IJOEAR

International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-3, Issue-12, December 2017

Preface

We would like to present, with great pleasure, the inaugural volume-3, Issue-12, December 2017, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

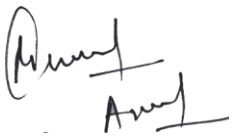
Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

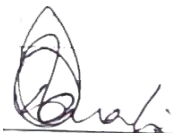
Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



Mukesh Arora
(Editor-in Chief)



Dr. Bhagawan Bharali
(Managing Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000- March 2002 and DBT oversease Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Table of Contents

Prediction of biodegradability ratios in wastewater treatment plant of Skhirat Morocco

Lakhlifi M.[1*], A. Elatmani[2], T. Elhammoumi[3], O. Elrhaouat[4], M. Sibari[5], Y. Elguamri[6], D. Belghyti[7], K. El Kharrim[8*]

[1,3,4,6,7,8]Laboratory of Agrophysiology, Biotechnology, Environment and Quality. University IbnTofail, Faculty of Science, BP 133, Morocco.
[1]Department of Environment, REDAL, Rabat, Morocco.
[2]Technical Study Office, Rabat, Morocco.
[5]National Office of Water and Electricity, ONEP, Kenitra

Abstract**—** *In this note, our study was carried out by physico-chemical monitoring in terms of organic matter. The mean objective is to evaluate the performance of the Skhirat wastewater treatment plant based on the ratios COD/BOD and BOD/COD in order to know the degree of biodegradability and to make the decision about the treatment model to choose. In fact, the performances acquired in terms of COD and BOD are respectively of the order of 87.26% and 88.35%; what is significant downstream of this station that we take advantage of to irrigate several green spaces by saving all conventional water resources. In addition, the organic material is easily biodegradable with ratios of COD/BOD and BOD/COD respectively of about 2.05 and 0.49. This allows us to adopt an appropriate biological treatment by design and sizing suitable structures.*

Keywords**—** ***Wastewaters, Organic matter, biodegradability, performance, Skhirat, Morocco.***

I. INTRODUCTION

At these last decades, Morocco has experienced a very significant development in the installation of the stations of purifications in several communes of the country. Because of development of the populations socio-economically the polluting load by equivalent-inhabitant has increased as rejection of domestic and industrial wastewaters to the network of collective cleansing arriving at the sewage treatment plant of the town of Skhirat located in the area Rabat-Sale-Kenitra.

Obviously, the composition of wastewaters reflects the life styles [1]. Pollution is an artificial phenomen on resulting either from the natural substance concentration or of the no natural synthetic compounds (xenobiotic) rejected into the environment [2].

The organic and inorganic substances rejected into the environment following domestic, agricultural and industrial activities of water lead to an organic and inorganic pollution [3,4]. At the horizon, these effluents pose a future problem because of the increase in the population which will generate very large quantities of pollutants what requires an appropriate treatment by correcting any specific anomaly. The objective of this study is of knowing the degree of biodegradability for the decision making of which type of treatment to follow, while being based on the ratios of COD/BOD and BOD/COD.

II. ZONE OF STUDY

The zone of study is spread out over a surface of approximately 600 ha and fact part of the urban district of Skhirat which is located at about twenty kilometer in the southwest of the town of Rabat. It is bounded by the communes of Harhoura and Ain Atiq to north and by the communes Essabah and Charrat to the south. The study area is part of the urban plan development of the city of Skhirat. In the North, there is a significant extension of the industrial zone which exceeds the surface delimited by the installation plan.

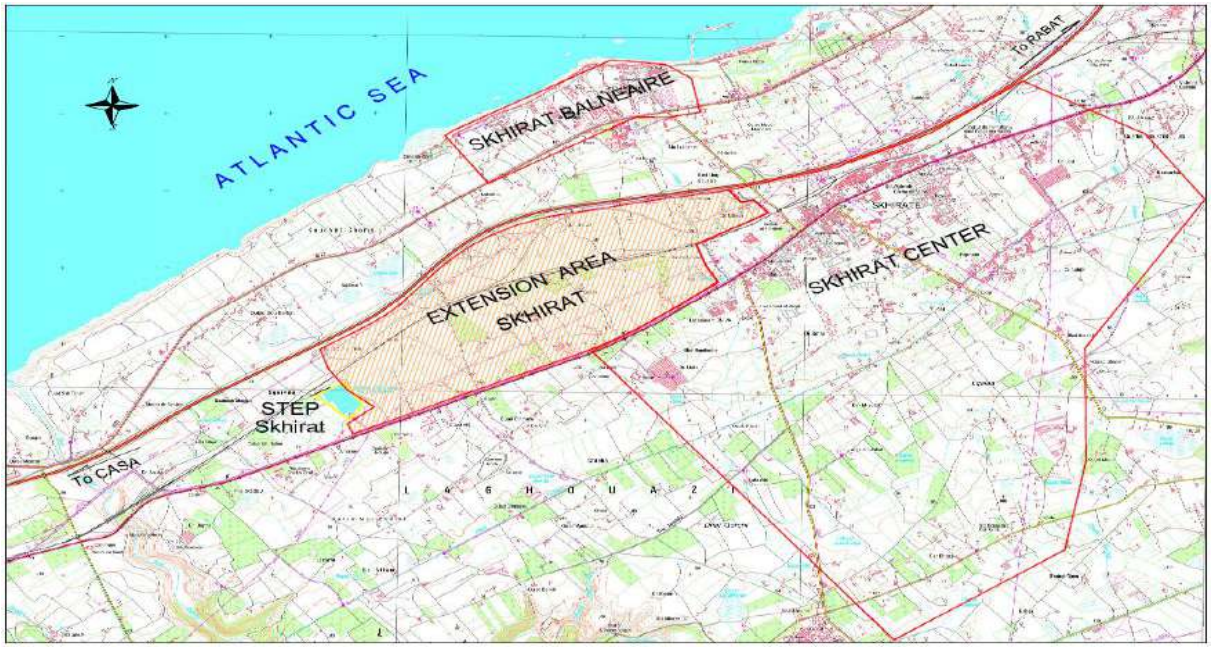


FIG.1: GEOGRAPHICAL LOCATION OF THE WASTEWATER TREATMENT PLANT (STEP) OF THE MUNICIPALITY OF SKHIRAT

The population of the urban district of Skhirat, according to the last censuses of 1994, 2004 and 2014, respectively counted 29599, 43025 and 59598 inhabitants with an inter annual average rate of increase in 3,8 %. The climate of the geographic area is of the subhumid mediteranean type with an annual average temperature of the area close to 18 °C and an annual rainfall average which is about 500 mm while varying from one year to another.

III. MATERIAL AND METHODS

Sampling was carried out by monthly taking away during one year with four taking away for each month as well as the sites of study were carefully selected at the entry and the exit of the wastewaters treatment plant of the city Skhirat (Area of Rabat-Sale-Kenitra)

After the conservation of the samples of wastewaters was made according to the general guide for the conservation and the handling of the samples according to[5]. The measurement of suspended matter (SM) was carried out by a cellulose membrane filter of diameter of 0.45 μm [6]. Moreover the BOD was measured by an oxytop DBOmeter at temperature 20°C during five days of incubation as well as the COD by a spectrophotometer.

IV. RESULTS AND DISCUSSION

4.1 Report COD / BOD

This report COD/BOD, informs us about the nature of pollution and it indicates the importance of the polluting matters more or little or no biodegradable [7].

The index of biodegradability (COB/BOD) proves also very useful for the fellow-up of the effectiveness of biological treatment; the report is increasing more especially as the biological treatment is more difficult. According to always the same reference, for an effluent with domestic predominance, this report generally is understood between 1.5 and 2.5. For industrial effluents, which may contain a significant fraction of non-biodegradable compound, it may be envisaged according to this ratio COD / BOD whose aptitude for degradation is more or less favorable to a biological treatment, according to the rules being generally adopted:

* COD/BOD< 3 : Easily biodegradable effluent

* 3 < COD/BOD<5 : Fairly biodegradable effluent

* COD/BOD > 5 : Not easily biodegradable effluent, even no biodegradable

Our report COD/BOD is of the order 2.92 for wastewaters of the sewage treatment plant of the Skhirat city. Thus this ratio is lower than 3 what indicates than these effluents need a biological treatment.

4.2 Report BOD / COD

The report BOD/COD, is an indicator of the biodegradability of the organic matter [8], and this ratio provides important indications on the origin of the pollution of wastewaters and the suitable treatment to realize [9].

In our obtained results, we watch that the average of report BOD/COD is about 0.48 is noted higher than ratio 0.47 [10] with a minimal value of 0.15 and one value maximum of 1.

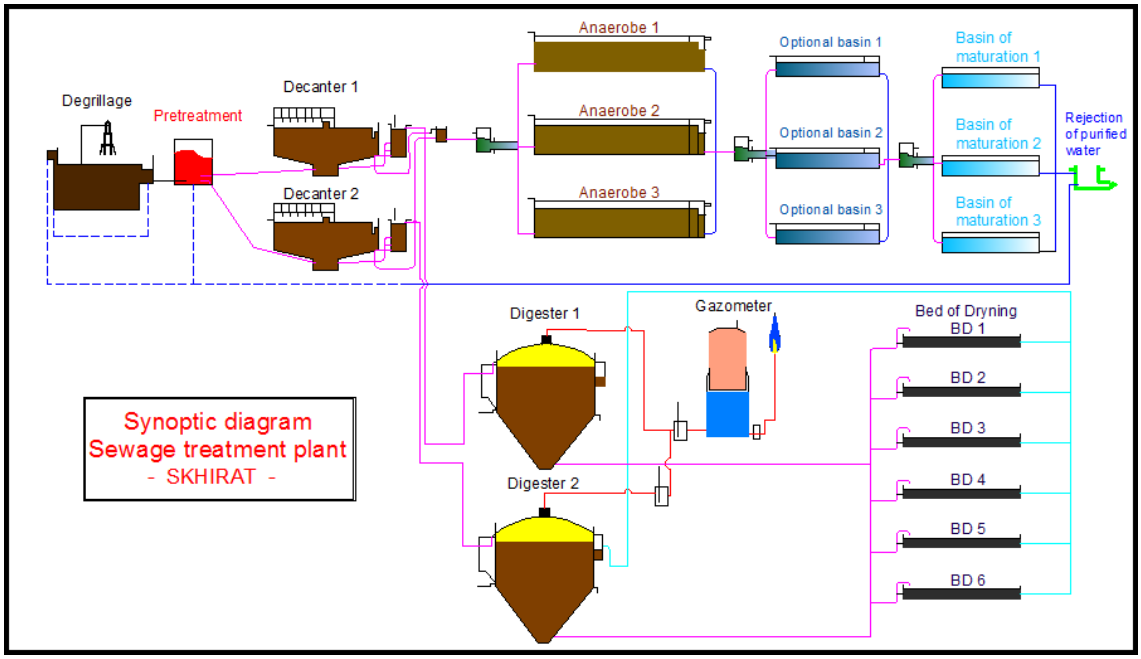


FIG.2: PRESENTATION OF THE SYNOPTIC DIAGRAM OF THE STEP OF SKHIRAT

Figure 2 represents really the stages of operation of sewage treatment plant of the town of Skhirat and the levels of treatment conveyed as of the entry of the effluents to their exit of this STEP. So we tracked and decompensated his decision-making allowance if there was a malfunction in one of the current phases or in the whole, correcting any anomaly that subsequently occurred.

TABLE 2
COD AND BOD RATIOS OF WASTEWATERS ENTERING AND LEAVING THE STEP SKHIRAT

Statistical	COD Entrance	BOD Entrance	COD purified	BOD purified
Nb. observation	12.00	12.00	12.00	12.00
Minimum	187.00	112.00	19.20	1.60
Maximum	1030.00	614.00	107.52	42.00
First Quartile	588.50	146.75	40.30	7.33
Median	783.50	245.00	53.76	10.00
Third Quartile	941.00	525.00	65.84	17.00
Average	722.67	326.75	56.86	14.67
Variance	70936.39	39390.52	753.87	160.36
Standard Deviation SD	266.34	198.47	27.46	12.66
Coefficient of variation	0.37	0.61	0.48	0.86
Standard deviation of average	80.30	59.84	8.28	3.82
Limit inf. of average (95%)	545.92	195.04	38.64	6.26
Limit sup. Of average (95%)	899.42	458.46	75.08	23.07

This station is characterzed by degriller/sand trap, as degriller, two decanters, 3 basin of anaerobes, 3 basin optional, 3 basins of maturation, 2 digesters and a gasometer which produces methane while finishing by beds for waste sludge drying. Finally the purified wastewaters are rejected into the Charrat river

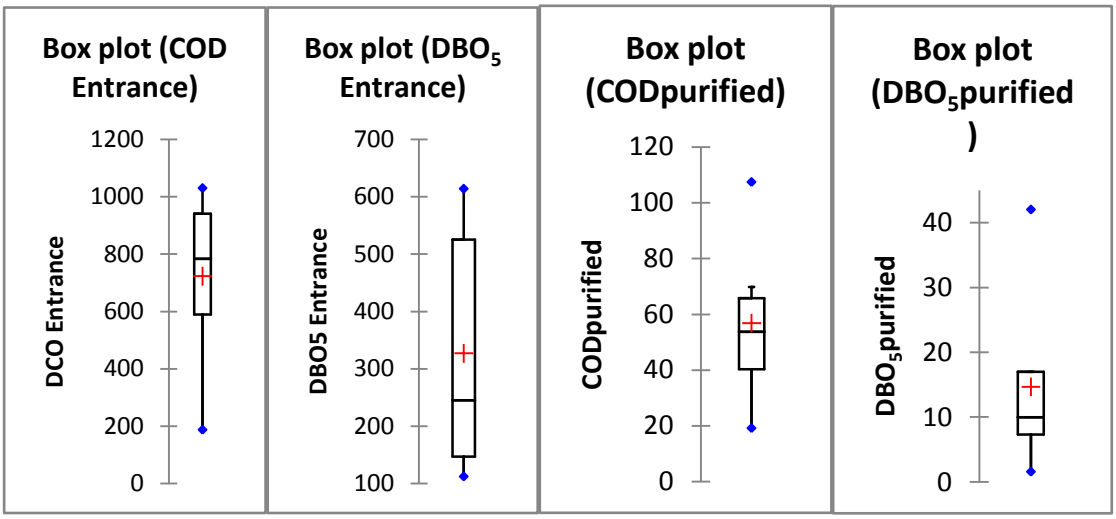


FIG.3: PRESENTATION OF THE BOXPLOTS OF COD AND BOD_5 AT THE ENTRY AND EXIT OF STEP

According to the figure 3 above us a difference between rough wastewaters and those purified within the STEP of the town of Skhirat being characterized by of Boxplots which present averages different of COD and BOD respectively at the entrance of the order of 722.67 and 326.75 mg of O2/L. At the exit those index are of about 56.86 and 14.67 mg of O2/L. These boxplots presents 1st, 3rd quartiles, Minimas, Maximas, Medians and Averages which are very different depending on wastewaters states brut or purified according to these graphs.

So they got results are in conformity with the standards of rejection in terms of COD limited to less than 250 mgO_2/L and BOD with 120 mg of O_2/L what makes it possible to re-use these purified wastewaters at several agricultural purposes such as forestry, irrigation of urban green spaces, lawn watering golf courses…etc.

With regard to the mean value of the COD is regarded as higher than that recorded at the sewage treatment plant of the center emplissor of the company Salam Gaz at the town of Skhirat (698 mg of O_2/l) on the other hand the mean value of the BOD is noted lower than this STEP of the company as [11].

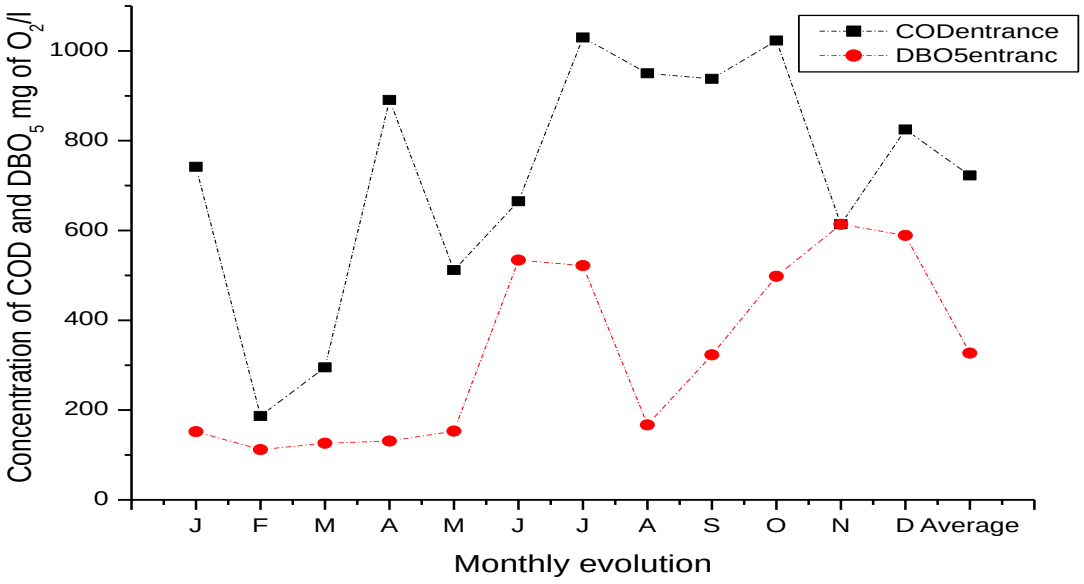


FIG.4: MONTHLY EVOLUTION OF THE CONCENTRATIONS OF COD AND BOD AT STEP ENTRANCE

The monthly mean value of the chemical demand for oxygen (COD) is about 722.67 mg of O_2/L what is lower than that recorded with the STEP of the town of Nouakchott (1806.76 mg of O_2/L) in the same way for [12-13]. Moreover, the biological oxygen demand (BOD_5) has a monthly mean value of about 326.75 mg of O_2/L which is also lower than that of the STEP of the town of Naoukchott (538.31 mg of O_2/L) at its entry [14] and those in [12-13].

