7QS was found to be 269.25°C, while the decomposition temperature of the 8H7QS was determined to be 283.07°C. The literature value for the melting point of 8H7QS is 269-270°C. The literature value is close to the laboratory determined value of 268-270°C using the capillary method.

The melting point of SMX on the other hand is 168.30°C which is close to the literature value of 166-171.5°C and to that determined in the laboratory which was 170°C. The decomposition temperature was determined to be 250.07°C.

The melting point of the co-crystal produced is lower than the melting points of the starting materials and decreases in the order 'Mixed'> Ground>Kneaded as given in Table 4.

The latent heat of the co-crystals formed using the different synthesis methods is approximately the same, though it decreases slightly in the order 'Mixed'> Ground>Kneaded. This implies that the products are the same, but with different impurities which may be remnants of the starting compounds.

Table 4: Melting point and Latent Heat of compoundsand co-crystals

Sample	Melting point (°C)	Latent Heat (J/g)
8H7QS	269.25	
SMX	168.30	-112.34
8H7QS + SMX 'Mixed'	167.50	-38.85
8H7QS + SMX Ground	166.32	-38.96
8H7QS + SMX Kneaded	156.92	-40.25

Co-crystals were formed under the various synthesis conditions with the highest yield being when the powders were kneaded. The different forms of co-crystals all decomposed at temperatures (between 193-196°C) far lower than the decomposition temperatures of the starting compounds. Table 5 below gives the latent heat of decomposition of the co-crystals.

Table 5: Decomposition point of compounds and co-crystals

Sample	Onset Decom- position Point (°C)	Latent Heat of Decomposition (J/g)
8H7QS	283.07	-375.87*
SMX	250.07	471.65
8H7QS + SMX 'Mixed'	195.81	159.01
8H7QS + SMX Ground	193.16	230.91
8H7QS + SMX Kneaded	194.66	298.60

* Latent heat of melting and decomposition

Powder X-Ray Diffractometry

Generally, the intensities of the peaks were lowered when the co-crystals were formed.

All the major peaks in the diffractogram of the co-crystal formed by 'mixing' of 8H7QS and SMX are found as peaks at the same angles, 2 θ , in the diffractograms of 8H7QS and/or SMX except for the peak at $2\theta = 6.88^{\circ}$. Peaks at $2\theta = 11.76^{\circ}$ and 20.56° found in the diffractogram of 8H7QS however did not appear in the diffractogram

of the co-crystal. Likewise, peaks at $2\theta = 42^{\circ}$, 7.36° and 35.20° in the diffractogram of SMX did not appear in the diffractogram of the co-crystal.

In the case of the co-crystal formed by grinding, the diffractograms showed fewer peaks at angles common to both 8H7QS and SMX on one hand and the co-crystal on the other. For example, the peaks at $2\theta = 28.80^{\circ}$, 17.76° and 21.52° of the diffractogram of the co-crystal had no corresponding peaks at the same angles for either 8H7QS or SMX. Conversely, most of the major peaks in the diffractogram of either 8H7QS or SMX did not appear in the diffractogram of the co-crystal.

In the case of the co-crystal from kneading, the five peaks with the highest intensities of 8H7QS were not found in the diffractogram of the co-crystal, though three of the highest peaks of SMX were visible. The co-crystal had characteristic peaks at $2\theta = 17.52^{\circ}$ and 22.56° . The characteristic intense peak of SMX at $2\theta = 24.08^{\circ}$ was also not visible in the diffractogram of the co-crystal.

When the diffractogram of the co-crystals from the different synthesis were compared, characteristic peaks were found at $2\theta = 9.92^{\circ}$, 17.52° , 27.60° , 28.60° and 40.30° .

This indicates the formation of a product, in this case, a cocrystal, when the two compounds are brought together, with the best method of synthesis being kneading.

Conclusion

Co-crystals of 8-Hydroxy-7-Iodoquinoline-5-Sulfonic acid and Sulfamethoxazole were successfully produced using Green Chemistry principles by applying mechano-synthesis methods.

Kneading of the compounds together gives the highest yield of the co-crystals compared to mixing which gave the least.

Using UV spectroscopy alone does not show clearly the formation of the co-crystals.

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Understanding the smallholder farmers' crop production choices in the forest-savanna transition zone of Ghana

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ABSTRACT

Crop production choices made by smallholder farmers in Sub-Saharan Africa may enhance food security in the face of biophysical and socio-economic constraints. The forest savanna transition zone of Ghana is traditionally characterized by a multiplicity of uncertainties, to which farmers respond by cultivating a diversity of crops including cereals, root crops, tuber crops and leguminous crops. There is, however, little understanding of the driving forces that underpin individual farmers' choices of the type of crops they cultivate. Using the environs of the Kogyae Strict Nature Reserve as a case study, this paper investigates the array of crops available to the farmer, and what guides the farmer household in the choice of crops to cultivate. The study employed field-based techniques, namely focus group discussions and administration of questionnaires, for the data collection. The findings reveal that a combination of factors including knowledge of local conditions, length of the rainy period, market forces, dietary habits and crop maturity time dictate the choice of crops. The findings further indicate that local adaptation to climate variability has resulted in a shift of the farming calendar in the area from March/April to May/June since 2008; and in the intensification of the production of some lesser-known crops such as cowpeas and rice. Another important observation is that beyond what pertains at the community level, individual choices are based on relative risks posed and opportunities offered by each of the constraining elements. The study concludes that to a large extent, smallholder farmers, regardless of their experience, have to operate within constraints imposed by the biophysical environment and market forces, which potentially render crop production vulnerable to unexpected risks. Crop production choices are therefore an effective risk spreading strategy as a response to environmental vagaries and socio-economic uncertainties.