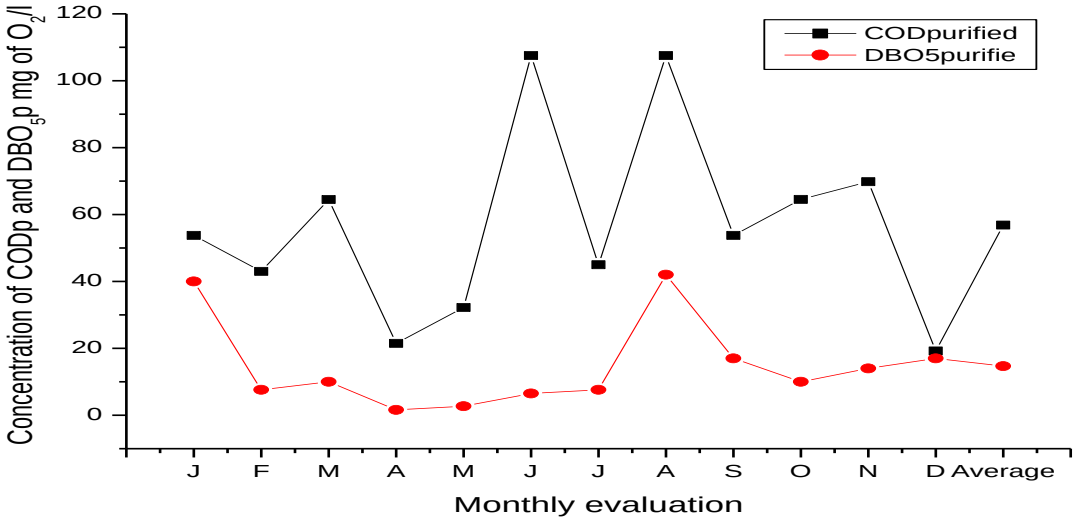


FIG.5: MONTHLY EVOLUTION OF THE CONCENTRATIONS OF COD AND BOD OF PURIFIED WASTEWATERS

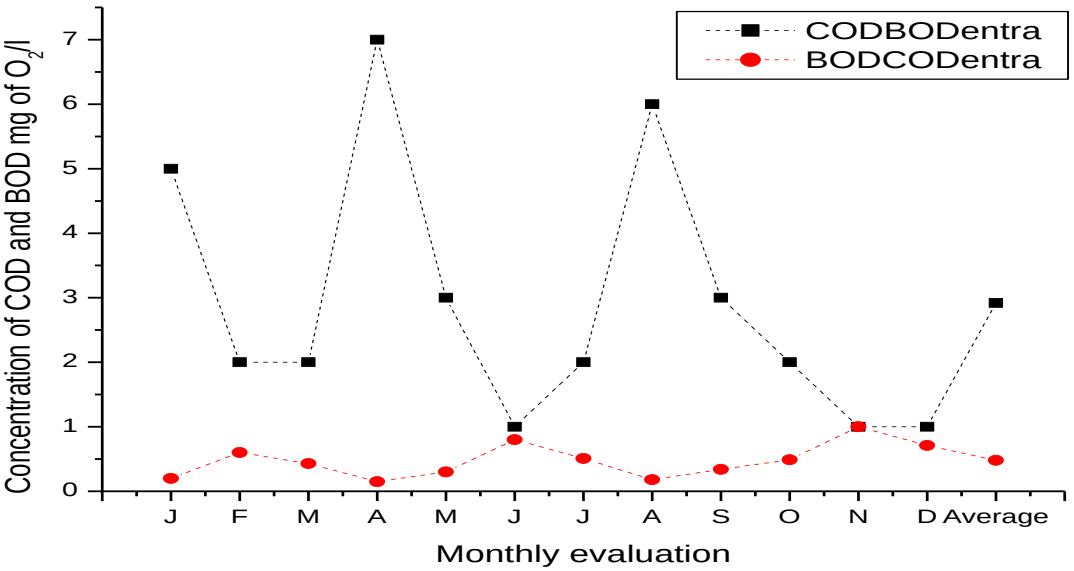


FIG.6: MONTHLY EVOLUTION OF RATIO OF COD/BOD AND BOD/COD OF ROUGH WASTEWATERS

However at rates of BOD/COD higher than 0.30, the biological processes are more effective than the physico-chemical processes [15]. In the case of the STEP of Skhirat, this ratio is about 0.48 and it is superior to the precedent report while implying to follow the advantage of a biological process of treatment.

- $DCO/DBO_5 < 2$: the effluent is easily biodegradable. = Effluents of food industry
- $2 < DCO/DBO_5 < 3$: the effluent is biodegradable with selected stocks. = Domestic Effluents dominant.
- $DCO/DBO_5 > 3$: the effluent is not biodegradable. = Effluents industrials

V. CONCLUSION

According to the hall above results, we can deduct a ratio from COD/BOD of monthly mean value of about 2.92 which is greater than 2 but less than 3 what mean that these effluents are of domestic predominance with easily biodegradable organic matters.

This result is confirmed by the report of BOD/COD which is about 0.48. So these ratios help us to make a good decision to carry out a biological treatment what is in conformity with the actual sewage treatment plant of biological lagoon type at the of the town of Skhirat.

REFERENCES

[1] NF. Gray.. 1989. Biology of Wastewater Treatment Oxford Univ. Press. Oxford. 828p.

[2] N. Abdel-Raouf.. AA. Al-Homaidan.. IBM. Ibraheem.. (2012). Microalgae and wastewater treatment. Saudi Journal of Biological Sciences. Vol 19. 3. pp. 257-275.

[3] P. Mouchet. (1986). Algal reactions to mineral and organic micropollutants. ecological consequences and possibilities for industrial scale application; a review Water Res. 20. pp. 399-412.

[4] S. Lim. W. Chu. S. Phang.. (2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. J. Bioresour. Technol. 101. pp. 7314-7322.

[5] ISO 5667/3. (1994). Qualité de l'eau - échantillonnage - guide pour la conservation et la manipulation des échantillons (Organisation internationale de normalisation).

[6] J. Rodier. (1996). L'analyse de l'eau naturelle. eaux résiduaires. eau de mer. 8éme édition. Denod. Paris. 1383 p.

[7] J. Rodier. (2009). L'analyse de l'eau. 9ème Edition. Dunod. Paris. 2009. 1529 p.

[8] C. Berthe. 2006. Etude de la matière organique contenue dans des lixiviats issus de différentes filières de traitent de déchets ménagers et assimilés. Thèse de doctorat. Université de Limoges. 186p.

[9] J. Suschka.. E. Ferreira.. (1986). Actived sludge respirometric measuremnts. Water Reserch. 20. 2. pp. 137-144.

[10] A.E. Kouassi.. K.E. Ahoussi.. Y.B. Koffi.. I.K. Kouame.. N. Soro.. J. Biemi. (2014). Caractérisation physico-chimique du lixiviat d'une décharge de l'Afrique de l'ouest : cas de la décharge d'akouedo (abidjan-côte d'ivoire). Larhyss Journal. ISSN 1112-3680. n°19. pp. 63-74

[11] I. Chaouki.. L. Mouhir.. S. Souabi.. M. Fekhaoui.. A. ELabidi. 2013. Etude de la performance de la STEP du centre emplisseur de la société Salam Gaz_Skhirat. Maroc. Afrique Science. 09 (3). pp. 91-102.

[12] D. Belghyti.. Y. Elguamri.. G. Ztit.. My. L. Ouahidi.. My b. Joti.. A. Harchrass.. H. Amghar.. O. Bouchouata.. K. El kharrim et H. Bounouira.. (2009). Caractérisation physico-chimique des eaux usées d'abattoir en vue de la mise en oeuvre d'un traitement adéquat : cas de Kénitra au Maroc. Afrique Science 05(2). pp. 153-216.

[13] O. El Rhaouat.. I. El Kherrati.. F. El khayyat.. H. Chiguer.. K. Ezziani.. A. Ibeda.. M. Fareh.. Y. Saidi.. K. El Kharrim.. D. Belghyti.. (2014). Physic-Chemical Evaluation of Urban Wastewater of the Town of Sidi Kacem. Computational Water. Energy. and Environmental Engineering. 3. 30.

[14] C. Ould Abdlkader.. M.A. Mohamed Vall.. O.D.T. Khyar.. E. Ould Mohamedou.. M. O. S. Ould Kankou.. O.M. Daha.. D. Mamadou.. M.M. Khadijetou.. O. El Rhaouat.. K. El Kharrim and D. Belghyti.. (2015). Caractérisation physico - chimique des eaux usées brutes de la ville de Nouakchott (Mauritanie). International Journal of Innovation and Applied Studies. ISSN 2028-9324 Vol. 11 No. 1. pp. 96-102.

[15] H. Alvares-Vazquez.. B. Jefferson.. SJ. Judd.. (2004). Membrane bioreactors vs. Conventionnel biological. Chem. technical. Biotechnol. 79. pp. 1043-1049.

Ecological and Agricultural monitoring of Sebou river waters at Kariat Bamohamed (Taounate-Morocco)

ELHAMMOUMI Tarik[1*], Saad ABOUBAKER[2], Ayoub ELATMANI[3], Mohamed SIBARI[4], Mostafa Lakhlifi[5], Driss BELGHYTI[6], Khadija EL KHARRIM[7*]

[1-7]Lab. Biotechnology & Environment, Faculty of Sciences, University Ibn Tofail, 14000 Kenitra, Morocco.
[3]Studies Technical Office, Rabat, Morocco.
[4]ONEP, Regional Office of Water and Electricity, Kenitra, Morocco

Abstract**—** *Kariat Bamohamed is an agricultural region located to the west of the city of Taounate and north of the city of Fez. The surface waters in the region are mainly composed by the Sebou river and many natural springs.*

The study concerns the analysis of hydrochemical parameters (T°, pH, CE, MES, O_2, BOD_5, COD, Cl-, PO_4^3, NH_4+ and NO_3-) along the middle Sebou river region of Kariat Bamohamed in order to establish a diagnosis of the state of pollution of the surface waters of this part of the river. Water sampling was carried out at four study stations during flood period and during low water period.

In the light of the results obtained on the surface waters of the Sebou river, it is concluded to a degradation of water quality in both the winter and summer periods. In particular, an increase in the values of ammonium, COD and BOD_5 in addition to an acidic pH due to margine discharges and leaching of fertilizers and other discharges of wastewaters from the cities of Fez and Kariat Bamohamed.

Keywords— Sebou River, Waters, Hydrochemistry, Irrigation, Kariat Bamohamed, Morocco.

I. INTRODUCTION

In Morocco, superficial flows are dependent on rainfall and exhibit high spatial variability [1-2]. In general, the water resources available to Morocco are limited and subject to extreme cyclical variations [3]. Similarly, the qualitative situation of the waters is far from satisfactory [4]. Indeed, population growth accompanied by rapid urbanization that causes many disturbances to natural environments [5]. Industrialization, the irrational use of fertilizers and pesticides and the lack of awareness of the population towards the protection of the environment, lead as much to an imbalance of the ecosystem and generate polluting elements that can affect the physico-chemical quality biological and aquatic receiving environments [6], but also alter the uses of water; water collection, swimming ... etc [7].

The sub-basin of Sebou river drains the Kariat Bamohamed region and is particularly affected by the problem of continuous development of the agricultural sector [8]. Indeed, discharges of wastewaters, pesticides and fertilizers drained by rainwater and irrigation, in addition to domestic discharges generated by the small town of Kariat Bamohamed and the city of Fez are dumped directly into the Sebou river [9].

The present research on the Bamohamed region is aimed at the prospection of the hydrochemistry of the waters of the Sebou river. The study will be based on a monitoring of the indicators of the physicochemical pollution of the water and the determination of the seasonal fluctuations of these parameters between the period of low water and the period of flood.

II. MATERIAL AND METHOD

2.1 Study area

Kariat Bamohamed is a region of Taounate province of high agricultural intensity. It is located at 34° 22 '08 " North and 5° 12' 35" West. 58 km northwest of the city of Fes and 90 km west of the city of Taounate (Fig. 1). Its population is estimated at about 20000 inhabitants. The area includes the town of Kariat Bamohamed surrounded by several rural communes (Bni Snous, Sidi El Abed, Sidi Daoud, Sebt Loudaya, Mkansa).

2.2 Study method

2.2.1 Sampling

Four stations S1 to S4, distributed along the Sebou wadi (Fig. 1), have been retained in such a way that they are accessible and reflect the real characteristics of the surface waters of Sebou river at the level of the Kariat Bamohamed study.

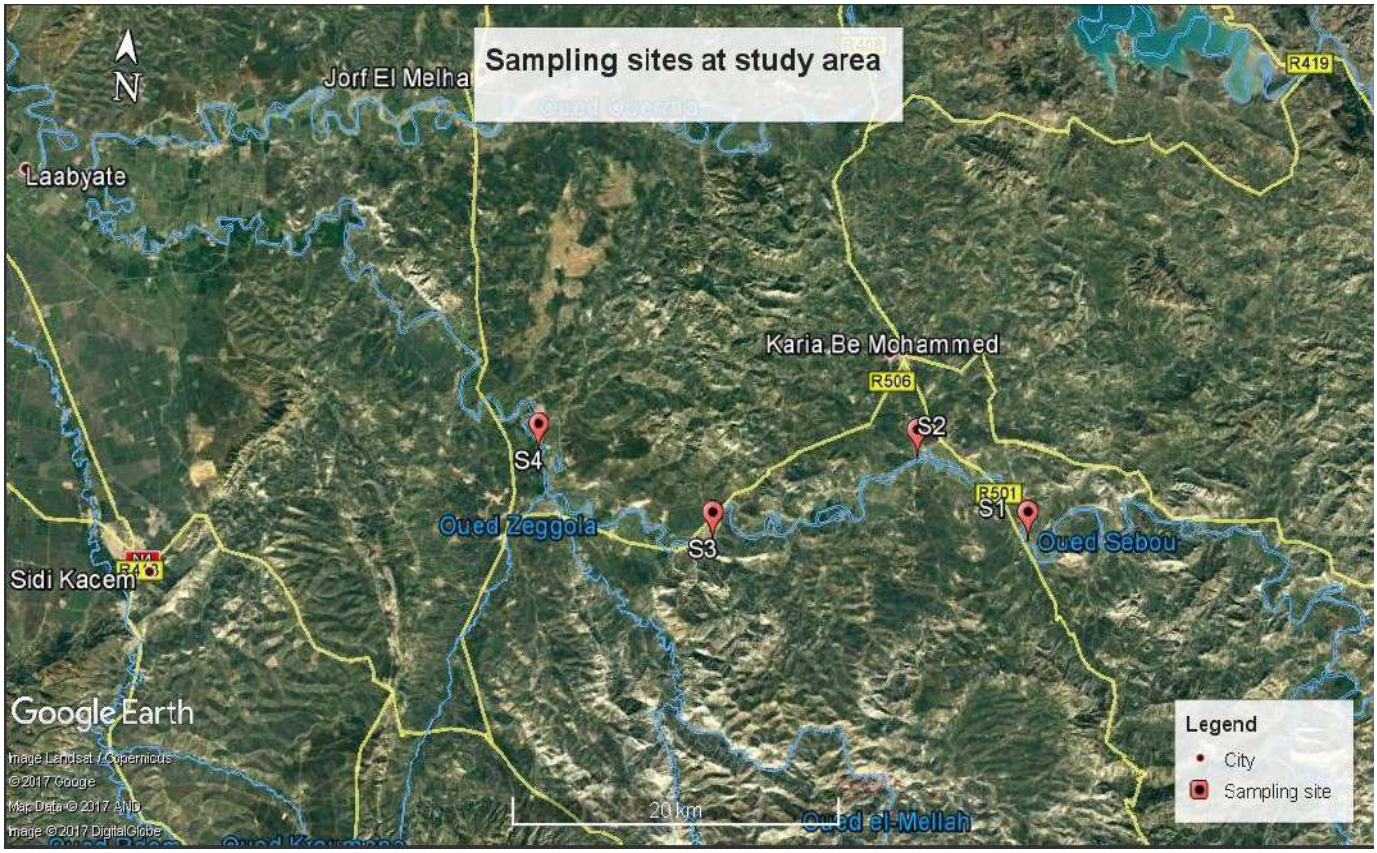


FIGURE 1: LOCATION OF SAMPLING STATIONS (S1, S2, S3 AND S4) AT THE SEBOU RIVER OF KARIAT BAMOHAMED

2.2.2 Sampling and analysis

Along the Sebou river and throughout the study area (**Fig.2**), water samples were taken during wet periods (December, January and February) and in dry periods (June, July and August). The wet period has an intense rain and a violent flood.

At each sampling, the samples were kept in plastic bottles, previously rinsed with water from the station. The bottles were then transported to the laboratory at 4 ° C.

The temperature is measured "in-situ" using a mercury thermometer graduated 1/10 from 0 to 50 ° C. The hydrogen potential (pH), the EC electrical conductivity, the dissolved oxygen (O2) are determined using a CONSORT-Model 835 multi-parameter analyzer, an oxymeter and a pH meter.

The suspended solids MES are determined by filtration of a volume of water on a 0.45 µm cellulose filter according to Rodier [10].

The BOD5 is determined by the respiratory method using a BOD-meter brand WTW, model 1020T according to the technique described by DIN [11].

The COD is determined by acid oxidation by the excess of potassium dichromate at the temperature of 148 ° C of the oxidizable materials under the conditions of the test in the presence of silver sulphate as catalyst and mercury sulphate according to DIN [12].

The chlorides are determined by a volumetric acid determination (HNO3) by a solution of mercuric nitrate in the presence of a pH indicator.

Nitrates, ammonium and orthophosphates are analyzed by colorimetric methods using a Visible Type 722 S Beijing UV spectrophotometer.

FIGURE 2: SAMPLING WATERS AT KARIAT BAMOHAMED SEBOU RIVER AREA

III. RESULTS AND DISCUSSION

3.1 Water temperature

In the study area, recorded temperatures (**Tab.1**) oscillate between 9 °C (station S1) and 12 ° C (stations S3 and S4) during wet periods, and between 25 ° C (S4) and 27.68 ° C (S4) in dry period. These variations follow the temperature of the climate of the region. With measured water temperatures of 9 to 27.6 ° C, the Sebou river belong to the middle class to excellent according to Moroccan standards [6, 13].

3.2 pH

The pH is a measure of the acidity of the water, that is to say the concentration of hydrogen ions (H +). The pH scale extends in practice from 0 (very acidic) to 14 (very alkaline); the median value 7 corresponds to a neutral solution at 25 ° C.

The observed values (**Tab.1**) reveal that the pH is acidic in the rainy period, and neutral to slightly alkaline in the dry period. The pH varies between 5.8 (S1) and 6.8 (S3) in winter and between 7.1 (S2) and 7.8 (S4) in summer. This is probably due to the discharge of vegetable water in December and January from several oil mills between Fez and Kariat Bamohamed.