Keywords: Smallholder farmer, Forest-savanna, Crop production choices, Kogyae Strict Nature Reserve

Introduction

Farming decisions made by smallholder farmers concerning crop production are often influenced by both production and consumption factors (Timmer *et al.*, 1983). On production, the factors relate to biophysical conditions such as soil quality, moisture availability, pests and diseases, climatic factors, yield and constraints posed by institutional arrangements. The factors regarding consumption relate to traditional dietary values, taste and market forces, though the factors may differ across cultures. Most of these factors constitute uncertainties that may be beyond the farmer's control, yet smallholder farmers had demonstrated their ability to adapt to these inherent challenges through time-tested traditional knowledge to sustainably manage their production systems (Gyasi *et al.*, 2004). Farmers' responses, as a form of adaptation, are dynamic and in accordance with changing biophysical conditions. These may involve engagement in diverse management techniques such as moisture conservation, erosion control, pest control and soil nutrient enhancement strategies (Altieri and Koohafkan, 2008). One important adaptation strategy that most researchers have overlooked in the past is crop production choices that farmers make in an attempt to maximize their livelihood benefits and to minimize the risk of crop failure. The sequential cultivation of a diversity of crops such as cereals, root crops, tubers and legumes on the same farm field and the relevance of such choices in the era of climate change deserve due attention. Unfortunately, there is paucity of information on, and little understanding of what informs individual farmer's choices of the type of crops they cultivate, as these may differ from one community to the other.

About 68% of Ghana's land area is arable, yet only about half of it is under cultivation (World DataBank, 2013). Smallholder rain-fed agriculture using simple tools, notably the cutlass and hoe, dominates the agricultural sector in the country, and accounts for about 80% of total agricultural production (MOFA, 2011). The rudimentary technologies associated with smallholder agriculture imply that any fluctuations in natural conditions, notably climate variability, would invariably have negative impacts on national food production targets. Understanding crop production choices made by the smallholder farmer therefore becomes relevant in explaining how the smallholder system keeps pace with growing national food demands. Using the environs of the Kogyae Strict Nature Reserve as a case study, this study investigated the array of crops available to the farmer, and what guides the farmer household in the choice of crops. The study also investigated gender roles in farm-household crop production choices and farmers' perspective of crops most suitable for food and income security against climate change.

Available literature on factors determining smallholder farmers' choice of crops shows that such choices may not be easily predictable. There is a large body of literature on general patterns of crop production based on agroecological zoning and soil suitability traits (Asiamah, 1995; Williams, Hook, & Hamblin, 2002; Agyei-Gyapong and Asiamah, 2002; Quddus, 2009). Such ecological areas have certain types of crops that they are best suited for, which are therefore recommended for cultivation (Asiamah, 1995). In a similar manner, in cases where communal farms are operated such as in Vietnam, collective agricultural decisions are made within a given environmental constraint (Timmer et al., 1983). However, with regard to smallholder farmers, decision making is done on individual initiative, and the incentives that induce them to work in a timely and careful fashion strongly influence the quality and quantity of output (Timmer et al., 1983). In view of this, there is hardly any clear pattern of crop production choices when it comes to smallholder agriculture. According to Mubanga (2015), although climatic factors influence crop yield and may seem to dictate crop production choices, this may not always be the case because such factors vary across local, regional and national boundaries. According to this author, although certain soil and climatic characteristics may be suitable for a particular crop and are more likely to produce higher yields as compared to randomly chosen crops, this in itself is not a sufficient reason for farming households to plant the most suitable crop. A study by Omamo (1998) in Siaya District of Kenya, for instance, revealed that smallholders could raise farm profits by at

Another study by Asrat *et al.* (2010) indicated that in the selection of crops among a certain group of farmers in Ethiopia, the highest premium was placed on environmental adaptability, followed by the yield stability of the particular crop. Fafchamps (1992) observed that in third world countries, large-scale farmers devote a larger share of their land to cash crops than do small farmers due to better access to credit, a greater ability to sustain risk and the lower share that staple foods represent in their total consumption expenditure. Earlier, Timmer *et al.* (1983) pointed out that agriculture is site

least one-third by growing more cotton and less maize

and sorghum. Yet they regularly devoted larger shares

of land and other resources to relatively low yielding

maize and sorghum than to cotton which had higher

market returns, for the reason that cotton production

was more capital intensive and beyond the reach of the

poor. Therefore, for the poor smallholder farmers who

lacked access to production credit, the preferred choice

would be the production of food crops to enhance their

nutritional needs.

specific and that what works in one location may not apply in another because farm households face different constraints on their decision making. Keleman *et al.* (2009) explored potential links between crop diversity and improved livelihoods and indicated that such links may be achieved when the sale of diverse crop varieties directly contributes to farmers' incomes, or when the crops themselves improve household nutrition.

Several other researchers have attributed famers' crop production choices to a variety of factors. These include the availability of land, labor, and capital (Collins, 1984; Coomes et al., 2000), the cultural context in which household land use decisions are made (Alcorn, 1984b; Geertz, 1984), the desire by farming households to secure consumption from own production, market failures, and the roles of state and non-state actors (Umar, 2011). Shiferaw and Bantilan (2004) referred to global or national level policies that may be transmitted to the local level through policy reforms, institutional changes and infrastructural investments. Such broad level policies in turn determine relative input-output prices and access to new technologies and markets at the local level (Shiferaw and Bantilan, 2004). Other studies suggest that collective action by the community may enhance and supplement individual production and investment possibilities (Sreedevi et al., 2006; Wani et al., 2006).

Materials and Methods

Geographical Setting

The forest-savannah transition zone of Ghana lies between the semi-deciduous forest zone at the southern fringes and the Guinea Savannah belt to the north. The zone runs across the country from east to west. It covers about 28% of the country's land area and occurs mostly in parts of the Ashanti, Brong-Ahafo and Northern regions of Ghana. The annual average temperature of the zone is about 28°C (Dickson & Benneh, 1995). It experiences annual average rainfall of between 1200mm and 1500mm, with a bimodal pattern which peaks in June–July and again in September–October, with a dry season in December–March. However, in the northern parts of the zone, the rainfall is uni-modal, with a peak in September (SRID, 2014; Tom-Dery *et al.*, 2013; Isaac *et al.*, 2014). Recent studies have shown that the average annual rainfall figures for some parts of the zone are decreasing, possibly as a result of climate change. Owusu and Waylen (2012), for instance, analyzed rainfall figures obtained from an agro-meteorological station in Ejura in the extreme northwestern part of the study area, and noted that from 1950 to 2000, rainfall averages had reduced from 1800mm to 1600mm. Some residents of the area also shared similar views based on their knowledge of the local rainfall regime (Ofori *et al.*, 2015; Ayivor *et al.*, 2015).

The vegetation is dominated by relics of forest patches and savannah woodland with tall trees and occasional riparian woodland along water courses. Soil types vary from Savannah Ochrosols within the wooded savannah areas to Forest Ochrosols along the forest margins (Dickson & Benneh, 1995). A common feature of the zone is its continuous southward expansion, resulting from anthropogenic drivers and climate change (Hagan, 1998).

The zone is a major destination for migrants from northern Ghana, who see internal migration as a livelihood strategy (Anarfi *et al.*, 2003; van der Geest *et al.*, 2010). Thus, decades of extensive agricultural practices in the zone by both the indigenes and a large migrant population have depleted most soils of their vital nutrients and affected crop productivity. Other environmental challenges of the zone include evidence of changing climate conditions (Klutse *et al.*, 2013, Codjoe and Owusu, 2011), annual bush fires, tree felling for both timber and charcoal production and extensive grazing. Visible signs of degradation have been observed and widely documented (e.g. Attua and Pabi, 2013).

The environs of the Kogyae Strict Nature Reserve, located in the heart of the forest-savannah transition zone in the Ashanti Region of Ghana (Fig. 1), was chosen for this study. The choice of this area was because it has numerous environmental challenges such as climate change and annual bush fires. The establishment of the Strict Nature Reserve has reportedly also reduced arable lands in the area, thus putting a lot of pressure on the available land (Ayivor and Ntiamoa-Baidu, 2015).

The relief of the study area is generally low-lying and undulating with average heights of about 120 m above mean sea level. There are few areas of higher elevation, attaining heights of between 215 m and 230 m. Two major streams, namely the Afram and Sene, and their network of tributaries drain the area. The Sene River drains the northern part of the area while the Afram River and its tributaries drain the southern parts into Lake Volta, located to the east. Most of the drainage channels have adjoining low lying areas that get flooded seasonally, thus creating agroecological niches which are used by farmers for rice cultivation (Hagan, 1998; Ofori *et al.*, 2014; Ayivor *et al.*, 2015).

The population of the area has increased very significantly over the past five decades through both natural means and the high rate of in-migration of farmer families (Ayivor *et al.*, 2015). The main livelihood activity of the area is agriculture. This involves crop farming, livestock raising, lumbering, hunting and fishing (Oduro-Ofori, *et al.*, 2015).

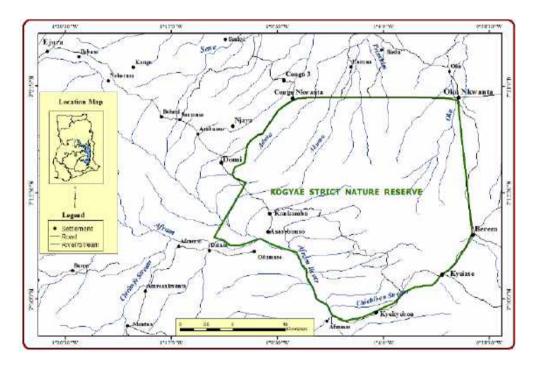


Fig. 1: The Environs of the Kogyae Strict Nature Reserve in the Forest-Savannah Transition Zone

Methods

The study employed field-based techniques in collecting data, namely focus group discussions and administration of questionnaires. A total of 27 communities (Fig. 1) were visited during the preliminary visit, which was also used to hold informal discussions with key community leaders on farming practices. Based on the preliminary observations, purposive sampling was designed for nine (9) communities for in-depth studies. The nine communities were Domi, Njaya, Congo Nkwanta, Oku Junction, Berem, Kyease, Kyekyebon, Aframso and Asasebonso. They were selected on the basis of their accessibility to road transport, relative size and number of households. This was to ensure that a good number of adult respondents were available for the questionnaire administration. A set of 11 questionnaires (more or less) were administered in each of the nine communities based on random sampling of households within each community. A total of 100 questionnaires were administered.