3.3 Suspended materials

For both flood and low water seasons, recorded values range from 128 mg / L to 3200 mg / L. The spatio-temporal evolution of suspended solids content (MES) (**Tab.1**) in Sebou river shows two distinct periods:

- A winter period, where heavy loads recorded in all stations between 3200 mg / L (S1) in December 2014 and 2450 mg / L (S3) in February 2015. These high levels may be the result of a sudden hydrological event (flood), whose load in alluviants can be attributed to an intense erosion of the watershed, following stormy rains, accentuated by discharges of water-gardens;

- A summer period, where concentrations drop in all stations from 128 mg / L (S1) in August 2015 to 165 mg/L (S3) in June 2015.

The comparison of the contents of suspended solids in the Sebou medium with the Moroccan standard set at 1000 mg / L places these waters in the medium to very poor grid [13].

3.4 Electrical conductivity

The measurement of the conductivity is a good appreciation of the degree of mineralization of water where each ion acts by its concentration and its specific conductivity.

The average recorded values of conductivity (**Tab.1)** show that they fluctuate between 240 µS / cm and 320 µS / cm during flood water periods. These low levels are due to dillution by the rains. In the dry period, the intensities of the electrical conductivity increase and vary between 2730 µS / cm and 3340 µS / cm exceeding the Moroccan standard for surface water (2700 µS / cm) [13]. This indicates excessive mineralization attributed mainly to wastewater from the city of Fez.

TABLE 1
STATISTICAL DATAS ON HYDROCHEMICAL ANALYSIS OF SEBOU RIVER AT KARIAT BAMOHAMED (MOROCCO)

	Stations	déc-14	janv-15	févr-15	juin-15	juil-15	Aout-2015
T°C	S1	9.14	9.12	11	25	26.24	27.44
	S2	10	9.67	10.68	25.34	26.42	28
	S3	10.42	9	10.14	26	27.12	27.68
	S4	11.12	10	12	26.22	27.14	27
pH	S1	5.8	6.2	6.7	7.1	7.6	7.4
	S2	6.1	6.3	6.8	7.1	7.7	7.4
	S3	5.9	6.3	6.6	7.3	7.8	7.6
	S4	6.2	6.4	6.7	7.2	7.5	7.7
O_2 mg/L	S1	2.12	2.86	4.22	3.22	3.66	2.18
	S2	2.08	3.1	4.48	3.44	2.88	2.34
	S3	3.24	2.64	4.84	2.98	3.22	2.48
	S4	2.84	2.42	4.78	4.18	2.22	2.1
CE µS/cm	S1	320	280	240	2830	3120	3320
	S2	290	284	250	2780	2950	3340
	S3	310	300	264	2980	3240	3325
	S4	295	265	268	2870	3180	3125
Cl- mg/L	S1	840	770	760	884	986	1420
	S2	1220	842	784	820	1120	1530
	S3	1620	920	820	790	1220	1600
	S4	980	910	910	834	1240	1130
MES mg/L	S1	3200	2860	2460	156	148	128
	S2	3140	2670	2480	162	152	130
	S3	2760	2630	2450	165	146	132
	S4	3050	2480	2520	157	140	129
DBO5	S1	8	6	5	14	18	22
	S2	10	8	5.8	16.2	19	21.8
	S3	9	9	6.2	16.4	20.4	20.6
	S4	8	10	6.4	16.8	20	21.9
DCO	S1	12	10	8	22	40	36
	S2	14	17	9.5	34	38	32
	S3	16	16	9	36	28	30
	S4	12	15	11	24	32	29
PO_4^{3-} mg/L	S1	0.58	0.53	0.56	0.82	0.86	0.92
	S2	0.6	0.72	0.53	0.84	0.88	0.89
	S3	0.62	0.76	0.58	0.86	0.84	0.91
	S4	0.7	0.68	0.61	0.9	0.85	0.9
NH_4+ mg/L	S1	2.66	2.7	2.44	2.54	2.77	2.76
	S2	2.68	2.76	2.48	2.58	2.78	2.8
	S3	2.7	2.78	2.56	2.62	2.76	2.78
	S4	2.78	2.82	2.52	2.57	2.79	2.81
NO_3- mg/L	S1	14.24	15	14	2.12	1.45	1.8
	S2	16.62	16.2	16.22	2.14	1.66	1.66
	S3	19.45	20.18	17.1	2.21	1.88	1.76
	S4	18.34	20.42	17.8	2.28	1.84	2

3.5 Chlorides

Chloride ion concentrations in the waters of Sebou river at Kariat Bamohamed (**Tab.1**) ranged from 760 mg / L (S1) to 1620 mg / L (S3) during flood periods and between 720 mg / L (S1) and 1600 mg / L (S3) in the low water period. This evolution indicates the influence of an anthropic contribution which can be of urban or industrial origin. For both periods of study, chlorides record levels that exceed the Moroccan standards set at 750 mg / L [13]. This makes it possible to classify these waters in the bad grid of the surface waters.

3.6 Dissolved oxygen

Oxygen is an excellent indicator of water quality. This is one of the most sensitive parameters to pollution. Seasonal evolution of dissolved oxygen shows low concentrations; in fact, the recorded levels (**Tab.1**) vary between 2.08 mg / L and 4.84 mg / L and between 2.1 mg / L and 4.18 mg / L respectively during the flood period and during the dry period.

The low levels recorded during the rainy season are mainly due to the rejections of the vegetable waters and also to the self-cleaning capacity which is inhibited. However, during the summer season, the warming of the water and the low flow of the river cause a decrease in the dissolution of dissolved oxygen, aggravated by an increase in oxygen consumption by living organisms in the water. River and a drop in wind speed in addition to the organic load of urban discharges from the city of Fes and the town of Kariat Bamohamed.

Our results show that the waters of the Sebou river at the level of the study area are of bad quality to very bad quality [13].

3.7 Biochemical oxygen demand

BOD_5 or biochemical oxygen demand is the amount of dissolved oxygen consumed by microorganisms in the dark and at 20 °C for 5 days to degrade organic matter. It allows the evaluation of the biodegradable organic matter overload.

BOD_5 values range from 5 mg / L to 10 mg / L in winter and from 14 mg / L to 22 mg / L in dry periods (**Tab.1**). The increase in BOD_5 levels in the dry period can be explained by the introduction of conditions of degradation of organic matter by microorganisms whose activity intensifies with the decrease of the flow rate and with the warming of water. This activity, which consumes oxygen, is at the origin of self-purification of water [13]. On the contrary, during wet periods, rainwater contributes to the dilution of the organic load emanating from urban wastewater.

3.8 Chemical oxygen demand

The chemical oxygen demand (COD) represents the amount of oxygen consumed by the chemical oxidation of the materials contained in the water. It represents most of the organic compounds but also oxidizable mineral salts (sulfides, chlorides, etc.).

The levels of COD recorded in the water studied range from 8 mg / L to 17 mg / L during the flood period and from 22 mg / L to 40 mg / L during the dry period (**Tab.1**). The grid of Moroccan standards makes it possible to classify these waters as of average quality [13].

3.9 Orthophosphate

Orthophosphates follow relatively marked spatial variation with a tendency to increase in the dry period. The recorded values (**Tab.1**) range from 0.53 mg / L to 0.76 mg / L in the flood period and from 0.82 mg / L to 0.92 mg / L. This availability of orthophosphates can be explained by urban discharges from neighboring agglomerations and the release of phosphorus trapped in large quantities in sediments. The atmospheric agents, wind and rain, also represent sources of phosphates especially when the river flow is weak [14].

The recorded concentrations remain below the Moroccan standard of 1 mg / L. These values then make it possible to classify these waters in the middle class with poor quality [13].

3.10 Ammonium

Ammonium is the product of the final reduction of nitrogenous organic substances and inorganic matter in water and soil. It also comes from the excretion of living organisms and the reduction and biodegradation of waste, without neglecting domestic, industrial and agricultural inputs.

This element exists in a small proportion less than 0.1 mg / L of ammoniacal nitrogen in natural waters. In the surface waters, it comes from nitrogenous organic matter, and gaseous exchanges between water and the atmosphere [15]. It is therefore a good indicator of the pollution of watercourses by urban effluents.

Usually, the ammonium values found during the flood period are clearly much lower than those of the dry period, especially downstream of the study area, thus reflecting the effect of the dilution and testifying to a good oxygenation of the waters. This causing oxidation of nitrogen in winter. But the analysis of the ammonium profile of the waters studied (**Tab.1**) shows that there is not a big difference between the two seasons: the contents vary between 2.44 mg/L and 2.82 mg / L in the flood period and between 2.54 mg / L and 2.81 mg / L in the dry period. This is certainly due to the rejections of the wastewater from oil mills (margine) during the month of December.

The values recorded in ammonium in the waters of Sebou river at the level of the Kariat Bamohamed area make it possible to place them in the bad class [13].

3.11. Nitrates

Nitrate (NO_3-) is the highest form of nitrogen in water. Their concentrations in natural waters are between 1 and 10 mg / L. However, their levels in untreated wastewater are low [15].The nitrate levels recorded (**Tab.1**) range from 12.5 mg / L to 20.18 mg / L and 1.45 to 2.28 mg / L respectively in flood and low water periods. The high levels of nitrates recorded in December 2014 may be due to the leaching of fertilizers used in agricultural soils located on the banks of Sebou river.

IV. CONCLUSION

In the light of cumulative results after monitoring the physicochemical parameters of the surface waters of the Sebou river in the Kariat Bamohamed (Midle Sebou) region, it was concluded that the quality of the water deteriorated both during the winter and the summer period [16-19].

Indeed, during the winter period, an increase in the values of ammonium, COD, BOD_5 and an acidic pH was noted. Discards of vegetable waters and leaching of fertilizers are held responsible in addition to the usual factors such as discharges of raw sewage from the cities of Fes and Kariat Bamohamed.

During the summer season, the low flow of the river, the increase of the water temperature and the activity of the microorganisms, explain the fact that the recorded values of the BOD_5 and the COD are more important than that recorded in winter. In addition, high values of electrical conductivity indicate excessive mineralization that exceeds the drinking norms [20].

ACKNOWLEDGMENTS

The authors acknowledge the Regional Office of Drinking Water Mkansa-Fez who allowed this physico-chemical monitoring.

REFERENCES

[1] MATUHE. Rapport sur l'Etat de l'Environnement du Maroc (REEM), Ministère de l'Aménagement du Territoire, de l'Eau et de l'Environnement, Département de l'Environnement, Observatoire National de l'Environnement du Maroc (ONEM), Octobre, Ed. 2, 296p. 2001.

[2] L Benaabidat. Caractérisation du bassin versant de Sebou, hydrologie, qualité des eaux et géochimie de sources thermals. Thèse en hydrologie à FST- Saïs, 250p. 2000.

[3] DRPE, Direction de la Recherche et de la Planification de l'Eau. Rapport National sur les ressources en eau au Maroc. 2004.

[4] DGH. Direction Générale de l'Hydraulique. Rapport sur Processus d'action et de renforcement des capacités; l'eau et l'environnement. 1996.

[5] A Agoumi, A Debbarh. Ressources en eau et bassin versants du Maroc: 50 ans de développement (1955-2005). 2006.

[6] D Belghyti, H Daifi, A Alemad, K Elkharrim, M Elmarkhi, Y Souidi, F Benelharkati, B Joti, Z Elmoukrifi, A Ibeda, Y Azami-Idrissi, S Baroud, F Elkhayyat, O Elrhaouat, S Sadeq, Y Taboz, H Sbai, R Naser, H Chigger, N Derwich. Groundwater management for sustainable production of drinking water quality in Maamora, 2nd International Conference on Water and Society, 4 - 6 September 2013, New Forest, UK, 2013, Vol 178, pp.242 -254, doi:10.2495 / WS130201, 2013.

[7] O.N.E.P. Alimentation en eau potable, Menaces de pollution. Rapport1999.

[8] ORMVAG: Office Régional de Mise en Valeur Agricole Gharb Maroc. Etude pédologique au1/20000 de la Troisième Tranche d'Irrigation (TTI) sur une superficie de 100000 ha, Zone M'nasra, Z1-Z2, Kénitra, Maroc, Rapport inédit, 180p. 1994.

[9] E Derwich, Z Beziane, L Benaabidate, D Belghyti. Evaluation de la qualité des eaux de surface des oueds fès et sebou utilisées en agriculture maraîchère au Maroc. Larhyss Journal, n° 07, 2008, 59-77.

[10] J. Rodier. L'analyse de l'eau, eaux naturelles, eau résiduaires, eau de mer, 7e edition, Dénod, 1383p. Paris, 1996.

[11] DIN 1992a. Détermination de la Demande Biologique en Oxygène (DBO) selon DIN Laboratoire National de l'Environnement, Rabat. 1992.

[12] DIN 1992b. Détermination de la Demande Chimique en Oxygène (DCO) selon DIN 38409-H52. Laboratoire National de l'Environnement - Rabat. 1992.

[13] CNS. Comité Normes et Standards. Ministère de l'Environnement du Maroc. Rabat, 1994.

[14] CSE. Conseil Supérieur de l'Eau. Aménagement optimal des eaux de l'oued Ouergha: Réalisation du barrage Mjara. Rabat, Maroc. 1988.

[15] A Krira, B Chakour, & H Fouta. Intensification de l'agriculture et son impact sur l'environnement. Cas des nitrates dans la nappe phréatique de M'nasra du Ghab, Actes 1er Colloq, Sur le Développement agric, Rech, Agron, Au niveau de la région du Ghab. 2001.

[16] M Fekhaoui. Recherches hydrobiologiques sur le moyen de Sebou soumis aux rejets de la ville de Fès, suivi d'une Macro-pollution et élévation de son incidence sur les composantes physiques, chimiques et biologiques de l'écosystème. Thèse d'état, Univ, Med V, Rabat, Maroc, 152p.1990.

[17] T El Hammoumi & D Belghyti. Caractérisation physicochimique des eaux potables produites par la station de traitement de Mkansa (Maroc). Scienceslib. 2012.

[18] O.N.E.P. Contrôle de la pollution des eaux destinées à l'alimentation en eau potable. Rapport 1998.

[19] O.N.E.P. Alimentation en eau potable, Menaces de pollution. Rapport 1999.

[20] WHO (eds), World Health Organization., Guideline for Drinking Water Quality, Health Criteria and Other Supporting Information. 2nd Edition, Vol.2, Geneva, pp.940-949, 2004.

Physiological Selectivity of Agrochemicals to Predatory Mites of *Tetranychus urticae* (Acari: Tetranychidae) on Rosebushes Growing in Greenhouse

Giselle Christiane Souza-Pimentel[1], Paulo Rebelles Reis[2], Patrícia de Pádua Marafeli[3], João Paulo Alves[4]

[1,3]D.Sc. Postgraduate Program, Universidade Federal de Lavras - UFLA, Lavras, MG, Brazil.
[2]D.Sc. Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG Sul/EcoCentro, Lavras, MG, Brazil and CNPq
[4]Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG Sul/EcoCentro. FAPEMIG Fellowship biologist.

Abstract— *The growing of rose (Rosa spp.) in a greenhouse provides favorable conditions for both, the plant and the pest mite Tetranychus urticae Koch (Acari: Tetranychidae), for which chemical control is still used. Consumers' demand has encouraged researches to use less aggressive agricultural practices, making the biological control as a viable option. The objective of the present study was to investigate the physiological selectivity of plant protection products, used on rosebushes for the control of T. urticae and other pests or diseases, to Phytoseiulus macropilis (Banks) and Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) both predatory mites of T. urticae on rosebush growing in a greenhouse. According to IOBC/WPRS, the residual method of spraying on a glass and leaf surface area was used for the physiological selectivity test of plant protection products for the predatory mites. The obtained results shown that with the exception of the acaricides-insecticide chlorfenapyr all other tested products - fungicides, acaricides and acaricides-insecticide - methiram + pyraclostrobin, thiofanate-methyl, boscalid + kresoxim-methyl, chlorothalonil, propargite, mandipropamid, mefenoxam, difenoconazol, bifenthrin and pyriproxifen, were innocuous (class 1) or only slightly harmful (class 2) to both species. Chlorfenapyr was highly toxic only for N. californicus (class 4), however after five days of its application no toxic residue of the product was detected on the glass surface and so the product has been classified as innocuous (class 1) as well for this predatory mite species after this period.*

Keywords— Neoseiulus californicus, Phytoseiulus macropilis, Rosa spp., Protected crop, Agricultural acarology.

I. INTRODUCTION

The flowers trade in Brazil is growing, so that it has been recognized as an emergent business sector of high profitability. In 2014, the sector gains were of approximately R$ 5.7 billion (US$ 1.42 billion, based on the exchange rate as R$4.00/dollar) and an 8% growth is estimated for the year of 2015 [1].

The state of Minas Gerais stands out in this sector, especially, for the production of rosebushes (*Rosa* spp., Rosaceae) and of other conventional cutting flowers. Although there is not available up-to-date data of this activity in Minas Gerais, the flower production in this state occurs especially in the municipalities of Barbacena, Andradas, Araxá, and Munhoz [2].

The rosebush from Asia has developed well in Brazil and is cultivated in various Brazilian regions. A large part of the production occurs in greenhouses. Although this environment offers better conditions for the control of pests and diseases, it is also a more favorable environment to the occurrence of these two issues with a special highlight to the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a pest mite that is one of the main problems that affect rosebushes in a protected cultivation system [3].

Pest control is one of the challenges found in the cultivation of flowers and others ornamental plants, being any damage caused by insects and other arthropods inacceptable by consumers, since it depreciates the final product, the flower and the foliage that will be commercialized [4].

The demand of producers and consumers for the use reduction of agrochemicals is very evident [5]. However, the spraying of plant protection products is still the main tactic used for the control of pests and diseases, being carried out in a preventive

way [6] that in many instances causes ecological disequilibrium and environmental contamination [7], especially as a function of the misapplication of these products.

Consumers' concern about human health and environmental preservation has encouraged researchers to investigate the use of less aggressive agricultural practices geared towards sustainability and agroecosystems [6].

The biological control of pest mites has been used in fruit trees, ornamental plants, and other crops for the control of the two-spotted spider mite with the use of predatory mites *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) (Phytoseiidae) among others. This biological control method reduces the amount of chemical defensives used in cultivations and holds other benefits such as a lesser exposure of the workers to chemicals and lower amounts of residues [8].

In addition, studies on the selectivity of plant protection products to predatory mites belonging to the Phytoseiidae family are of great importance, since mites of this family have been more widely used for the biological pest control [9] [10].

In a program of integrated pest management - IPM, the use of selective products is highly desirable under certain conditions, as when the pest is at excessively high levels [11].

Therefore, the objective of the present study was to investigate the physiological selectivity of plant protection products, used on rosebushes for the control of *T. urticae*, and other pests or diseases, on *P. macropilis* and *N. californicus* both predatory mites of the *T. urticae* on rosebush growing in greenhouse.