In addition to questionnaires, one focus group discussion (FGD) was conducted in each of the nine selected communities. Members of the groups were selected with the assistance of the local Assembly members and agents of the community leadership. Each group was made up of nine (9) to 15 adult participants between the ages of 18 and 72. In total, 102 farmers made up of 52% males and 48% females participated in the focus group discussions. The discussions were guided by a check list with a set of questions on general farming practices, types of crops cultivated, reasons for the choice of crops cultivated and adaptive practices that were introduced as coping strategies against changing environmental conditions.

The mode of questioning was similar to the "choice experiment" approach which Asrat *et al.* (2010) used to evaluate farmers' preferences for the various attributes of crop varieties. Based on this approach, the research team inquired about the types of crops cultivated by each farmer and the crop variety attributes that informed the choices. The attributes used in the choice experiment included food security, income security, market forces, environmental conditions, climate change, migrants' experience and the influence of friends and neighbours. The responses were later tallied and ranked by relative frequency for discussion.

The study also made use of secondary data culled from published and unpublished sources including books, peer reviewed journals, technical reports and national and other relevant state documents.

Analysis of the data from the focus group discussions was done qualitatively using a thematic technique through a three-step approach of identification of themes, descriptive accounts and interpretative analysis. Responses received by means of questionnaires were analyzed using non-probability/non-parametric descriptive techniques such as the use of relative frequencies and bar charts.

Results

Background of Respondents

The majority of the respondents, comprising about 76% of the sampled population, were within the age range of 26 to 50, with as many as 45% between the ages of 31 and 40, implying that most of the farming population was youthful. Eighteen percent (18%) of the sampled population was 51 years and above, whereas only 6% were 25 years and below.

Several ethnic groups were identified in the study area. Apart from the indigenous Asante who constituted less than 40% of the sampled population, most of the residents were migrants of diverse ethnicity such as Kokomba, Dagomba, Dargati, Moshie, Sissala, Gruma, Gonja and Frafra from northern Ghana. Due to the migrant status of most of the residents, the length of stay of respondents in the communities varied from person to person as indicated in Table 1. It is remarkable to note from the table that as much as 51% of the respondents had lived in the area for not more than 20 years, underscoring the high rate of in-migration in the area in recent years. Though the length of stay alone may not be sufficient proof of one's migrant status, the fact that about 60% of the population were migrants may seem to validate the use of length of stay to confirm residents' migration status.

Table 1: Length of Stay in Community

No. of years	Percentage (%)
Less than 5 years	3
5-10	17
11-15	17
16-20	14
21-25	9
26-30	11
31-35	10
36-40	5
Over 40	15

The high migrant population in the area was enhanced by a very attractive land tenure system that easily met famers' needs. The findings revealed that about 74% of the farmers engaged in share cropping or other forms of leasehold agreements on favourable terms. For instance, at Njaya, the residents claimed that they paid virtually nothing for farmland; rather, they made annual contributions voluntarily to the landlord, Kwamanhene (the paramount chief of Kwaman), towards the celebration of his annual traditional festival.

The findings further revealed that all respondents in the study area undertook farming either as the first or second major economic activity. About 3% of the population, however, practiced farming only as a second occupation and were engaged in the local artisanal industry such as carpentry, or petty trading. The results also revealed that about 13% of the respondents, mostly women and the aged, engaged in food crop production for subsistence, whilst the rest of the population engaged in both food crop and cash crop farming. It was further noted that about 95% of the respondents were able to produce enough to take care of their basic food needs, whilst 5% were not and had to depend on their families and/or external relations to make up for the deficit. A few foodinsecure farmers engaged in waged labour to purchase food from the community markets.

The use of agro-chemicals to enhance farm productivity and to clear weeds and pests was very common in the study area. The results indicated that as many as 64% of the respondents applied chemicals on their farms. The cycle of chemical application by farmers started with the use of weedicides to clear the land (mostly degraded farmlands) for new planting. The non-selective herbicides, Glyphosate 480g/l and Gramokal super, which the farmers nicknamed "condemn", were used at this stage. This was followed by planting, then application of fertilizers (mostly NPK and Sulphate of Ammonia) and the use of Herbextra, a selective herbicide used mostly to clear weeds in rice and maize farms. Finally, pesticides were applied on vegetable farms a few weeks before harvest or on other crops earlier in the cycle, depending on the prevailing circumstance. About 34% of the farmers who did not apply agro-chemicals on their

farms practiced rotational fallow. However, with the rapid rate of population increase over the past decades and the shortening of fallow period, lower yields were being recorded by this category of farmers.

About 2% of the farmers used exclusively organic manure or adopted other soil fertility enhancement methods. These were mostly crop farmers who, as part of their management strategy, planted on raised-mounds or ridges to enhance the water infiltration capacity of the soil. They also used organic material for mulching to preserve moisture and as a way of adding organic residue to the soil.