II. MATERIAL AND METHODS

The present study was conducted in the Acarology Laboratory of the *Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG-Sul / Centro de Pesquisa em Manejo Ecológico de Pragas e Doenças de Plantas - EcoCentro*, Lavras, MG, Brazil, under controlled conditions, at temperature of 25 ± 2ºC, RH of 70 ± 10%, and 14 hours of photophase.

The predatory mites, as well as the pest mite, *T. urticae*, were obtained from the *Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas - Campus de Inconfidentes*, Minas Gerais, Brazil.

2.1 Lab rearing of the two-spotted spider mite

Uncapped Petri dishes (15 cm in diameter) were used and 1 cm thick foam, which was maintained moist with distilled water, occupying the entire bottom surface of each Petri dish. A Jack-bean leaflet [*Canavalia ensiformis* L. (DC), Fabaceae] was placed on top of the foam and surrounded by strips of hydrophilic cotton that were also in contact with the damp foam in order to prevent the mites from escaping and to better conserve the leaflet. The pest mites, *T. urticae*, were put on top of the Jack-bean leaflets, which were switched out weekly.

2.2 Lab rearing of predatory mites

The predatory mite *P. macropilis* was reared in rectangular arenas of black flexible PVC plastic sheets (26 x 22 cm) were used. These were put on Styrofoam™ of equal size, and in turn these were placed in water on plastic trays (32 x 26.5 x 5.5 cm). Cotton was placed around the Styrofoam™ and the arena, and it was in contact with the water from the tray. The cotton was used to prevent the mites from escaping in addition to preserving the Jack-bean leaflets. The leaflets were placed on the arenas with the petiole under the damp cotton and were infested by *T. urticae*, which served as food for predatory mites; as the leaflets withered other new and infested by *T. urticae* were placed on the old leaflets [12].

The predatory mite *N. californicus* was reared in arenas of flexible PVC plastic 3 cm in diameter floating in distilled water in Petri dishes 15 cm in diameter [13] and the predatory mite were fed with pollen of castor bean plant (*Ricinus communis* L., Euphorbiaceae) [14].

2.3 Physiological selectivity assays

A survey on listed plant protection products, used by producers for the control of pests and diseases on rosebushes in the region of *Campos das Vertentes* in the state of Minas Gerais, Brazil, was carried out for the physiological selectivity tests, and 11 of these products were tested in this work (Table 1).

TABLE 1
CHARACTERISTICS OF SELECTED PLANT PROTECTION PRODUCTS USED ON ROSEBUSHES AND TESTED ON *NEOSEIULUS CALIFORNICUS* AND *PHYTOSEIULUS MACROPILIS*, UNDER LABORATORY CONDITIONS

Active ingredient	Commercial product	Chemical group	Dose per 100 liters of water	Agronomic class	Toxicological Classification[1]
Methiram + Pyraclostrobin	Cabrio Top®	Dithiocarbamate + strobilurins	200 g	Fungicide	III
Thiofanate-Methyl	Cercobin 700 WP®	Benzimidazole	70 g	Fungicide	I
Boscalid + Kresoxim-methyl	Collis®	Anilide + strobilurins	50 mL	Fungicide	III
Chlorothalonil	Daconil BR®	Isoftalonitrils	200 g	Fungicide	I
Propargite	Omite 720 EC®	Alkyl sulphite	30 mL	Acaricide	I
Chlorfenapyr	Pirate®	Pyrazole analog	50 mL	Acaricide-insecticide	III
Mandipropamid	Revus®	Mandelamide ether	60 mL	Fungicide	II
Mefenoxam (Metalaxyl-M) + Mancozeb	Ridomil Gold MZ®	Acilalaninate + dithiocarbamate	300 g	Fungicide	III
Difenoconazol	Score®	Triazole	80 mL	Fungicide	I
Bifenthrin	Talstar 100 EC®	Pyrethroid	30 ml	Acaricide-insecticide	III
Pyriproxifen	Tiger 100 EC®	Pyridyloxypropil Ether	75 mL	Insecticide	I

[1]Toxicological classification used in Brazil: Class I - Extremely toxic; Class II - Highly toxic; Class III - Average toxicity Class IV - Low toxicity

The experimental design used was the completely randomized with 12 treatments, being 11 products and one control group sprayed with only distilled water, seven repetitions for *N. californicus* and five repetitions for *P. macropilis*. The spraying of products was carried out in a laboratory Potter tower at pressure of 15 lb/pol^2, with an amount of 1.5 ± 0.5 mg/cm^2 of surface [15]. The maximum dosages recommended by manufacturers, to use in the control of pests and diseases on rosebushes, were also used in the selectivity assays.

2.4 Physiological selectivity assays to *Neoseiulus californicus*

The residual spraying method on glass surface was used for the predatory mite *N. californicus*, recommended method as a standard under laboratory conditions to test adverse or side effects of plant protection products to predatory mites [16]. Glass coverslips for microscopy of 20 x 20 mm, floating in water on an uncapped Petri dish 5 cm in diameter x 2 cm in depth, were used as a surface for the application of the products and as a support for the mites [15]. Under these conditions, the coverslip was relatively in the center of the dish, not touching the edge and making it difficult for mites to scape. After the application of the products the coverslip were set aside for one hour to dry in room temperature. Then, five female of *N. californicus* were transferred, from the lab rearing, with a fine-tipped brush to each of coverslip. Only predatory mite females were used in the assay because this is the stage responsible for the species perpetuation; also because one of the objectives was to evaluate the effect of the products on the predatory mite reproduction. Castor bean pollen was offered as food to the survivor's predatory mites [14].

2.5 Physiological selectivity assays to *Phytoseiulus macropilis*

The residual method of spaying on leaf surface was used for the physiological selectivity tests to the predatory mite *P. macropilis*. This method is also recommended as standard to test adverse effects of pesticides to predatory mites under laboratory conditions [16]. Arenas 3 cm in diameter were made up from Jack-bean leaflets and placed on Petri dishes 5 cm in

diameter filled with agar-water at 3%. The Petri dishes that contained the leaf discs were sprayed with the products and set aside for one hour to dry in room temperature.

After that, five female of *P. macropilis* were transferred, from de lab rearing, with the use of a fine-tipped brush to each arena. A mixture of all stages of the two-spotted spider mite was offered as food to the survivors. The Petri dishes were sealed with plastic wrap to impede two-spotted spider mites from escaping.

This methodology was not used for *N. californicus* because since they are white mites in color, it would be more difficult to find them and their eggs in the agar-water and on the Petri dish, and also because *T. urticae* was offered as food to the survivor's *P. macropilis* rather than pollen, once the possible survivor's pest mites need plant leaves to feed.

2.6 Selectivity assessment

During six days after the application of plant protection products, daily assessments were carried out for the verification of the residual effect of these products on predatory mites, with the aid of a stereomicroscope. The number of predatory mite females alive, of laid eggs, and of hatched larvae were evaluated in order to obtain information on the number and eggs' viability.

The adverse or total effect (E %) was calculated taking into account the corrected mortality in the treatment (Mc) as a function of the control group mortality [17]. The reproduction effect (Er) was calculated based on IOBC/WPRS [16], being E % = 100% - (100% - Mc) x Er.

Daily, during six days, it was counted the number of alive female mites as well as the number of viable eggs (larvae hatched), and removed the dead females. The reproduction effect (Er) was obtained by dividing the female eggs production (R) in the treatment by the production of eggs in the control group ($Er = R_{Treatment} / R_{Control\ group}$).

The average production of eggs for each female (R) was obtained by the relation: R = number of viable eggs/number of alive females. The tests considered valid were the ones in which the mortality of the control group was at the most 20% [16].

Values of the total effects found for each tested product were grouped in classes of 1 through 4, according to the criteria established by IOBC/WPRS in order to categorize agrochemicals based on the adverse effect caused to beneficial organisms in laboratory tests [16] [18], being: class 1 = $E < 30$ % (innocuous); class 2 = $30\ \% \leq E \leq 79\ \%$ (slightly harmful); class 3 = $80\ \% \leq E \leq 99\ \%$ (moderately harmful), and class 4 = $E > 99$ % (harmful).

A new experiment was carried out with the goal of evaluating the duration of the residual effect of products grouped in classes 3 and 4, moderately harmful and harmful, which acted as harmful to predatory mites.

For this new bioassay, approximately 50 glass cover slips will be sprayed with the products, all coverslips in the same day, and experiments with 5 repetitions and 5 female of predatory mite each, were set up daily. The coverslips were evaluated daily, until no more female mortality was found. In the control group, the coverslips were sprayed with distilled water. These coverslips when free of residue, were evaluated during another six days, so the product residual effect could be verified in the reproduction of females; and only at that time a new classification was attributed to the product based on the IOBC/WPRS scale [16].

III. RESULTS AND DISCUSSION

3.1 Physiological selectivity assays

From all the tested products, only the acaricide-insecticide chlorfernapyr (Pirate®) was highly toxic (class 4) and only for *N. californicus* (Table 2). The other tested products were innocuous or slightly harmful for *N. californicus* (Table 2) as well as for *P. macropilis* (Table 3).

In the present study, since only the product chlorfenapyr (Pirate®) was highly toxic to *N. californicus* (Table 2**)**, another experiment was carried out in order to determine the residual effect of this product until all females remained alive.

Based on the results of the new experiment, after five days of evaluation the product residue did not have any more effect on the mortality of *N. californicus* females (Table 4) and after another six days of evaluation to verify the effect on the predatory mite reproduction and the calculation of the total effect, the product that was initially categorized in class 4 (Table 4) was changed to class 1 as innocuous (Table 5).

TABLE 2

EFFECT OF ACARICIDES, INSECTICIDES, AND FUNGICIDES USED ON ROSEBUSHES IN THE REGION OF CAMPO DAS VERTENTES IN THE STATE OF MINAS GERAIS ON ADULT FEMALES OF *NEOSEIULUS CALIFORNICUS*, UNDER LABORATORY CONDITIONS, AT TEMPERATURE OF 25 ± 2°C, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE

Treatment	Number of specimen evaluated	Mortality (%)	Mc (%)[1]	Er[2]	E (%)[3]	Class of toxicity[4]
Control group	35	14.30	-	-	-	-
Bifenthrin	35	22.90	10.00	0.61	44.97	2
Boscalid + Kresoxim-methyl	35	25.70	13.30	1.23	0.00	1
Chlorfenapyr	35	100.00	100.00	-	100.00	4
Chlorothalonil	35	48.60	40.00	0.98	41.27	2
Difenoconazole	35	22.90	10.00	0.46	58.73	2
Metalaxyl-M + Mancozeb (Mefenoxam)	35	34.30	23.30	0.42	67.72	2
Mandipropamid	35	31.40	20.00	0.40	67.72	2
Methiram + Pyraclostrobin	35	20.00	6.70	0.61	42.86	2
Propargite	35	17.20	3.30	0.59	42.86	2
Pyriproxifen	35	14.30	0.00	0.34	66.14	2
Thiofanate-Methyl	35	5.40	0.00	0.17	79.00	2

[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful).

TABLE 3

EFFECT OF ACARICIDES, INSECTICIDES, AND FUNGICIDES USED ON ROSEBUSHES IN THE REGION OF *CAMPO DAS VERTENTES* IN THE STATE OF MINAS GERAIS ON ADULT FEMALES OF *PHYTOSEIULUS MACROPILIS*, UNDER LABORATORY CONDITIONS, AT TEMPERATURE OF 25 ± 2°C, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE

Treatment	Number of specimen evaluated	Mortality (%)	Mc (%)[1]	Er[2]	E (%)[3]	Class of toxicity[4]
Control group	25	4.00	-	-	-	-
Bifenthrin	25	40.00	37.50	0.72	54.88	2
Boscalid + Kresoxim-methyl	25	28.00	25.00	0.62	53.56	2
Chlorfenapyr	25	72.00	70.80	0.93	72.82	2
Chlorothalonil	25	28.00	25.00	0.98	26.65	1
Difenoconazole	25	68.00	66.70	1.29	56.99	2
Metalaxyl-M + Mancozeb (Mefenoxam)	25	32.00	29.20	1.04	26.65	1
Mandipropamid	25	16.00	12.50	0.57	50.13	2
Methiram + Pyraclostrobin	25	48.00	45.80	1.30	29.55	1
Propargite	25	72.00	70.80	1.59	53.56	2
Pyriproxifen	25	40.00	37.50	1.14	29.02	1
Thiofanate-Methyl	25	36.00	33.30	0.47	68.87	2

[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful)

TABLE 4
RESIDUAL EFFECT OF THE ACARICIDE - INSECTICIDE CHLORFENAPYR ON ADULT FEMALES OF *NEOSEIULUS CALIFORNICUS* AT 24, 48, 72, 96, AND 120 HOURS AFTER THE APPLICATION OF THE PRODUCT, AT TEMPERATURE OF 25 ± 2°C, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE

Hours after the application	N[1]	Number of mites alive	Percentage of mortality
24	25	0	100
48	25	0	100
72	25	0	100
96	25	11	44
120	25	25	0

[1]N = Number of specimens of N. californicus studied

TABLE 5
EFFECT OF THE ACARICIDE - INSECTICIDE CHLORFENAPYR ON THE MORTALITY AND REPRODUCTION OF FEMALE ADULTS OF *NEOSEIULUS CALIFORNICUS* AFTER 120 HOURS OF THE APPLICATION OF THE PRODUCT, AT TEMPERATURE OF 25 ± 2°C, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE

Treatment	Number of specimens evaluated	Mortality (%)	Mc (%)[1]	Er[2]	E (%)[3]	Class of toxicity[4]
Control group	25	0	0	-	-	-
Chlorfenapyr	25	0	0	1,51	0	1

[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful).

The predatory mite *N. californicus* was highly tolerant to the acaricide-insecticide bifenthrin in CL50 equal or higher than the recommended concentration for the use in citrus (*Citrus* spp., Rutaceae) [19].

Regarding the mortality of mites by the product, it was observed in the present study that the CL50 value was also below 50% and bifenthrin was grouped in class 2, slightly harmful for both predatory mites, therefore being considered selective (Table 2 and Table 3).

Other studies that took into account only adult females of *N. californicus* and *P. macropilis*, alike in the present study, have reported that the fungicide boscalid + kresoxim-methyl caused mortality lower than 20% for both mites, being *N. californicus* less susceptive to the product [20]. The same result was observed in the present study and although this product has been considered selective to both predatory mites, the mortality and total effect of the product was lower for the *N. californicus*, which was classified as innocuous (class 1) to this species. The same product was classified as slightly harmful to the predatory mite *P. macropilis* (class 2) (Table 2 and Table 3).

It has been reported that the fungicide chlorothalonil has caused lower mortality than 20% for *N. californicus* and *P. macropilis*; therefore, being selective [20]. In the present study, this fungicide was also considered selective to both predatory mites; however; *P. macropilis* suffered a lesser effect and this product was considered innocuous (class 1) to this species. The effect of this fungicide was slightly greater for *N. californicus* and it was considered slightly harmful (class 2) (Table 2 and Table 3).

The fungicide difenoconazole was slightly harmful (class 2) to predatory mites *N. californicus* and *P. macropilis*, and considered selective (Tables 2 and Table 3). Although studies on the selectivity of this product to predatory mites have not been found in the literature, the difenoconazole was considered innocuous to other group of natural enemies as adults of the *Trichogramma pretiosum* Riley and *Trichogramma atopovirilia* Outman & Platner (Hymenoptera: Trichogrammatidae) in apple tree crops [21] and to adult parasitoids of *T. atopovirilia* found in citrus [22].

In the present study, the fungicide metalaxyl-M + mancozeb was slightly harmful (class 2) to *N. californicus* (Table 2), and innocuous to *P. macropilis* (class 1) (Table 3), as well as to adults of the predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) [23].

The fungicide mandipropamid was slightly harmful (class 2) to *N. californicus* and to *P. macropilis* (Tables 2 and 3). Although reports on the selectivity of this fungicide to beneficial organisms have not been found in the literature, this product is widely used for the control of mildew on rosebushes.

In the present study, the fungicide methiram + pyraclostrobin was slightly harmful (class 2) to *N. californicus* (Table 2) and innocuous (class 1) to *P. macropilis* (Table 3). Different results have been reported that *N. californicus* was less susceptive to this product than *P. macropilis*, when taking into account only the female mortality [20]. In the present study, considering only the corrected mortality, it was observed that *N. californicus* was also less affected (Mc = 6.7) than *P. macropilis* (Mc = 45.8). However, regarding also the predatory mite reproduction effect or total effect, it was observed that *N. californicus* was more affected (Table 2) than *P. macropilis* (Table 3); because of that there is a difference when the total effect is examined, although this fungicide is considered equally selective to both predatory mites.

The fungicide thiofanate-methyl in this work was classified as slightly harmful (class 2) to both predatory mites (Tables 2 and Table 3), therefore, it can be considered selective to both; although it has been also classified as innocuous to *P. macropilis* (class 1) [24].

The acaricide propargite was classified as slightly harmful (class 2) to both predatory mites in the present study (Tables 2 and Table 3). These results are different than that found in other studies, in which the propargite was highly toxic to *N. californicus* and *P. macropilis* [25] [24] [19] [26]. However, in those studies only the mortality of the mites by the product was considered, which could have led to the differences between the results of both works. When the total effect on *P. macropilis* was considered, alike in the present study, the propargite was also classified as slightly harmful (class 2) [27].

Still describing the acaricide propargite, this product was classified as innocuous (class 1) to *N. californicus* and it was suggested that the resistance of the predatory mite to this active ingredient was probably selected in the strawberry cropping, in which the mite was collected for these tests, since this acaricide is frequently used in this type of cropping or due to a natural tolerance of the predatory mite [28]. This fact could have also occurred in the present study, in which propargite was considered slightly harmful to the predatory mite *N. californicus*.

The insecticide pyriproxifen was considered selective to both predatory mites, slightly harmful (class 2) to *N. californicus* (Table 2) and innocuous (class 1) to *P. macropilis* (Table 3). However, when only the mortality by the product is observed, *N. californicus* is more resistant than *P. macropilis.* In contrast, although this product has not caused mortality in *N. californicus*, it has reduced the oviposition by the female, which made this insecticide be classified as slightly harmful (class 2). For *P. macropilis* there was mortality of females due to the application of this product; however, the product did not cause decrease in the oviposition of females, which was larger than the oviposition of the control group. Therefore, the product becomes less harmful to the predatory mite because the females will leave their descendants (Table 3).

In the present study, the acaricide-insecticide chlorfenapyr was the only tested product that caused mortality of 100% of the predatory mite *N. californicus* and, therefore, this product was considered harmful (class 4) to this predatory mite (Table 2). Chlorfenapyr was also considered harmful to *N. californicus* in other studies found in the literature [29] [28] [19] as well as to *Iphiseiodes zuluagai* Denmark & Muma and *Euseius alatus* DeLeon (Phytoseiidae) in the tested doses of this product for its use in citrus [20]. Different than that, chlorfenapyr was considered slightly harmful to *P. macropilis*, in the present study (class 2) (Table 3).

Chlorfenapyr did not exhibit a toxic residue to *N. californicus* only after five days of its application (Table 4). After this period, the residue of this acaricide-insecticide in the leaves also stimulated the oviposition of females, and an Er value above 1 was found (Er = 1.51) (Table 5). The phenomenon of hormoligosis has probably occurred, in which sub lethal dose of this product produce a direct stimulus in the reproduction [31] [32].

The results of the residual effect of up to seven days after the application of the chlorfenapyr to the mite *N. californicus* have also been reported in others studies [29] [33]. The high toxicity of chlorfenapyr to *N. californicus* on the strawberry plant could be associated to the fact that this product had not yet been used in the field where the study was carried out. Thus, the mites probably had not yet suffered any pressure of selection to resistance at this product until that moment [29].

Chlorfenapyr or Pirate® is a product widely used by flower growers in the control of *T. urticae* and other pests. If the two-spotted spider mite infestation is very high, the strategy of the integrated management of pest mite might be used, by first apply the product to reduce mite infestation and only after five days can the predatory mite *N. californicus* be released to continue the control.

IV. CONCLUSIONS

The following products, bifenthrin, boscalid + kresoxim-methyl, chlorotalonil, difenoconazole, metalaxyl-M + mancozeb, mandipropamid, methiram + pyraclostrobin, propargite, pyraclostrobin, and thiofanate-methyl are selective to *N. californicus*

and *P. macropilis* and could be used as a strategy in the integrated management of insects, mites and diseases in rosebushes without affecting both predatory mite species.

The acaricide-insecticide chlorfenapyr is non-toxic to the predatory mite *P. macropilis* and, therefore, might be used as a tactic to the integrated management of the two-spotted spider mite in rosebushes. However, this product may not be used together the predatory mite *N. californicus* because this mite is sensitive to the product.

The predatory mite *N. californicus*, in integrated management program using the acaricides-insecticide chlorfenapyr, should only be released after five days of this product application.

ACKNOWLEDGMENTS

To the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* - CNPq for the financial support and scholarships granted, and *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* - FAPEMIG for the grant of scholarship

REFERENCES

[1] Ibraflor - Instituto Brasileiro de Floricultura. Available from: <http://www.ibraflor.com/publicacoes/vw.php?cod=246>. Access in: 15 Jun 2015

[2] Landgraf, P.R.C. and Paiva, P.D.D. 2009. Production of cut flowers in the state of Minas Gerais. Ciência e Agrotecnologia **33** (1):120-126.

[3] Barbosa, J.G.; Grossi, J.A.S.; Santos, J.M.; Pivetta, K. and Finger, F.L. 2007. Rosa (*Rosa* spp.). p. 675-682. In: Paula Junior, T.J. and Venzon, M., Eds., 101 Culturas: manual de tecnologias agrícolas. EPAMIG, Belo Horizonte. 800 p.

[4] Carvalho, L.M.; Bueno, V.H.P.; Santa-Cecília, L.V.C; Silva, R.A. and Reis, P.R. 2009. Pragas na floricultura: identificação e controle. Informe Agropecuário **30** (249): 36-46.

[5] Severino, C.A.M. 2007. Controle biológico de pragas e doenças em floricultura. Salvador, Rede de Tecnologia da Bahia. 24p. (Dossiê Técnico).

[6] Carvalho, L.M.; Almeida, K.; Taques, T.C.; Soares, C.S.A.; Almeida, E.F.A. and Reis, S.N. 2012. Manejo de pragas em cultivo de roseira de sistema de produção integrada e sistema convencional. Bioscience Journal **28** (6):938-944.

[7] Torres, F.Z.V.; Carvalho, G.A.; Souza, J.R. and Rocha, L.C.D. 2007. Seletividade de inseticidas a *Orius insidiosus*. Bragantia **66** (3):433-439.

[8] Barbosa, M.F.C.; Demite. P.R.; Moraes, G.J.; Poletti, M. 2017. Controle biológico com ácaros predadores e seu papel no manejo integrado de pragas. Engenheiro Coelho, Promip Holding S.A., 69p. Available from: www.promip.agr.br. Accessed in: 29 Nov 2017.

[9] Pilkington, L.J. Messelink, G.; Van Lenteren, J.C. and Mottee, K.L. 2010. "Protected biological control" - Biological pest management in the greenhouse industry. Biological Control **52**:216-220.

[10] McMurtry, J.A.; Sourassou, N.F. and Demite, P.R. 2015. The Phytoseiidae (Acari: Mesostigmata) as Biological Control Agents. p. 133-149. In: Carrillo, D.; Moraes, G.J. and Peña, J.E., Eds., Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms. Switzerland, Springer, 328 p.

[11] Silva, F.R.; Vasconcelos, G.J.N.; Gondim Júnior, M.G.C. and Oliveira, J.V. 2006. Toxicidade de acaricidas para ovos e fêmeas adultas de *Euseius alatus* DeLeon (Acari: Phytoseiidae). Revista Caatinga 19 (3): 294-303.

[12] Souza-Pimentel, G.C.; Reis, P.R.; Silveira, E.C.; Marafeli, P.P.; Silva, E.A. and Andrade, H.B. 2014. Biological control of *Tetranychus urticae* (Tetranychidae) on rosebushes using *Neoseiulus californicus* (Phytoseiidae) and agrochemical selectivity. Revista Colombiana de Entomologia **40** (1):80-84.

[13] Reis, P.R. and Alves, E.B. 1997. Criação do ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) em laboratório. Neotropical Entomology *formerly* Anais da Sociedade Entomológica do Brasil **26** (3):565-568.

[14] Marafeli, P.P.; Reis, P.R.; Silveira, E.C.; Souza-Pimentel, G.C. and Toledo, M.A. 2014. Life history of *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) when laboratory fed with castor bean plant (*Ricinus communis* L.) pollen. Brazilian Journal of Biology **74** (3): 691-697.

[15] Reis, P.R.; Chiavegato, L.G. and Alves, E.B. 1998. Seletividade de agroquímicos ao ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae). Neotropical Entomology *formerly* Anais da Sociedade Entomológica do Brasil **27** (2):265-274.

[16] Bakker, F.M.; Grove, A.; Blümel, S.; Calis, J. and Oomen, P. 1992. Side-effect test for Phytoseiidae and their rearing methods. IOBC /WPRS Bulletin **15** (3):61-81.

[17] Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology **18**:265-267.

[18] Hassan, S.A.; Bigler, F.; Bogenschütz, H.; Boller, E.; Brun, J.; Calis, J.N.M.; Coremans-Pelseneer, J.; Duso, C.; Grove, A.; Heimbch, U.; Helyer, N.; Hokkanen, H.; Lewis, G.B.; Mansour, F.; Moreth, L.; Polgar, L.; Samsøe - Petersen, L.; Sauphanor, B.; Stäubli, A.; Sterk, G.; Vainio, A.: Van De Veire, M.; Viggiani, G. and Vogt, H. 1994. Results of the sixth joint pesticide testing programme of the IOBC /WPRS - Working Group "Pesticides and Beneficial Organisms". Entomophaga **39** (1):109-119.

[19] Silva, M.Z.; Sato, M.E.; Oliveira, C.A.L. and Rais, D.S. 2011. Toxicidade diferencial de agrotóxicos utilizados em citros para *Neoseiulus californicus*, *Euseius concordis* e *Brevipalpus phoenicis*. Bragantia **70** (1):87-95.

[20] Poletti, M.; Collette, L.P. and Omoto, C. 2008. Compatibilidade de agrotóxicos com os ácaros predadores *Neoseiulus californicus* (McGregor) e *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae). BioAssay **3** (3):1-14.

[21] Manzoni, C.G. Grützmacher, A.D.; Giolo, F.P.; Härter, W.R.; Castilhos, R.V. and Paschoal, M.D.F. 2007. Seletividade de agroquímicos utilizados na produção integrada de maçã aos parasitóides *Trichogramma pretiosum* Riley e *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogrammatidae). BioAssay **2** (1):1-11.

[22] Matos, M.M. 2007. Seletividade a *Trichogramma atopovirilia* Oatman & Platner, 1983 de agroquímicos utilizados na citricultura paulista para o controle do bicho-furão-dos-citros, *Gymmandrosoma aurantianun* Lima, 1927. 54p. Dissertação (Mestrado em Entomologia) - Escola Superior de Agricultura Luiz de Queiroz - ESALQ-USP, Piracicaba, 2007.

[23] Rocha, L.C.D.; Carvalho, G.A.; Moura, A.P. and Torres, F.Z.V. 2006. Toxicidade de produtos fitossanitários para adultos de *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Bragantia **65** (2):309-315.

[24] Amin, M.M.; Mizell, R.F. and Flowers, R.W. 2009. Response of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) to pesticides and kairomones of three spider mite species (Acari: Tetranychidae), and non-prey food. Florida Entomologist **92** (4):554-562.

[25] Ruiz, M.G. and Moraes, G.J. 2008. Mortalidade do ácaro predador *Neoseiulus californicus* (Acari: Phytoseiidae) em testes de toxicidade residual de inseticidas e acaricidas usuais em pomáceas. Revista Brasileira de Fruticultura **30** (4):919-924.

[26] Veronez, B.; Sato, M.E. and Nicastro, R.L. 2012. Toxicidade de compostos sintéticos e naturais sobre *Tetranychus urticae* e o predador *Phytoseiulus macropilis*. Pesquisa Agropecuária Brasileira **47** (4):511-518.

[27] Costa, R.; Rocha, L. C.D.; Freitas, J.A.; Coura Júnior, G.M.; Santos, O.M. and Couto, E.O. 2012. Efeito de agrotóxicos usados na cultura do morangueiro sobre o predador *Phytoseiulus macropilis* (Banks) em laboratório, semicampo e campo no sul de Minas Gerais. Revista Agrogeoambiental **4** (3):1-12.

[28] Silva, M.Z. and Oliveira, C.A.L. 2006. Seletividade de alguns agrotóxicos em uso na citricultura ao ácaro predador *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). Revista Brasileira de Fruticultura **28** (2):205-208.

[29] Sato, M.E.; Silva, M.; Gonçalves, L.R.; Souza Filho, M.F. and Raga, A. 2002. Toxicidade diferencial de agroquímicos a *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) e *Tetranychus urticae* Koch (Acari: Tetranychidae) em morangueiro. Neotropical Entomology **31** (3):449-456.

[30] Reis, P.R. and Sousa, E.O. 2001. Seletividade de chlorfenapyr e fenbutatin-oxide sobre duas espécies de ácaros predadores (Acari: Phytoseiidae) em citros. Revista Brasileira de Fruticultura **23** (3):584-588.

[31] Reis, P.R. and Teodoro, A.V. 2000. Efeito de oxicloreto de cobre sobre a reprodução do ácaro-vermelho-do-cafeeiro, *Oligonychus ilicis* (McGregor, 1917). Ciência e Agrotecnologia **24** (2):347-352.

[32] Reis, P.R. and Zacarias, M.S. 2007. Ácaros em cafeeiro. Belo Horizonte: EPAMIG 76p. (Boletim Técnico, 81).

[33] Silva, M.Z. and Oliveira, C.A.L. 2007. Toxicidade residual de alguns agrotóxicos recomendados na citricultura sobre *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). Revista Brasileira de Fruticultura **29** (1):85-90.

Status of Methane Emission in Sewage Treatment Process and Emissions Estimate in Henan Province

Bingtao Liu[1], Weiyan Zhao[2], Yalong Zhang[3]

School of Environment and Municipal Engineering, North China University of Water Resources and Electric Power, China

***Abstract*—** *Methane produced during sewage treatment is a source of greenhouse gases which cannot be ignored. The estimate of methane emissions during wastewater treatment has an important significance for proposing methane reduction measures on the technical and economic feasibility. In this paper, the status of CH_4 emissions during sewage treatment of Henan Province is described. By obtaining the sewage treatment-related data and emission factor in Henan Province in 2010, CH_4 emissions in the year is estimated. The results show that CH_4 net emissions during domestic wastewater treatment is 21,764.10 tons in 2010 and that can evaluate the greenhouse effect and provide a basis for environmental management.*

***Keywords*— Methane, emissions, estimate, sewage treatment.**

I. INTRODUCTION

Methane (CH_4) emissions caused by human activities include those energy activities, industrial manufacturing, agriculture activities, forest planting, animal husbandry and the process of living sewage and industrial wastewater treatment. Sewage biological treatment technology was oxidation and decomposition of the organic pollutants and plant nutrients in water and putting them into stable inorganic substance through Microbial metabolism of biological function itself. Sewage biological treatment technology is mainly composed of aerobic and anaerobic method. During the processing of the sewage aerobic method, mainly is use artificial aeration or natural aeration to provide oxygen for aerobic microorganisms, so that the organic matter is absorbed and decomposed, such as activated sludge, biological filter, rotating biological contactors and oxidation ponds. In the process of anaerobic wastewater treatment, organic matter in anaerobic conditions is a common action of anaerobic microorganisms, eventually be converted to CH_4, H_2S, and CO_2, such as sludge anaerobic digestion, anaerobic bioreactor and anaerobic pond. Organics in the condition of anaerobic or anoxic to produce CH_4, the most important emissions place is the aeration tank and anaerobic tank.

There are three methods to estimate methane emissions such as measured method, material balance algorithm and emission factor method. They have different features and complement each other. Emissions coefficient method is also known as emission factor method. The activity level data of coefficient method is the amount of emissions of unit product emission quantity under normal production conditions. It can generally be obtained by measurement, investigation and material balance method .In 2010, It has built 146 municipal sewage treatment plants and 27 town sewage treatment plants in Henan province and the provincial industrial and urban sewage emission is 3.587 billion tons. The industrial wastewater emission is 1.504 billion tons accounting for 42% of the wastewater emission and the urban sewage emission is 2.083 billion tons accounting for 58%. Chemical oxygen demand (COD) of the main pollutants is 619700 tons. In this paper, we adopt IPCC emission factor method and calculate the emissions of methane in the sewage treatment process in Henan and the results are analyzed and discussed.

II. ESTIMATE OF METHANE EMISSIONS IN THE PROCESS OF SEWAGE TREATMENT

2.1 Estimation method

By acquiring the sewage treatment-related data and emission factors, CH_4 emissions in Henan Province in 2010 was estimated according to the method recommended by IPCC. The specific formula is as follows,

$$E_{CH4}=(TOW\times EF)-R \quad (1)$$

Where,

E_{CH4}, total amount of sewage treatment methane emissions (tons CH_4 / year)

TOW, total organic matter emissions of living sewage treatment (kg BOD / year)

EF, emission factor (kg CH_4 / kg BOD)

R , amount of recovered methane（ kg CH_4 / year)

$$EF=B_0\times MCF \quad (2)$$

Where,

B_0, the maximum production capacity of methane (kg CH_4/kg BOD);

MCF, the correction factor of methane

2.2 Activity level data

Activity levels data of methane emissions is the total amount of organic matter biochemical oxygen demand (BOD) as important indicators, including BOD discharged into the environment oceans, rivers or lakes and the part of removed in the sewage treatment plant processing system. Because only own the statistics data of chemical oxygen demand (COD). So we use the conversion correlation between the regional BOD and COD and shown in table 1.

TABLE 1
DIFFERENT REGION BOD/COD AVERAGE VALUE

Different region	Northern China	Northeast	Eastern China	Central China	Southern China	Southwest	Nationwide
BOD/COD	0.45	0.46	0.43	0.49	0.47	0.51	0.46

Amount of emissions of municipal wastewater is 2.083 billion tons In Henan in 2010.We know only Zhengzhou Wangxinzhuang sewage treatment plant has sewage methane recovery and recycled methane was 7 million cubic meters one year. According to methane density 0.00067 t/m^3, we can calculate the amount of methane recovered was 4,690 tons in Henan in 2010.

2.3 Emission factor data

MCF indicates the degree of achieve methane maximum production capacity through different treatment and disposal system (B_0) and also reflects the anaerobic degree of system.MCF can use the following formula,

$$MCF = \sum_i WS_i \times MCF_i \quad (3)$$

Where,

WS_i, the proportion of type i wastewater treatment system for municipal sewage treatment

MCF_i, the correction factor of methane of type i wastewater treatment system

According to the actual situation and using the related parameters, it is concluded that the national average MCF is 0.165.

TABLE 2
URBAN LIVING WASTEWATER EMISSION FACTOR

Category	MCF	B_0 (kg CH_4/kg BOD)	EF (kg CH_4/kg BOD)
Sewage treatment system	0.165	0.6	0.099
Discharged into natural water bodies	0.100	0.6	0.06

Methane maximum capacity which means that organic matter in wastewater can produce the biggest methane emissions, per kilogram of living wastewater BOD can produce methane 0.6 kg. Also emission factor should be determined in accordance with the specific circumstances. Because Henan province is in central China, we can know the recommended conversion value between BOD and COD value is 0.49. Other emission factors detail in table 2 and according to the formula (2) calculate EF of the sewage treatment system and EF discharged into natural water.

2.4 Estimate the result and analysis

According the mechanism of methane emissions, CH_4 emissions estimate from sewage can be divided into two parts, one produced in the process of sewage disposal and CH_4 produced in the process of wastewater discharge, the other one produced from untreated living sewage discharged into the environment. Therefore methane emissions estimate from the maximum living waste water. From values in Table 1 between BOD and COD, we can emissions 254604 tons of the living sewage treatment plants and remove BOD 21805 tons of natural water.

According to the activity levels data and emission factors in table 1, and BOD of processing system. We can calculate the methane emissions of sewage treatment in 2010 in Henan province show in Table 3

TABLE 3
LIVING WASTEWATER TREATMENT METHANE EMISSIONS STATEMENT IN 2010

Sewage treatment	Total Organic Matter (TOW),tons,BOD	Emission Factor(EF) (kg CH_4/kg BOD)	Methane Recovery(R),tons
Sewage treatment plant	254604	0.099	4690
Directly discharged into natural water	21805	0.06	0

According to table 3, methane emissions estimates of sewage treatment plant was 25205.80 tons in Henan province in 2010. Methane recycled capacity of sewage treatment plant was 4690 tons. Methane emissions of sewage treatment discharged into natural water was 1248.3 tons. We can also know that sewage treatment produced 26,454.1 tons CH_4. The net methane emissions of Henan province sewage treatment is 21,764.1 tons in 2010 and equivalent to 457,046.1 tons carbon dioxide.