Household Decision Making on Crop Production Choices

According to the findings, farmer decision on what to produce is largely the preserve of the head of family, mostly men. Based on the percentage frequency of responses in male-headed households in relation to who the decision maker was, it was noted that 87% of the decisions were made by husbands whilst 13% were jointly made by husbands and their wives. There was no instance where a decision was made by a woman in a male-headed household. This explains male dominance in these farming communities, which is a major feature in most agrarian communities in Ghana. Instances where women made decisions on the choice of crop included:

- cases of female-headed households;
- cases where husband and wife operated separate farms;
- cases where a wife identified a niche within a joint family farm to cultivate specialized crops such as vegetables;
- occasions when married women established additional farms separate from joint family farms; and
- situations where a husband (head of household) was absent for one reason or the other, and the wife had to mobilize the children to carry out farming operations.

The farmers had a wide range of crops from which they made their choices. The most common staples included maize, yam, beans, groundnuts, cowpeas, rice, cassava, cocoyam and plantain. In general, farm-household choice of crops on an annual basis depended on a combination of factors including sequence of crop in the season, knowledge of local conditions, length of the rainy period, market forces, dietary habits and crop maturity time.

Crop rotation

It was noted during fieldwork that the period between March and May was devoted to the production of fast yielding drought resistant crops, notably cowpeas. This adaptation strategy emerged from several outreach programmes that the Crop Research Institute of the Centre for Scientific and Industrial Research (Ejura) had extended to farmers through the Agricultural Extension Agents and the World Vision International. Through the programme, farmers had adopted the 42-day strain of cowpeas which they harvest before the commencement of the main planting season.

Fig. 2 shows the normal cropping sequence on annual basis as reported by the farmers. The figure depicts several cropping patterns, which the farmers had adopted based on the length of the rainy season and the maturity period of the crop. It shows also that cowpea and maize feature the most in the cropping sequence. The respondents explained that cowpea can withstand the drought spell of about one and half months that follows the first rains in March. Thus, climate variability over the past decade influenced farmers' decisions to start the annual cropping sequence with cowpeas, a practice which had been introduced less than a decade ago. The dual advantage of cowpea was its early maturation period and its ability to fix atmospheric nitrogen in the soil, thus enhancing soil fertility for the maize crop which followed in the sequence. An additional advantage of this innovation, as reported by the farmers, is that the produce from cowpea cultivation was sold for re-investment during the main

cropping season. Leafy vegetables and okra, both of which mature quickly, may also be cultivated alongside cowpea for home consumption and for income.

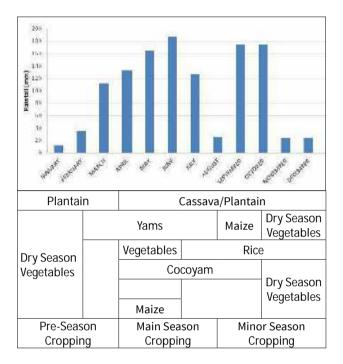


Fig. 2: Annual Sequence of Cropping in the Study Area

A variety of crops was cultivated during the main cropping season based on local knowledge. This knowledge gave farmers enough assurance of the availability of adequate moisture to ensure the growth of the various crops. The crops, which were cultivated mostly either as mixed crops or in combination with other crops, included maize, yam, groundnuts, cassava and vegetables.

The third cropping period in the annual sequence starts in July/August and ends normally in November/ December. The crops cultivated during this period included maize, rice and to a lesser extent, cowpeas. According to the findings, this period coincided with the peak rainy season characterized by abundant moisture to support crop growth. Rice was cultivated extensively in swampy areas not suitable for maize production. According to respondents who were practicing rice farming, the activity gained momentum about a decade ago in response to market forces. Though most farmers considered maize as the most important crop to meet their food security needs, cropped area for rice had increased by 95% from 2005 to 2014 whereas maize had increased by only 30% and yam 17% (Ayivor *et al.*, 2015).

The last period in the sequence of cropping commenced in November and ended around March of the following year. This involved mostly dry season vegetable farming practiced by a handful of farmers encountered at Chichibon. The study also revealed that paramount to the crop production choices was the fact that farmers had a good understanding of the vulnerability to weather extremes of each of the crops they cultivated and were therefore guided accordingly.

With regard to vulnerability to drought, the responses from farmers showed that maize and rice were most vulnerable, followed by cowpeas, yam and groundnuts (Fig. 3). Therefore, during dry spells most farmers preferred to cultivate cowpeas, which are more tolerant of drought, rather than maize and rice. On vulnerability to excessive rainfall, the percentage responses for cowpea ranked the highest, followed by maize, rice, yam and groundnuts.

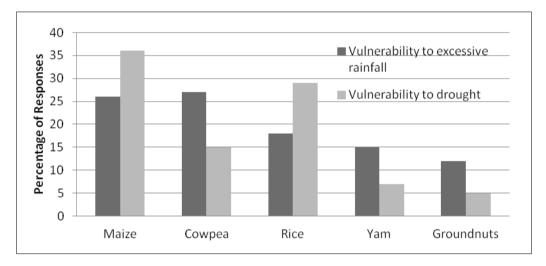


Fig. 3: Vulnerability of Crops to Excessive Rainfall and Drought

Maize and rice appeared to be most vulnerable to both drought and excessive rainfall, yet these two crops were the most cited by farmers for meeting food and income security needs (Fig. 4). Thus, despite the vulnerabilities of these crops to extreme weather conditions, their cultivation was very important in meeting the farmer's food and income needs.

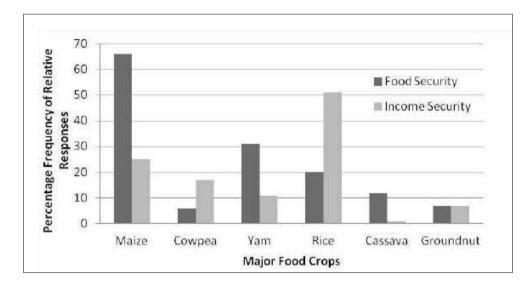


Fig. 4: Crops Meeting Food and Income Security Needs

On the whole, about 96% of the respondents were able to produce enough to meet their food security needs. It was noted further that majority of the remaining 4% who could not produce adequate crops to meet their food security needs, were between the ages of 65 and 70. About 20% of this category of respondents depended on their relations to make up for the food deficit.

Production Capital, Traditional Values and Market Trends

According to the respondents, though rice cultivation guaranteed their income security, its cost of production was higher than that of maize. The cost included the preparation of land and nurseries, transplanting, cost of different agrochemicals needed at various stages of the production process, cost of scaring away grain-eating birds during the fruiting stage and cost of harvesting. The process was therefore capital intensive and in the absence of production credit, most farmers had to rely on middle persons who pre-financed the production process and were paid back in kind soon after harvest. Unfortunately, such arrangements often left such farmers at a disadvantage, as unit prices to the middle persons were pegged lower than the prevailing market price at any point in time. Additionally, such arrangements did not allow farmers to store the produce for a longer period to attract higher market prices. This tends to erode farmer revenue, with most of them only able to break even.

Socio-cultural factors that drove crop production choices by farmers were mainly traditional dietary values, ethnicity and migration. It was observed that certain crops such as yam, plantain and cocoyam which were vulnerable to the current environmental conditions were still being cultivated. The respondents explained that such crops featured prominently in the preparation of their traditional dishes, which explains why they could not stop producing them. For example, plantain and cassava are combined in the preparation of the traditional dish fufu which is very popular among the Asante. However, in the past few years, even though erratic weather conditions, especially windstorms, negatively affected the productivity of plantain, most farmers continued to cultivate the crop in every available niche including back yard gardens. In a similar manner, though the yield from yam cultivation had not been impressive over the past years due to soil exhaustion, migrant farmers, mostly from northern Ghana, continued to cultivate the crop because it is an integral part of their diet. Most farmers no longer produced these crops in commercial quantities but just for home consumption.

A cocoyam farmer in Oku Junction in the northern part of the study area claimed that he introduced cocoyam to the area as a result of his cocoyam production experience in the forest zone of Ghana where he migrated from. According to the farmer, his net income from the sale of cocoyam on the local market far outweighed what he got from the maize crop. A few other farmers had adopted cocoyam farming in the area. It is worthwhile to note that the weather condition in the Oku area was mostly the tropical savannah type and offered little opportunity for the cultivation of broad-leafed crops. Motivated by taste and market forces, the farmer in question defied the crop production pattern based on broad agroecological zone and practiced 'niche farming', much to his advantage.

Opinion Ranking on Crop Production Choices

After evaluation of the factors that influenced crop production choices, the study attempted to rank the most preferred production choices based on farmer-responses. The analyses revealed that for the majority of farmers, the main driving force for the choice of crops they cultivated was income and food security. Farmer-responses, as shown in Table 2, indicate that 42% of the respondents were driven by income considerations whilst 27% were influenced by food security needs. 4% of the respondents stated market forces, which also relates to income, as the main driving force in their crop choices. This implies that about 46% of respondents were driven by a combined income and market factor. Field investigations revealed that despite the bad nature of the roads, truck-loads of assorted food items destined for urban markets such as Kumasi, the regional capital and Ejura, the district capital, were a common site, particularly on market days. Other factors of importance that dictated farmer-choices included environmental conditions, migrants' experience and influence by neighbours and friends (Table 2). Eighteen percent (18%) of farmers were influenced by environmental conditions including climate variability.

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Table 2: Driving forces for crop production choices

Item of motivation	Percentage responses
Income security	42
Food security	27
Environmental conditions including climate variability	18
Influence of neighbours and friends	6
Market	4
Migrants' experience	3

A further probe into farmers' knowledge of climatic trends and crop adaptability revealed that cowpea recorded the highest number of percentage responses as the most robust crop regarding climate variability in the area (Fig. 5). The crop with second highest percentage of responses was maize. According to the farmers, though maize cultivation was highly vulnerable to climatic uncertainties, the Crop Research Institute of the Centre for Scientific and Industrial Research had developed an early-maturing variety that could also withstand drought. Thus, the earlier variety, obatampa, which has a maturation period of 120 days, was being replaced by omankwa which takes only 90 days to mature. Therefore, the farmers believed that the production of the new variety of maize could be a good coping strategy against climate variability. The farmers were also of the view that since rainfall peaks around the time rice is cultivated in the area, proper timing of the crop would enhance its ability to cope with climatic uncertainties.