III. SEWAGE TREATMENT METHANE EMISSIONS ANALYSIS

Comparing the methane emissions of sewage treatment plant in 2010 and in 2005, we can found that emission of sewage was 1.39088 billion tons in 2005 while sewage treatment plant recycled and disposed 930.75 million tons. It is concluded that sewage treatment rate was 66.92%. In 2010, emission was 2.01468 billion tons while sewage treatment plant recycled and disposed 1.85560 billion tons that sewage treatment rate was 92.10%. We can see that the sewage treatment rate greatly promoted in 2010 relative to 2005.It should thank to investment and construction of sewage treatment plant. By the end of 2010, It has built 146 city level sewage treatment plants and 27 township level sewage treatment plants. But 31 municipal sewage treatment plants have been built in 2005 in Henan. Comparing 2010 and 2005, the methane net emissions increased from 14676.41 tons to 21764.1 tons and sewage treatment system produced 25205.8 and 9030.14 tons CH_4. Because of the construction of sewage treatment plants in Henan Province in recent years to speed up . Treatment capacity increased significantly and total BOD remove obviously. Sewage directly into natural water and treated sewage discharged into natural water produce 1248.3 tons CH_4. There was a substantial decrease compared with 5646.27 tons in 2005. The total quantity of CH_4 produced in the process of sewage treatment was 26454.10 tons in 2010. CH_4 net emission in the sewage treatment process was 21,764.10 tons and 82.27% proportion. Recovery amount of CH_4 was 4690 tons, accounting for 17.73% of the total amount. It was a major breakthrough compared with the zero recycled in 2005.Methane emissions comparing 2010 and 2005 show in table 4.

TABLE 4
METHANE EMISSIONS COMPARISON 2010 WITH 2005

Years	Sewage volume (million tons)	Into natural water CH_4 (tons)	CH_4 produced in Processing system (tons)	Recovery amount of CH_4 (tons)	Total CH_4 capacity (tons)	Net CH_4 emissions (tons)
2005	13.91	5646.27	9030.14	0	14676.41	14676.41
2010	20.15	1248.30	25205.80	4690	26454.10	21764.10

IV. CONCLUSION

This paper chose activity level data in the sewage treatment process in recent years and the relevant emission factor which think the actual status of CH_4 emissions. According to the method recommended by IPCC guidelines for national greenhouse gas inventories 2006, a calculation model was established to assess methane emissions of wastewater treatment in Henan.

Methane emission from sewage and industrial wastewater treatment process was estimated. In 2010, net CH_4 emissions from sewage treatment process are 21764.1 tons. Through the analysis of CH_4 emission from sewage treatment, we can see that the net emissions of CH_4 in 2010 was 7087.69 tons more than that of 2005. Clean development mechanism (CDM) of anaerobic denitrifying of sludge in sewage treatment system will be the next focus.

ACKNOWLEDGEMENTS

Thanks to the financial support provided by the Henan development and Reform Commission

REFERENCES

[1] DING Weixin, CAI Zucong.The influence of temperature on the methane production and oxidation, Journal of applied ecology, 14(4),604—608 ,2003

[2] LIU Yan,SUN Dezhi,LUN Xiaoxiu.Advanced research of the produce,emission character and influence factor of CH4 discharged from the sewage transport and treatment.,Environment Pollution and Control, 34(5), 91-95,2012

[3] IPCC. Climate Change 2007: Mitigation of climate change, Contribution of working group to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, UK and New York, USA: Cambridge university press, 2007.

[4] Otte S, Seviour R J, Kuenen J G, etal. Nitrous oxide production by alcaligenes faecalis during feast and famine regimes. Water Res, 34(7) ,2080-2088,2000.

[5] Hanaki K, Zheng H, Matsuo T. Production of nitrous oxide gas during denitrification of wastewater. Water Science and Technology, 526(26),1027—1036,1992.

[6] Garrido J M, Moreno J, Mendez-Pampin R. Nitrous oxide production under toxic conditions in a denitrifying anoxic filter,Water Research,32(8), 2550—2552,1998.

[7] Schulthess R V, Wild D, Guger W. Nitric and nitrous oxide from denitrifying activated sludge at low oxygen concentration,Water Science and Technology, 30(6),123—132,1994.

[8] YU Yang, CUI Shenghui,Lin Jianyi,etal,Study on greenhouse gas emissions from urban waste disposal system: A case study in xiamen,,Environmental Science, 33(9), 3288-3294,2012.

[9] Takaya N, Catalan M A B, Sakaguchi Y. Aerobic dentrifying bacteria that produce low levels of nitrous oxide. Applied and Environmental Microbiology, 69(6),3152-3157,2003.

[10] Gianfranco Chicco, Pierluigi Mancarella. Assessment of the greenhouse gas emissions from cogeneration and trigeneration systems. Part I: Model and indicators ,Energy, 33(6),410-417,2008.

[11] Tsao Chou Chen, Cheng Feng Lin. Greenhouse gases emissions from waste management practices using life cycle inventory model, Journal of Hazardous Materials.155,23-31,2008.

Efficiency Production Cost of Goat Farming in the Lowland and the Highland Areas in Mojo Sub-district of Kediri Regency

Elok Setyorini[1*], Koesnoto Soepranianondo[2], Sri Hidanah[3]

[1]Student of Agribusiness Veterinary Faculty of Veterinary Medicine, Postgraduate School, Airlangga University
[2,3]Department of Animal Science, Faculty of Veterinary, Airlangga University' Kampus C Mulyorejo 60115 Surabaya, Indonesia

Abstract— *This research aims to determine the level of efficiency production cost of goat farming in the lowland and the highland areas in Mojo Sub-district of Kediri Regency. Location determination and 60 respondents based on purposive sampling method from the total of all farmers in Mojo Sub-district of Kediri Regency. Data collection was done by questionnaire technique, interview technique and documentation. To analyzed the data in this research used Data Envelopment Analysis (DEA) approach Measuring the efficiency production cost of goat farming used input variable that consist of the cost of shrinkage of cages, shrinkage tools, seeds, feeds, medicines and labor. Variable output was the revenue from the goats sale. The results showed that the efficiency of production cost of lowland goat farming was varies, farmers who achieve efficiency value equal to 1 was 9 farmers or 30% of the total respondents and 70% inefficient. Farmers in the highland who achieved the same level of efficiency as 1 was 15 farmers or 50% and the remaining 50% was inefficient. The use of input production costs in lowland and highland areas had a slack input value. Farmers in the lowland area could reduce the use of production costs in the form of 18% shrinkage of cages, 13% tool shrinkage, 6% seed, 2% feed, 6% medicine and 1.4% labor whereas breeders in the highland could reduce the use of costs production of 12% shrinkage of cages, 23% tool shrinkage, 11% seed, 10% feed, 12% medicine and 7% labor.*

Keywords— Data Envelopment Analysis, efficiency of production cost, goat farming, highland, lowland.

I. INTRODUCTION

Goats are the most cultivated meat livestock commodities. Meat consumption has increased with population growth and changes in consumption patterns and tastes of the people (Wahyudi *et al.*, 2017). The population of goat in Mojo Sub-districts in 2016 is 11.011 goats. Goat population increase in Mojo Sub-district from 2011 was recorded 8.499, in 2013 was recorded 10.530, and in 2015 was recorded 10.849 goats (Department of animal husbandry Kediri Regency, 2016). Most farmers of goats in Mojo Sub-district are small-scale farms in rural area and have limited control over resources (land, income, innovation and technology).

Goats are animals that have good living ability with various climatic conditions and can live on land with a vary topography. Topography of Mojo Sub-district is hilly covering lowland and highland with the lowest altitude of 74 mean sea level area Ngadi Village and highest altitude 573 mean sea level area Jugo Village (*Central Bureau of Statistics* Mojo Sub-district, 2015).

A livestock business, maintenance sites is one of the main things that must be considered so that the business can operate effectively and efficiently. Lowlands are generally areas with hot air temperature, low humidity and limited feed source condition. The highlands have low temperature, so the livestock tend to consume more feed, while in lowland temperature tend to be high so that the goat will try to maintain their body temperature in a relatively constant state through increased the frequency of respiration, the amount of drinking water consumption, and decreased in feed consumption (Apriliani *et al.*, 2016).

The activity of a goat farming production requires the sacrifice of economic resources such as seeds, feeds, labor, medicines and cages called the cost of production. Production costs are all goods that producers must incur in order to obtain production factor and other supporting materials that can be used for certain products that can be realized properly (Taufik *et al.*, 2013). Production activities in processing goat farming should be able to control production cost. Control of production cost is necessary in order to achieve cost efficiency of production so that it will obtain the optimal profit. Efficiency is the relative ratio between the output (the result in physical size or rupiah) with the input (the cost factor used to obtain the result) to the ratio of output to input at optimal conditions (Kalangi *et al*., 2014).

Based on the problems above, the purpose of this research is to know and analyze the efficiency level of the production cost of goat farming in lowland and highland area in Mojo Sub-district Kediri Regency. This research is expected to be useful for the development and improvement of goat productivity as well as the use of efficient production cost of goat farming based on the location of altitude in Mojo Sub-district Kediri Regency.

II. MATERIAL AND METHOD

The study was conducted at the goat farming in Mojo Sub-district Kediri Regency. This sub-district was determined with the consideration that this sub-district had the largest number of goat population compared to other sub-districts in Kediri Regency that is 11.011 goats in 2016. In addition Mojo Sub-district had a hilly topography so that 6 villages were in the highland and the rest were in the lowland. The research was conducted from September 2017 to October 2017.

Sampling of this research using technique of *purposive sampling*. Samples by selecting one village in the lowland with a population of 1,035 goats that was Ngadi village was 30 farmers and one village in the highland with a population of 1,171 goats that was Jugo village was 30 farmers. Sampling of each farmer's were divided into 3 groups based on goat ownership, group I (1-4 goats) 70% of the farmers, group II (5-8 goats) 20% of the farmers and group III (> 8 goats) 10% of the farmers.

The research method used is a survey method that was research taken sample by using questionnaires as a data collection tool. The data taken consisted of primary data obtained through direct interviews with sample farmers using questionnaires covering the identity of sample farmers (age, number of livestock and the long experience of farming) and costs incurred during production (shrinkage of cages, tools, seeds, feeds, medicines and labor), and the resulting revenue.

The cost efficiency analysis method of the production of goat farming used DEA model developed by Coelli et al. (2005). This model was used to analyze the efficiency production cost of goat farming. An efficiency value equal to one indicates that the cost of producing goat was relatively efficient, while the efficiency values of less than one indicates that the cost of producing goat was relatively inefficient (Gül *et al.*, 2016).

DEA model used is an input-oriented model, because the farmers had more control over the inputs used than the output produced. This research also used *Variable Return Scale* approach because goat farming in the research location was almost impossible to reach optimum scale. Mathematically, the calculation of the efficiency of using the model of *variable returns to scale* was expressed as follows:

$$\text{Min } \Theta, \lambda \Theta,$$

$$st - y_i + Y\lambda \geq 0,$$

$$\Theta x_i - X\lambda \geq 0,$$

$$N1'\lambda = 1$$

$$\lambda \geq 0$$

In which Θ was a scalar, N1' was convexity constraint, λ was N x 1 vector of constants, Y represents output matrix; and X represent input matrix. The value of Θ would be the efficiency score for firm I (Gül *et al.*, 2016).

The model used in this study consists of input and output variables. The input variables used are the cost of shrinkage of cages, tools, seeds, feeds, medicines and labor. Meanwhile, the output variable used in this research was revenue from the sale of the goats. To calculate the value efficiency production cost of goat farming studied, then this research using software STATA version 13.1.

III. RESULT AND DISCUSSION

3.1 Efficiency Production Costs of Folk Goat Farming

The results of data processing used software STATA version 13.1 given efficiency value for each goat farmers. The frequency distribution of efficiency production cost of goat farming of lowland and highland in Mojo Sub-district Kediri Regency could be seen in Table 1. The table shown that the average efficiency of production cost of lowland goat farming was 0.74 and the highland was 0.81, which was less than 1, which means that the production costs incurred inefficient goat farmer. The distribution of the efficient used of production costs in the lowland amount 30% of farmers had been efficient used of production costs and 70% of farmers were inefficient use of production costs. The distribution of the efficient used of

production costs in the highland amount 50% of farmers had been efficient used of costs of production and 50% of farmers were inefficient use of production costs.

TABLE 1
FREQUENCY DISTRIBUTION OF EFFICIENCY PRODUCTION COSTS OF GOAT FARMING IN LOWLAND AND HIGHLAND IN MOJO DISTRICT KEDIRI REGENCY

Efficiency Level	Lowland		Highland	
	Number of farmers (person)	Percentage [a)]%	Number of farmers (person)	Percentage[a)]%
1.00	9	30	15	50
< 1.00	21	70	5	50
Total	30	100	30	100
Minimum	0.04		0.09	
Maximum	1		1	
Average	0.74		0.81	

Calculated from the data on the number of respondent who adopt of by the total number of respondent multiplied by one hundred precent. Number of respondents = 30 farmers

The value of efficiency production cost with *Data Envelopment Analysis* (DEA) approach shown the average value efficiency goat farming in the of 0.74 which means that majority of goat farmers in lowland area were not achieved efficiency production cost. Farmers who had achieved efficiency was 30%, while inefficient farmers in using production cost was 70%, it shown that there were many goat farmers in lowland areas that could not maximize the input used in folk goat farming. Inefficiency of production cost in the form of excess or wasted part of input used. Achievement of an excellent level of efficiency could not be separated from the human resource element. The potential of human resource based on the ownership of knowledge, skills, technology and productivity of labor greatly contribute to the achievement of optimal output (Sugiharto & Syariffudin, 2013). Efficiency of production cost of goat farming lowland area could be achieved if farmers had ability and skill used of inputs used. While the average value of efficiency of goat farming in highland area of 0.81, which means that the majority of goat farmers in highland area had also not reached the efficiency production cost yet. Some 50% of farmers in the highland were efficient in managing production cost and 50% of farmers were not efficient in using production cost. Inefficiency of production cost in the form of excess or wasted part of input used. *Input* that was wasted if they could be streamlined would reduce production cost for farmers and could increase profits. According to Kalangi (2014) the opportunities of farmers in improving efficiency in the highland could be shown by the potential of natural resources and human resources. Natural resources such as agricultural land and large plantations in the highlands were potentials that need to be utilized optimally by utilizing the skill of human resources to take advantage of the abundant feed processed using the latest feed technology so that the expenses of feed production cost could be reduced.

3.2 Input Slack Production Cost of Folk Goat Farming

The average value *input slack* production cost of all goat farmers in the lowland and the highland could be seen in Table 2. *Input slack* was the level of inefficiency in the respondent farmer who was inefficient because the performance was not optimal from the input, output or both. Input slack was a number of inputs that could be reduce by the respondent farmer to produce the same level of output. The assumption of DEA model used in this research was oriented to input, so the calculation was only focused on input slack.

TABLE 2
THE AVERAGE VALUES INPUT SLACK PRODUCTION COST OF ALL FARMER IN THE LOWLAND AND HIGHLAND

Variable	Average value input slack (%) and number of respondents (%)			
	Lowland		Highland	
	input slack	respondents	*input slack*	respondents
Shrinkage of cage	18	56	12	50
Shrinkage of tools	13	50	23	43
Seed	6	30	11	47
Feed	2	13	10	43
Medicines	6	20	12	30
Labor	1.4	10	7	23

The used of excess input in input production cost in lowland areas was quite large. 56% of farmers were inefficient at the cost of shrinkage of cages. The cost was wasted 18% of the cost incurred. The excess value of input from the cost of shrinkage of the cages was inseparable from investment cost of the enclosure. Farmers could not plan for good enclosures, the capacity of the enclosures made mostly in exceeds the need of the existing livestock so that it made the cost of cages inefficient and excess costs incurred. Farmers should make a cage with a spacious enclosure for the need of the number of goats that were kept so that the cost of shrinkage of the cage issued as needed.

The majority of goat cages owned by lowland made of bamboo, asbestos roofed and wooden floors. Cages made of bamboo only survive in the not too long, especially goats that many moves made quickly broken cages so that the cost to renovate the cages should be issued by the farmer. The efficiency of production cost in the form of shrinkage of cages circumvented by making the cages with stronger construction with cast cement, although the cost of manufacture which was relatively expensive but could survive for decades and strong. The cages should be strong so that it could be worn for a long time, the size according to the number of livestock, clean, get the morning sunlight and the cages ventilation should be enough (Badriyah & Ika, 2011).

Fifty percent of farmers excess the cost of shrinkage of tool, the value of the excess cost in the amount of 13% of the cost incurred, although the average value of expenditure for equipment investment was small enough at Rp.100.000 but by saving 13% of the value would be able to lower production costs, as much as 30% of farmers overage seeds cost, the value of excess cost was 6% of the cost incurred. Excess value occurred because the purchase price would be quite high seedlings and farmers bought seeds with reproductive status ugly so that when expected to give birth 3 times in 2 years, it would not happen. Farmers who bought expensive female goat would be harmed by the current low price to sell.

The used of excess input in input production cost in highland area was quite large. 40% of farmers were inefficient at the cost of shrinkage of cages, seeds and feeds. 50% of farmers were inefficient at the cost of shrinkage of cage. The wasted cost was 12% of the cost incurred. The excess value of input from of shrinkage of cages was inseparable from the investment cost of the enclosure. Farmers could not plan for good enclosures, the capacity of the enclosure were made mostly exceeds the need of the livestock so that it made the cost of cages inefficient and excess cost incurred. Farmers should make a cage with a spacious enclosure for the need of the number of goats that were kept so that the cost of shrinkage of cages issued as needed.

The used of seeds input was still excessive, 47% of farmers inefficiently used the seeds cost. The excess cost of seeds issued by farmers was 11% of the cost incurred seed. The excess costs because farmers buying seeds from village traders. Village traders went around the village to find and sell goats to be bought and sold by farmers. The cost of buying and selling of goat seeds from seed village traders vary, depending on the age and quality of the goat. Village traders bought and sold with the desired price, while farmers had never considered the value and quality of seeds in general they were rather difficult to get the goats were good quality so the price to buy relatively high and sell it at a verily low price because when the farmers need money for urgent needs of families like them for the payment of school fees (Budiarsana *et al.*,2016). Farmers need to get information about the price buying and selling goats in the market, information about buying and selling price an buy goats could be used as reference farmers to optimize other production costs in order to get optimal profit. In addition, farmers could get around the purchase of seeds in groups with other farmers in order to get cheaper seed price. Need to be established and enforced institutional goat farmers were healthier, so that both farmers and traders mutually benefit.

The used of feed was still excessive because of the abundant of forage in the highlands to make farmers take as much feed but not eaten by all the goats or a lot of feed was wasted and scattered in the cage. Feed is one of the input components that determine success of business financially. Livestock feed was one of the important factor in the livestock business that determines the productivity of the livestock itself.