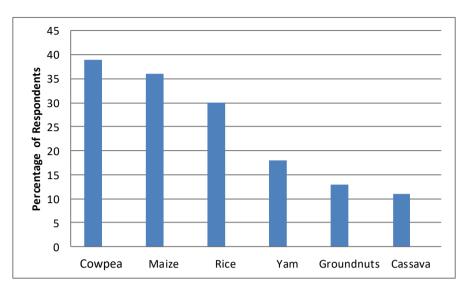


Fig. 5: Crops Suitable for Food Security against Climate Change

Discussions

According to Timmer *et al.* (1983), growing food is a decision-intensive undertaking. Most farmers are occupied routinely with decisions on what crops to plant; what inputs to use; when to plow, seed, cultivate, irrigate, and harvest; how much to keep for home consumption, sell or store for later sale. With regard to the choice of crop to be produced by the smallholder farmer as revealed by the current study, the decision is often subjective and based on the farmers' preferences such as taste and the traditional value placed on the crop. It was however very clear that income and food security needs were the main driving forces.

The sequence of crops in the season and the crop combination grown by the farmers were also very important in the crop production choices. According to Timmer *et al.* (1983), cropping patterns vary among smallholder farms because of differences in soils, irrigation, prices and proximity to markets. In addition to the cropping pattern, the farmers' knowledge of the local environment, particularly the changing climatic conditions and the subsequent change in the planting season since 2008, also played a major role in their crop production choices. For instance, Ayivor *et al.* (2015) reported that there has been a one- to two-month shift

in the farming calendar in the area over the past decades. According to Ayivor *et al.* (2015) before the 1980s, there were early rains in mid-March, which used to usher in the planting season, but this is no longer the case. Our findings underscore this observation and confirm the shift in the farming calendar from March/April to May/ June since 2008 due to delays in the onset of the rainy season. As an adaptation measure, farmers had adopted the 42-day strain of cowpeas which they harvested before the commencement of the main planting season. This observation suggests that the choice of cowpea at this stage was influenced principally by rainfall availability. Since the farmers practiced rainfed agriculture, rainfall amounts and seasonality were crucial in farming decisions.

Rice cultivation saw a tremendous boom in the area over the last decade. The findings showed that most farmers considered maize as the most important crop to meet their food security needs, but cropped area for rice increased by as much as 95% from 2005 to 2014 whereas maize increased only by 30% and yam 17% (Ayivor *et al.*, 2015). This may be the result of what Ofori *et al.* (2015) described as the use of agro-ecological niches to expand the cultivation of rice as an adaptation to climate change. It may also be in response to a general trend in West Africa where rice is becoming a staple crop as a result of urbanization. Elbehri *et al.* (2013) estimated that in West Africa, urban markets represent a growing marketing outlet for local staple food, with rice constituting about 60% of local staples consumed. Indeed, most of the rice produced in the study area finds its way to Kumasi, the regional capital.

The high percentage of responses for cowpea followed by maize, rice, yam and groundnuts as most vulnerable to excessive rainfall implies that in the cultivation of maize and cowpeas, for instance, farmers who have alternative sites will look for well drained areas to avoid losses. This partly explains why leasehold and share cropping arrangements were so rampant in the area. Similarly, farmers would ensure that the timing of maize and rice cultivation coincided with periods of adequate rainfall to avoid losses.

Thus, despite the vulnerabilities of these crops to extreme weather conditions, their cultivation was very important in meeting food and income needs. This implies that though farmers might have been mindful of the level of vulnerability of crops they cultivated to environmental stressors, this alone did not influence their crop production choices. Other factors such as production capital, personal taste and preferences, market value of the crop and cultural underpinnings guiding the cultivation of certain crops played a significant role in farmers' decisions (Timmer et al., 1983; Fafchamps, 1992; Keleman et al., 2009). The farmer therefore had to weigh the options to determine which crops were best suitable for certain adaptation measures such as a shift in the sequencing of the crop in the season and the adoption of early maturing varieties.

As Omamo (1998) argued, when the production of a crop from which farmers could raise more income is capital intensive, poor peasant farmers who lack access to production credit would prefer crops that are cheaper to produce and which would enhance their consumption needs.

From the foregoing, it is obvious that most of the crop production choices smallholder farmers made were based on 'crop suitability evaluation' through timetested indigenous traditional knowledge borne out of the ingenuity of the local farmer. According to Gyasi *et al.*, (2004) such knowledge of local conditions can offer a realistic basis for developing a more locally adaptive resource management model in line with the grassroots bottom-up paradigm. However, beyond the suitability evaluation is a combination of factors such as production capital and markets which may compel the farmer to make alternative choices as a trade-off.

Conclusion

Crop production choices constitute one of several important decisions that smallholder farmers with limited resources make as a strategy against biophysical and socio economic constraints. In the forest-savannah transition zone of Ghana, often referred to as 'the bread basket' of the country, tonnes of foodstuffs including cereals, tubers, roots, fruits and pulses are produced for the urban market. Given the fact that most smallholder farmers engage in the cultivation of a wide range of crops in order to spread risks, a good understanding of all the variables that affect the productivity of each crop is vital. The case study of the environs of the Kogyae Strict Nature Reserve, which offers a classical example of conditions in the forest-savannah transition zone of Ghana, revealed that a combination of factors including knowledge of local environmental conditions, length of the rainy period, market forces, dietary habits and crop maturity time dictate crop production choices made by farmers. Farmers, therefore, need a good understanding of the interplay of these factors to guarantee their food and income security needs.

An important revelation was that between food security and income security, the majority of farmers regarded income security as most critical for their crop production choices. They believed that though food security is important, it is not enough to protect them against the vicious cycle of poverty that farmers find themselves in the contemporary exchange economy. Therefore, farmers One major conclusion, therefore, is that smallholder agriculture has shifted from mere subsistence to a market oriented activity, with most farms operated as small business estates aimed at meeting demands of the exchange economy in which we are. Thus, for most farmers crop production choice is influenced primarily by income security needs. The good thing about this is that as farmers strive to achieve income security through food production, national food security needs are also being addressed.

The study concludes also that, to a large extent, smallholder farmers, regardless of their knowledge and experience, have to operate within constraints imposed by the biophysical environment, income demands and taste, which potentially render crop production vulnerable to unexpected risks. Crop production choices are therefore an effective risk spreading strategy in response to environmental vagaries and socio-economic demands.

The study recommends effective diffusion of innovations introduced by the Crop Research Institute to help farmers overcome productivity constraints. One such innovation is the production of yam along evenly-spaced constructed ridges with planting done one meter apart, instead of the current technique whereby relatively wider-spaced yam mounds are used for planting the crop. Research has shown that this new technique, if adopted, could raise output by 100% at no extra cost (Ayivor *et al.*, 2015). Agricultural Extension Agents should be wellresourced to spearhead the adoption of this innovation.

It is recommended also that the cultivation, marketing and consumption of cowpeas be enhanced and promoted by the Ministry of Food and Agriculture, through identifiable farmer-groups in all areas of similar changing climate conditions in the country. The ability of the crop to fix atmospheric nitrogen and its potential to generate income needed by the farmer for reinvestment in the major farming season makes it one of the most appropriate crops for the future in the face of climate change.

Acknowledgments

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The introduction should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary.

Methods

The methods section should include sampling, experimental design and setting of the study, a clear description of all analytical and experimental methods, processes, interventions and the type of statistical analysis used. When proprietary brands are used in research, include the brand names in parentheses.

Results

This should comprise the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as Tables and Figures.

Discussion

For research articles, this section should discuss the implications of the findings in context of existing research and highlight limitations of the study. For study protocols and methodology manuscripts, this section should include a discussion of any practical or operational issues involved in performing the study and any issues not covered in other sections

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study to the field.

Sections numbering

You may number the various sections/sub-sections of your article (excluding the abstract) using the format: 1, 2... (then 1.1, 1.2; 1.1.1, 1.1.2, ...) etc. The subsections may be given a brief heading, with each heading appearing on a separate line.

Presentation

Font, Times New Roman, 11; 1.5 spacing, maximum of 15 pages (including References but excluding Figures and Tables).

Declarations (if any)

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use.

Ethics approval and consent to participate

Manuscripts reporting studies involving human participants, human data, human tissue or animals, must:

- include a statement on ethics approval (even where the need for approval was waived) and consent (in case of human subjects);
- include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

Referencing

The APA (American Psychological Association) references style must be used. For detailed information, please see the Publication Manual of the American Psychological Association, 6th edition, http://www.apastyle.org/ and http://blog.apastyle.org/. or the Taylor and Francis Summary given here. http://www.tandf.co.uk/journals/ authors/style/reference/tf_APA.pdf

Preparing figures

When preparing figures, please follow the formatting instructions below.

- Figures should be provided as separate files, not embedded in the main manuscript file (maximum five figures).
- Each figure of a manuscript should be submitted as a single file that fits on a single page in portrait format. Each figure should be closely cropped to minimize the amount of white space surrounding the illustration.
- Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.
- Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order.
- Figures should be uploaded in the correct orientation.
- Figure titles (max 15 words) and legends (max 150 words) should be provided in the main manuscript, not in the graphic file.
- Figure keys should be incorporated into the graphic, not into the legend of the figure.
- Individual figure files should not exceed 10 MB. If a suitable format is chosen, this file size is adequate for extremely high quality figures.
- Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere. In order for

all figures to be open access, authors must have permission from the rights holder if they wish to include images that have been published elsewhere in non open access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Figure file types

We accept the following file formats for figures:

- PDF (suitable for diagrams and/or images)
 - Microsoft Word (suitable for diagrams and/or images)
 PowerPoint (suitable for diagrams and/or images,
 - figures must be a single page) TIFF (suitable for images)
- JPEG (suitable for photographic images, less suitable for graphical images)
- PNG (suitable for images)
- BMP (suitable for images)
- CDX (ChemDraw suitable for molecular structures)

Figure size and resolution

Figures are resized during publication detailed below. *Figures on the web:*

width of 600 pixels (standard), 1200 pixels (high resolution).

Figures in the final PDF version:

- width of 85 mm for half page width figure
- width of 170 mm for full page width figure
- maximum height of 225 mm for figure and legend
- image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

Preparing Tables

When preparing Tables, please follow the formatting instructions below.

- Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).
- Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files.
- Table titles (max 15 words) should be included above the table, and legends (max 150 words) should be included underneath the table.
- Tables should not be embedded as figures or spreadsheet files, but should be formatted using
- 'Table object' function in your word processing program.
- Tables should NOT be submitted as figures but should be included in the main manuscript file
- Colour and shading may not be used in tables. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.
- Commas should not be used to indicate numerical values.

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