Farmers need to know about fresh forage for goats. According to Wicaksana *et al.,* (2015), fresh forage was given as much as 10-20% of the weight of life. In fact there were still many farmers who feed without regard to the quality, quantity and manner of giving. As a result, the productivity of livestock maintained was not optimal even among many farmers who suffered losses due to inappropriate feeding. Farmers who could take advantage of feed excess amount in the form of feed technology. In addition to utilizing into the form of technology, feed quality would be maintained rather than wasted and feed production cost could be suppressed.

IV. CONCLUSION

The conclusion that could be drawn from the research results entitled "Efficiency Production Cost of Goat Farming in the Lowland and the Highland Areas in Mojo Sub-district of Kediri Regency" is goat farming of lowland and highland in Mojo Sub-district Kediri Regency is inefficient. Thirty percent of farmers of lowland efficiently use production costs and 70% were inefficient. Fifty percent of farmers of highland farmers efficiently use production cost and 50% were inefficient. The use of input production costs in lowland and highland areas had a slack input value. Farmers in the lowland area could reduce the use of production costs in the form of 18% shrinkage of cages, 13% tool shrinkage, 6% seed, 2% feed, 6% medicine and 1.4% labor whereas breeders in the highland could reduce the use of costs production of 12% shrinkage of cages, 23% tool shrinkage, 11% seed, 10% feed, 12% medicine and 7% labor.

REFERENCES

[1] E.D. Apriliani, Dudi, and H. Inrijani. 2016. Morphology analysis of local Ewes in highland. Journal of Padjadjaran University. Available online at http://jurnal.unpad.ac.id/ejournal/article/download/9635/4336

[2] N. Badriyah, and N.F. Ika. 2011. Studi Manajemen Pakan Domba di Peternakan Jaya Mandiri Di Desa Campurejo Kecamatan Panceng Kabupaten Gresik. Jurnal Ternak Vol. 02 No.01

[3] I.G.M.Budiarsana, W. Broto, and P. Dwi. 2016. Productivity and Supply Chain Sheep and Goat A Case Studying Tegal District. Journal of Animal Science. Vol.16, No.2

[4] Central Bureau of Statistics. Mojo Subdistrict 2016. In Figures. Catalog BPS 1102001.3506010

[5] T.J. Coelli, D.S.P. Rao, C.J. O'Donnell, and G.E. Battese. 2005. *An Introduction to Efficiency and Productivity*Analysis,Second Edition, New York: Springer.

[6] Department of animal husbandry Kediri Regency. 2016. Annual Report of Department of animal husbandry Kediri Regency 2016

[7] M. Gül, D. Vecdi, Y. Hasan, and Y. Hilal. 2016. Technical Efficiency of Goat Farming in Turkey: a case study of Isparta province. Revista Brasilleira de Zootecnia. 45 (6): 328-335

[8] L.S. Kalangi, Y. Shaukat, S.U. Kuntjoro, and A. Priyanti. 2014. Technical Efficiency of Beef Cattle Breeding Business in East Java Province. Media Peternakan. 37 (2): 136-142

[9] M. Sugiharto, and N. Syarifuddin. 2013. Pengembangan Potensi Sumberdaya Peternak Sebagai Upaya Peningkatan Daya Saing Peternakan Kambing Skala Mikro di Kabupaten Banyumas. Journal & Proceeding FEB Unsoed. Vol 3 No.1.

[10] D.K. Taufik, Isbandi dan M. Dyah. 2013. Analisis pengaruh sikap peternak terhadap pendapatan pada usaha peternakan itik di kelurahan pesurungan lor Kota Tegal. Fakultas Peternakan. Universitas Dipenegoro. Semarang. Jurnal Ilmu Teknologi Peternakan. 2 (3).

[11] E. Wahyudi, C.Gatot, dan B. Agus. 2017. Studi Kasus Tingkat Pemotongan Kambing Berdasarkan Jenis Kelamin, Kelompok Umur Dan Bobot Karkas Di Tempat Pemotongan Hewan Kota Malang. Jurnal Ternak Tropika Vol. 18, No. 1 : 69-76

[12] K. Wicaksana, Muhtarudin, and W.Joseph. 2015. Nutritional Status of Nuts Goat in Tanjung Bintang District South Lampung Regency.Scientific Journal Integrated LivestockVol 3 (4): 208-211

Evaluation of mass trapping for control of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Satsuma mandarin in Hatay province of Turkey

Nihat DEMİREL[1], Eda AKYOL[2]

Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31034 Hatay-Turkey.
*Corresponding author: Dr. Nihat DEMİREL, Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31034, Hatay-Turkey. [Tel: 90 (326) 245 58 45; Fax: 90 (326) 245 58 32; email: ndemirel@mku.edu.tr]

***Abstract*—** *The Mediterranean fruit fly (Medfly), Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), is one of the most important pests of citrus in Turkey. The objective of this study was to evaluate mass trapping for the control of Medfly in Satsuma mandarin in Hatay province of Turkey. The studies were conducted in 2011-2012 using eostrap® invaginada traps baited with % 95 Trimedlure impregnated in a polymeric plug-type dispenser. In the first year, 48 traps per 0.7 ha were placed in an experimental site from 1st August to December. In the second year, 23 traps per 0.7 ha were placed in the same site from 14 August to December. After two years of the study, the population density of medfly varied in each of the sampling year. In the first year, a total of 8968 medfly adults were caught by traps. The largest mean of catches per trap were recorded on 31 October (64.21), followed by 24 October (31.29), 17 October (22.48), 7 November (20.64), 3 October (17.60) and 10 October (16.71). In the second year, a total of 1307 medfly adults were caught by traps. The largest mean of the catches per trap were recorded on 25 September (7.35), followed by 13 November (5.83), 6 November (5.52), 18 September (5.43) and 30 October (4.26), respectively. The percentages of damage rates of medfly observed in both years. The damage rates of Medfly were 10.91 and 8.56 % in 2011 and 2012, respectively. In conclusion, the population density of medfly on satsuma mandarin increased in September and October due to high temperature. The mass trapping was not enough to control medfly on satsuma mandarin. Therefore, the mass trapping should be used with pesticides to decrease the population density of medfly during September and October in Hatay province of Turkey.*

Keywords*— *Medfly, trimedlure, traps, satsuma mandarin, Turkey.

I. INTRODUCTION

Satsuma mandarin, *Citrus unshiu* Marc., (Rutaceae: Sapindales) is one of the main cultivated *Citrus* variety in Turkey. Satsuma mandarin production is consisting of approximately 23,413.1 ha with a total produce of 795.050 tons of fruit per annum in Turkey, and Hatay province's share is 10,466.1 ha and 402.601 tons [1]. The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is the most destructive pest among economically important fruit fly species [2,3]. The medfly is a highly adaptive polyphagous tropical fruit fly attacking more than three hundred and fifty plant species [4,5]. The female flies lay eggs below the skin of fruits which are destroyed by larval feeding [6].

Protein bait sprays mixed with malathion or low toxicity insecticides, spinosad, lambda-cyhalothrin are successfully used to control medfly populations [7,8]. Traps baited with trimedlure are important tools for detection, monitoring and controlling of the medfly [2]. The mass trapping has proved to be effective in suppression of the Medfly and advantage of reduced environmental impact when comparing with toxic bait sprays [9,10]. In addition, mass trapping has been developed in several countries using traps baited with trimedlure, hydrolysed proteins or food-based attractants [11,12,13, 14, 15, 16,17]. This method has been conducted on different fruit trees like peach [18], cherimoya [19], citrus [14,20,21], fig [22], persimmon and coffee [7] and apple[23]. The purpose of the current study was to evaluate mass trapping for the control of Medfly in Satsuma mandarin in Hatay province of Turkey.

II. MATERIALS AND METHODS

The study was conducted in 2011-2102 at a satsuma mandarin orchard in Hatay province of Turkey. The study was carried out using the Eostrap® invaginada traps (Sanidad Agricola Econex, Santomera, Murcia, Spain) baited with % 95 Trimedlure, (formulated in a polymeric plug-type dispenser) (Sanidad Agricola Econex, Santomera, Murcia, Spain) and dichlorvos or 2.2- dichlorovinyl dimethyl phosphate (DDVP) tablet (Sanidad Agricola Econex, Santomera, Murcia, Spain).

In the first year, the study was conducted with 48 traps/0.7 ha and placed in the experimental site from 1st August to December. In the second year, the study was carried out with 23 traps/0.7 ha and placed in the same site from 14th August to December. The traps were placed 1.5 m above ground and checked weekly, trapped medfly adults were counted and removed from the traps. The trimedlure and DDVP tablet in traps were replaced with the new ones in every 90 days. The fruit damage assessment was measured by the percentage of medfly punctures during the harvest. For this purpose, all fruits from satsuma mandarin orchard were harvested and checked for medfly punctures and the percentage of the infested fruits were measured as weight at the packaging house.

III. RESULTS

The population density of *C. capitata* varied in each of the sampling year. In the first year, the mass trapping was conducted with 48 traps/0.7 ha in satsuma mandarin orchard and a total of 8968 medfly adults were caught by traps (Figure 1). The first adults were caught by the traps on 8 August, and the population density of this pest was gradually low from 8 August through 12 September, while it increased from 19 September to 31 October. The largest mean of catches per trap were recorded on 31 October (64.21), followed by 24 October (31.29), 17 October (22.48), 7 November (20.64), 3 October (17.60) and 10 October (16.71).

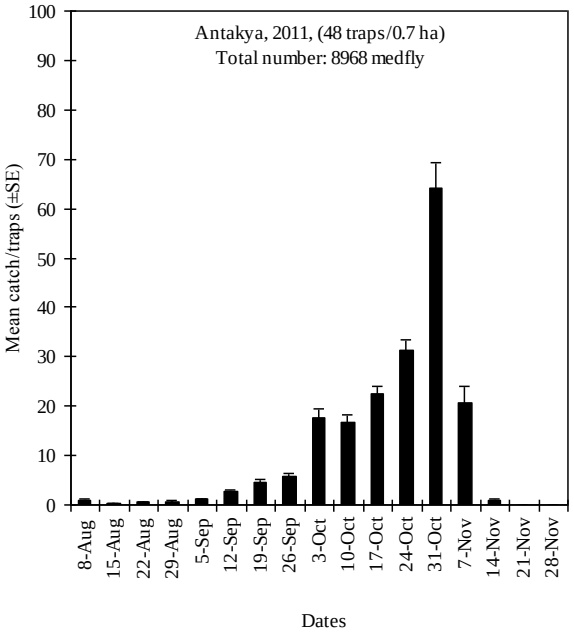


FIGURE 1. MEAN (±SE) CATCHES OF MEDFLY ADULTS IN TRAPS BAITED WITH TRIMEDLURE (1AUGUST–28 NOVEMBER, 2011) AT SATSUMA MANDARIN ORCHARD IN ANTAKYA DISTRICT

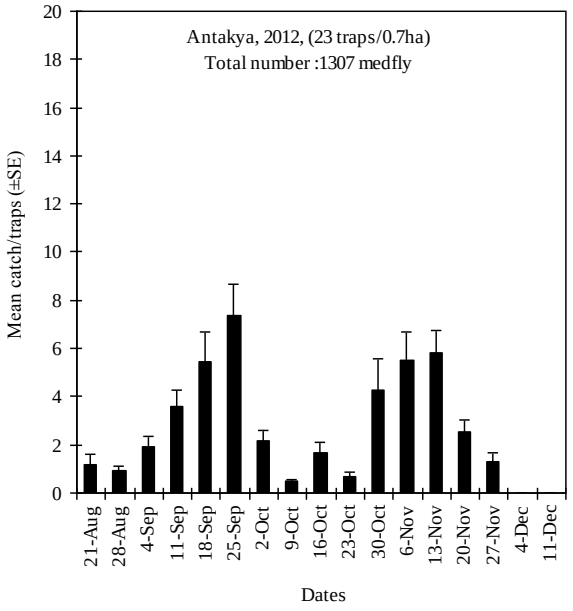


FIGURE 2. MEAN (±SE) CATCHES OF MEDFLY ADULTS IN TRAPS BAITED WITH TRIMEDLURE (15 AUGUST–11 DECEMBER, 2012) AT SATSUMA MANDARIN ORCHARD IN ANTAKYA DISTRICT

In the second year, the mass trapping was conducted with 23 traps/ha at the same orchard and a total of 1307 medfly adults were caught by traps (Figure 2). The population density of medfly was very low comparing with previous year. The first adults were caught by the traps on 21 August, and the population density of this pest was significantly high from 4 September to 25 September and 30 October through 13 November and yet it was significanly low on 2-23 October due to heavy rain in the sampling orchard. The largest mean of catches per traps were recorded on 25 September (7.35), followed by 13 November (5.83), 6 November (5.52), 18 September (5.43) and 30 October (4.26).

The percentage of the total caught over this period varied for each of the sampling month in both years. The distribution of the total caught of this pest according to the months as percentages were 1.17 (August), 6.55 (September), 71.32 (October) and 10.14 (November) in 2011 (Figure 3). These percentages were 3.67 (August), 32.13 (September), 16.37 (October) and 26.70 (December) in 2012 (Figure 4). The percentages of damage rates of medfly observed in both years. The damage ratios of medfly were recorded at 10, 91 percent in 2011, and 8, 56 percent in 2012 (Figure 5).

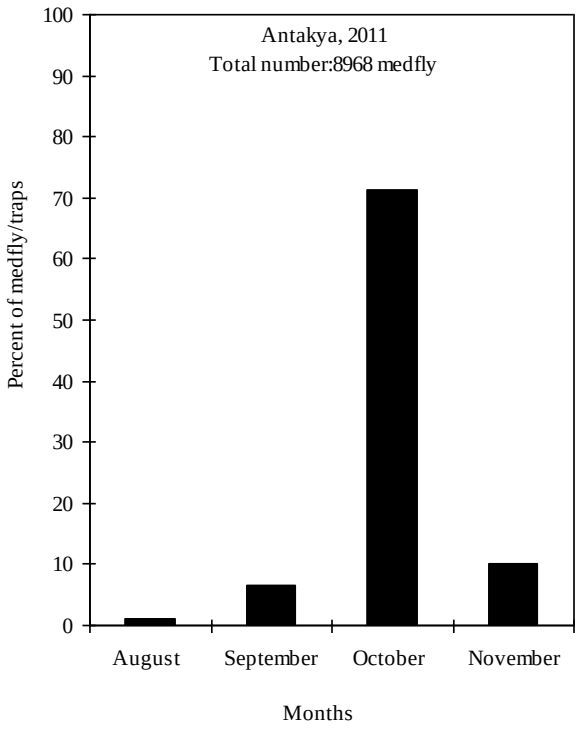


FIGURE 3. PERCENTAGE OF THE TOTAL MEDFLY ADULTS CAUGHT OVER THE SAMPLING PERIOD AT SATSUMA MANDARIN ORCHARD IN ANTAKYA DISTRICT.

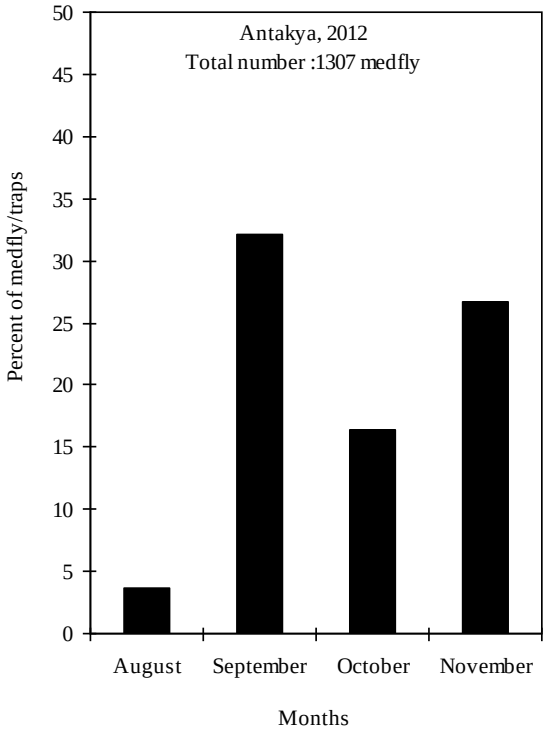


FIGURE 4. PERCENTAGE OF THE TOTAL MEDFLY ADULTS CAUGHT OVER THE SAMPLING PERIOD AT SATSUMA MANDARIN ORCHARD IN ANTAKYA DISTRICT.

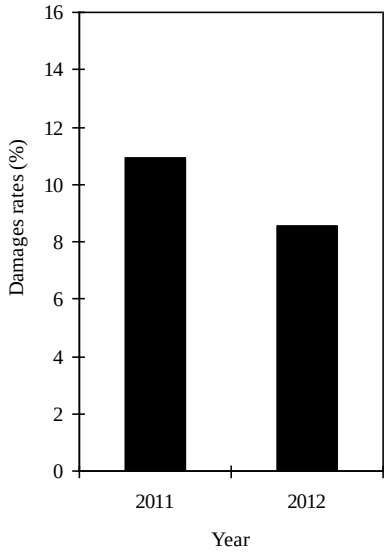


FIGURE 5. PERCENTAGE OF THE DAMAGED FRUITS BY MEDFLY IN SATSUMA MANDARIN ORCHARD IN ANTAKYA DISTRICT

IV. DISCUSSION

The mass trapping has shown significantly effective pest management tool for the Medfly and developed in several countries using traps baited with trimedlure, hydrolysed proteins or food-based attractants [2, 7, 9, 12, 13, 14, 15, 16, 17, 24, 25, 26, 27, 28, 29]. The synthetic food-based attractants, trimethylamine (TMA), ammonium acetate (AA) and putrescine (P) were the more appropriate for mass-trapping of the medfly [9, 11, 14, 15, 30,31, 32].

A various number of traps were used with mass trapping to control medfly on different host plants. Martinez-Ferrer et al. [10] suggested that 25 trap per ha (Maxitrap Model baited with Ferag. CC D TM® attractant) can be a good stand-alone control method against the Medfly in citrus groves in Spain. In addition, IAEA [2] suggested that 20-25 traps per ha density was required by using a mass trapping technique for medfly. Martinez-Ferrer et al. [10] reported that for the Clemenules variety, a 25 traps per ha were enough to capture adults flying within the grove and attract the foraging medflies, as low percentage of fruits were attacked. A number of researchers reported that if the population density of medfly increase, the number of trap per ha will be increased. Therefore, during September-October Medfly population is high, a 50 trap per ha density has been widely accepted as appropriate for citrus orchards [9,32,33,34,35,36,37]. Martinez-Ferrer et al. [10] reported that for the early-season varieties, 50 traps per ha captured as many adults as did 75 and 100 traps per ha, but not enough to diminish the adult medflies foraging in the grove under accepted levels.

Several studies were conducted by many researchers to evaluate the population density of medfly on various host plants. The present results indicated that the population density of medfly was high in September and October in both year. Our results are similar to those of [10,38,39] reported that the population density of medfly was significantly high in September and October due to high temperatures in citrus- producing area.

The percentages damage ratios of medfly varied on different host plants. Martinez-Ferrer *et al.* [37] found that on early-season varieties (Loretina and Marisol), mass trapping at a density of 50 Maxitrap® (Probodelt®) traps/ha baited with Ferag CC D TM® (SEDQ) and chemical treatments with Malafin® (malathion) or SpintorCebo® (spinosad) of the entire groves or the perimeter, can protect well the fruits from the Medfly attack as <2% were damaged in the harvest. However, on the mid-season variety (Clemenules), the adult population decline, and the number of traps could be lowered to only 25/ha and this tactic applied alone was efficient enough to obtain less than 0.5% of punctured fruits. Boulahia- Kheder *et al.* [40] reported that combination of 4 aerial spinosad sprays, sanitation and female mass trapping with Moskisan® + Biolure® Unipack, the damage on Navel oranges reached only 5% at harvest. However, Boulahia-Kheder et al.[41] reported that mass trapping as a single technique it wasn't enough to protect the Navel oranges with more than 30% of damaged fruits at the harvest.

V. CONCLUSION

The present study was conducted by traps baited with trimedlure to evaluate of mass trapping for the control of Medfly in Satsuma mandarin in Hatay province of Turkey. As a result of two-year investigations, the population density of this pest varied in each of the sampling year. In the first year, 48 trap per 0.7 ha were used with mass trapping to control of the medfly. A significant number of medfly were caught by traps and yet the medfly caused significant damages on satsuma mandarin due to high population density in September and October. In the second year, 23 trap per 0.7 ha were used with mass trapping to control of this pest. The population density of medfly was significantly low comparing with that of the previous year. However, the mass trapping was not enough to control this pest because of high population density

ACKNOWLEDGEMENTS

This project was supported by University of Mustafa Kemal of Scientific Research Projects (BAP) (project number: 1105 Y 0105 (191).

REFERENCES

[1] Anonymous, 2016. The summary of Agricultural Statistics (21.10.2017 version) https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul.

[2] International Atomic Energy Agency, 2003. Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency. Vienna, Austria. 47 p. Retrived from : http://www-pub.iaea.org/MTCD/Publications/PDF/TG-FFP_web.pdf

[3] Thomas, M.C., Heppner, J.B., Woodruff, R.E., Weems, H.V., Steck, G.J., Fasulo, T.R. 2007. Mediterranean Fruit Fly. Ceratitis Capitata (Wiedemann) (Insecta: Diptera: Tephritidae). University of Florida Ifas Extension. http://edis.ifas.ufl. edu/pdffiles/IN/IN37100.pdf Accessed on 13 October 2017.

[4] Weems, H.V., Jr. 1981. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Entomology Circular, Division of Plant Industry, Florida Department of Agriculture and Consumer Services**.** 12 pp.

[5] Liquido, N.J., Shinoda, L.A., and Cunningham, R.T. 1991. Host plants of the Mediterranean fruit (Diptera,Tephritidae) an annotated world review, Miscellaneous Publications 77. Entomol. Soc. Am., Lanham, MD. 1863-1878.

[6] Christenson, L.D., and Foote, R.H. 1960. Biology of fruit flies. Annual Review of Entomology. 5: 171-192.

[7] McQuate, G.T., Sylva, C.D., and Jang, E.B. 2005. Mediterranean fruit fly (Diptera: Tephritidae) suppression in persimmon through sprays in adjacent coffee plantings. Journal of Applied Entomology. 2: 110-117.

[8] Urbaneja, A., Chueca, P., Monton, H., Pascual-Ruiz, S., Dembilio, O., Vanaclocha, P., Abad-Moyano, R., Pina, T., and Castanera, P. 2009. Chemical alternatives to malathion for controlling *Ceratitis capitata* (Diptera: Tephritidae), and their side effects on natural enemies in Spanish citrus orchards. J. Econ. Entomol. 102: 144-151.

[9] Navarro-Llopis, V., Alfaro, F., Domínguez, J., Sanchis, J. and Primo, J. 2008. Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. J. Econ. Entomol. 101:126-131.

[10] Martinez-Ferrer, M.T., Campos, J.M., and Fibla, J.M. 2010. Field efficacy of *Ceratitis capitata* (Diptera: Tephritidae) mass trapping technique on clementine groves in Spain. Journal of Applied Entomology. 136: 181-190.

[11] Epsky, N.D., Hendrichs, J., Katsoyannos, B.I., Vasquez, L.A., Ros, J.P., Zumreoglu, A., Pereira, R., Bakri, A., Seewooruthun, S.I. and Heath, R.R. 1999. Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. J. Econ. Entomol. 92: 156-164.

[12] Katsoyannos, B.I., Papadopoulos, N.T., Heath, R.R., Hendrichs, J. and Kouloussis, N.A. 1999. Evaluation of synthetic food-based attractants for female Mediterranean fruit flies (Dipt., Tephritidae) in McPhail type traps**.** J. Appl. Entomol. 123: 607-612.

[13] Cohen, H. and Yuval, B. 2000. Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. J. Econ. Entomol. 93: 721-725.

[14] Miranda, M.A., Alonso, R. and Alemany, A. 2001. Field evaluation of Medfly (Diptera, Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). J. Appl. Entomol. 125: 333-339.

[15] Heath, R.R., Epsky, N.D., Midgarden. D. and Katsoyannos, B. 2004. Efficacy of 1,4-Diaminobutane (Putrescine) in a food based synthetic attractant for capture of mediterranean and mexican fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 97:1126-1131.

[16] Ortu, S., Lentini, A. and Cocco, A. 2005. Strategie di lotta per il contenimento di *Ceratitis capitata* (Wied.) in agrumicoltura. Informatore Fitopatologico. 55: 28-34.

[17] Alemany, A., Miranda, M.A., Alonso, R. and Escorza, C.M. 2006. Changes in the spatial and temporal population density of the Mediterranean fruit fly (Diptera: Tephritidae) in a citrus orchard. Span. J. Agric. Res. 4:161.

[18] Sastre, C. 1999. Eficacia de los productos fitosanitarios en el control de la mosca de la fruta *Ceratitis capitata* (Wied.). Phytoma España. 114: 75-77.

[19] Ros, J. P., Escobar, I., García Tapia, F. J. and Aranda, G. 2000: Pilot experiment to control Medfly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) using mass trapping technique in a Cherimoyer (*Annona cherimola* Miller) orchard. In: Area-wide control of fruit fly and other insect pests (ed. K. H. Tan): 639-643. Penerbit University Sains Malaysia, Penang.

[20] Alonso Muñoz, D., Soler, J. M., García Marí, F., and Blas Correas, M. 1999. Frutect®: control de la mosca de la fruta *Ceratitis capitata* Wied. en el cultivo de los cítricos. Levante Agrícola. 347: 204-211.

[21] Llorens, J. M., Vinaches, P., Marco, F. and Llorens, J. 2004: Control de *Ceratitis capitata* Wied. using mass trapping with Tephri traps and Biolure-3 component Lure (Tripack) in fig trees. 5º Meeting of the working group on fruit flies of the Western Hemisphere, 16-21 May, Florida. Abstract: 38.

[22] Escudero, A., Vilajeliu, M. and Batllori, L. 2005. Captura masiva para el control de a mosca mediterránea de la fruta (*Ceratitis capitata* Wied.) en manzano. Phytoma España. 171: 26-31.

[23] Ortu, S. and Prota, R. 1988. Biotechnical control means adopted against Ceratitis capitata Wied. in clementine groves. IOBC/wprs Bull. 11:14-19.

[24] Avery, J.W., Chambers, D.L., Cunningham, R.T. and Leonhardt, B.A. 1994. Use of ceralure and trimedlure in Mediterranean fruit fly (Diptera: Tephritidae) mass-trapping tests. J. Entomol. Sci. 29:543-556.

[25] Sastre C, Melo J.C. and Borreli G. 1999. La captura de hembras: una posible salida en el control de la mosca de la fruta (*Ceratitis capitata* Wied.) en melocotonero. Phytoma. 113: 42–46.

[26] Ros, J.P., Gomila, J., Reurer, M., Pons, P. and Castillo, E. 2002. The use of mass trapping against Medfly (*Ceratitis capitata* (Wied.)) in a sustainable agriculture system on Minorca Island, Spain. Proceedings of the 6th International Symposium on fruit flies of economic importance, Stellenbosch, South Africa, 6-10 May 2002, pp. 361-364.

[27] Garcia, G., Wong, E., Marquez, A.L., Garcia, S., Olivero, J. and Garcia Mari, F. 2003. Evaluation and comparison of mass-trapping methods for the control of *Ceratitis capitata* Wied., in citrus orchards. Bull. OILB SROP. 26:85.

[28] Jemâa, J.M.B., Bachrouch, O., Allimi, E. and Dhouibi, M.H. 2010. Field evaluation of Mediterranean fruit fly mass trapping with Tripack® as alternative to malathion baitspraying in citrus orchards. Spanish Journal of Agricultural Research. 8: 400–408.

[29] Ros, J.P., Castillo, E., Crespo, J., Latorre, Y., Martin, P., Miranda, M.A., Moner, P. and Sastre, C. 1997. Evaluación en campo de varios atrayentes sintéticos para la captura de hembras de la mosca mediterránea de la fruta Ceratitis capitata Wied. (Díptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas. 23**:** 393-402.

[30] Heath, R.R., Epsky, N.D., Dueben, B.D., Rizzo, J. and Jeronimo, F. 1997. Adding methylsubstituted ammonia derivates to a food-based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 90: 1584-1589.

[31] Alemany, A., Alonso, D. and Miranda, M.A. 2004. Evaluation of improved Mediterranean fruit fly attractants and retention systems in the Balearic Islands (Spain). Pages 355-359. In Proceedings 6th International Symposium on Fruit Flies of Economic Importance, 6-10 May, 2004, Stellenbosch, South Africa.

[32] Primo, E. 2004. Plan Valenciano de actuacio´n contra la mosca de la fruta (*Ceratitis capitata* Wied.) campanya 2004. Agricultura: Revista agropecuaria. 867: 790–793.

[33] Ros, J.P., Wong, E., Olivero, J., Rubio, J.R., Ma´rquez, A.L., Castillo, E. and Blas, P. 2005. Desarrollo de atrayentes y mosqueros para su integracio´n en los programas de trampeo masivo contra la mosca de la fruta (‘‘*Ceratitis capitata*’’ Wied.) y la del olivo (‘‘*Bactrocera oleae*’’ Gmel). Bol. Sanid. Veg. Plagas. 31: 599–607.

[34] Navarro-Llopis, V., Sanchis-Cabanes, J., Ayala, I., Castana Giner, V. and Primo-Yu´ fera, E. 2004. Efficacy of lufenuron as chemosterilant against *Ceratitis capitata* in field trials. Pest Manag. Sci. 60: 914–920.

[35] Leza, M.M., Juan, A., Capllonch, M. and Alemany, A. 2008. Female-biased mass trapping vs. bait application techniques against the Mediterranean fruit fly, *Ceratitis capitata* (Dipt.: Tephritidae). Journal of Applied Entomology 132: 753–761.

[36] Martinez-Ferrer, M.T., Campos, J.M. and Fibla, J.M. 2012. Field efficacy of *Ceratitis capitata* (Diptera: Tephritidae) mass trapping technique on clementine groves in Spain. Journal of Applied Entomology. 136:181-190.

[37] Martınez-Ferrer, M.T., Campos, J.M. and Fibla, J.M. 2006. Population dynamics of *Ceratitis capitata* on citrus in northeastern Spain: influence of adjacent host fruit trees. IOBC/WPRS Bull. 29: 77–84.

[38] Martınez-Ferrer, M.T., Alonso Mun˜ oz, A., Campos Rivela, J.M., Fibla Queralt, J.M. and Garcia-Mar, F. 2007. Dina´mica poblacional de la mosca de la fruta *Ceratitis capitata* en tres zonas citrı´colas mediterra´neas. *Levante Agrı´cola, Num*. 385: 92–98.

[39] Boulahia-Kheder, S., Trabelsi, I. and Aouadi, N. 2012. From chemicals to IPM against the Mediterranean fruit fly *Ceratitis capitata*. In Larramendy, M.L. and Soloneski, S. (eds.) Integrated Pest Manage. Pest Control, Curr. Future Tactics. Croatie: In Tech.

[40] Boulahia-Kheder, S., Jerraya, A., Fezzani, M. and Jrad, F. 2010. First results in Tunisia on the mass-trapping an alternative way to control the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae). *Annals INRAT*. 82: 168-180.

Survey of Household Solid Waste Management and Waste Minimization in Malaysia: Awareness, Issues and Practices

Zarifah Abdullah[1], Salniza Md Salleh[2], Ku Nor Izah Ku Ismail[3]

[1,3]Tunku Puteri Intan Safinaz School of Accountancy (TISSA-UUM), Universiti Utara Malaysia, MALAYSIA
[2]School of Business Management, Universiti Utara Malaysia, MALAYSIA

***Abstract*—** *Effective solid waste management (SWM) is very crucial in every nation as it determines the sustainability of the environment and ensures the health of the society. This study examines households' awareness on household solid waste management and their opinion on the services provided by a solid waste management concessionaire in one of the regions in Malaysia. A survey on 398 respondents among households finds that the awareness on the privatization of SWM is acceptable and a majority of them are ignorant about the frequency of waste collection. However, the respondents' knowledge on waste and their understanding on the health consequences of waste are relatively good. The main problem expressed by respondents with respect to waste disposal is that collection schedules are not adhered to by the collectors. Disorderly disposal of rubbish is also perceived to be a problem, and a majority of the respondents believe that lack of awareness, knowledge and enforcement are the major causes of the problem. As far as waste minimization is concerned, respondents feel that the facilities and services provided are not adequate. Other than using the waste collection service, the households rarely practice other means of waste minimization such as to reuse, recycle, and compost. The role of regulators and concessionaires in educating the community so that the latter can play their role effectively is very crucial.*

***Keywords*— solid waste management, waste minimization, awareness.**

I. INTRODUCTION

Malaysia is among the successful developing economy in terms of political stability and economic growth. However, the increase in urban population and rapid economic growth lead to the increase in solid waste generation (Tarmiji, Usman & Hassan, 2011). Similarly, the characteristics of solid waste have changed in the country due to the rapid industrialization and urbanization (Manaf, Samah & Zukki, 2009). It has also been noted globally that developing Asia are among the largest solid waste generators (UNCRD, 2011). Hence, there is a need for an effective practice of solid waste management to control the current waste generation in the region. This is because in any economy, the solid waste management is an important aspect of citizens' lifestyle and country's economic status (Baud, 2001). According to the United Nations Development Programme in 2008, waste management is a crucial aspect of sustaining the national development.

Not until 2007, when Solid Waste and Public Cleansing Management Corporation Act 2007 (SWMPC Act 2007) came into place, solid waste management was the responsibility of local authorities in Malaysia. The Act, enforced in 2011, vests executive power to the Federal Government to implement solid waste management and public cleansing. It was enacted to ensure the uniformity of law relating to the management of solid waste and public cleansing throughout Peninsular Malaysia and the Federal Territories of Kuala Lumpur, Putrajaya and Labuan. On 20th September 2011, the Federal Government and several state governments sealed the 22-year concession agreement for solid waste management with three companies across Malaysia. All solid waste collection and urban cleansing services would now be under the purview of three concessionaires overseeing three zones, Federal Territory of Kuala Lumpur and Putrajaya and Pahang; Kedah and Perlis; and Johor, Malacca and Negeri Sembilan.

The privatization of solid waste management in Malaysia is aimed to solve the challenges faced by local authorities in managing solid waste, namely finance and cost management, lack of expertise and advanced technology, illegal dumping and lack of management skills on disposal and landfill system. The concessionaires are expected to improve and ensure high-quality services in solid waste management, and provide recommendation and implementation policies and strategies pertaining to solid waste management services, as well as promoting participation and awareness among the public.

In order to achieve the abovementioned goal, there is a need for enhancement in solid waste management practices (Zeeda & Keng, 2014). For example, several waste recycling campaigns have been launched by the Malaysian government to involve the participation of different community groups and non-governmental organizations. However, the campaigns received very low responses from the public. Therefore, public awareness and enlightenment are the key factors of reducing the solid waste (Zainu & Songip, 2017).There have also been complaints from the general public about the services provided by the

concessionaires. One of the complaints is that the household waste has not been collected as scheduled and was not properly managed (*Kedah Hari Ini*, May 3rd, 2012). The uncollected household wastes, resulted in a disgusting phenomenon and nauseating smell, further welcomes the unwanted flies and garbage worms. This phenomenon can always be spotted during long public holidays, school holidays or during festive seasons. The potential causes for such phenomena are limited number of garbage trucks, limited number of contractors, improper collection schedules and solid waste disposal dump that are situated too far.

One of the concessionaires is FreshAir Sdn Bhd (not the real name), being the case organization of this study. This study focuses on household awareness and their perception on household solid waste management practices in Malaysia, particularly in FreshAir. The first objective of this study is to examine household awareness of the privatization of solid waste management, waste issues and concerns, and waste management. Next, we examine household satisfaction on waste collection and customer service, and finally we examine household perception and practice on waste minimization

II. LITERATURE REVIEW

Solid waste management is a way of controlling, collecting, storing, generating, transferring and transporting, processing and disposing solid waste (Tchobanoglous, 1993). Previous studies have identified the organizations or stakeholders that may have concern in effective and efficient system of solid waste management. These include authorities of national and local government (Shekdar, 2009); non-governmental organizations, municipal authorities, households (Sujauddin, Huda, & Hoque, 2008); Ministry of Health, private contractors (Geng, Zhu, Doberstein, & Fujita, 2009) and recycling companies (Tai, et al., 2011). Despite the involvement of these stakeholders in the waste management practice, there are factors that influence the effectiveness of solid waste management. For instance, Sujauddin et al. (2008) argued that waste generation is influenced by the size of the family, their income and their level of education. Similarly, the location of household, land size, peer influence, gender and separation behavior are also the factors affecting the effective management of solid waste (Ekere, Mugisha, & Drake, 2009). In a related study, Abdullah (2013) highlighted some of the challenges associated with the management of solid waste by local authorities in Malaysia; they include squatter villages, uncollected area, lack of finance and human resources as well as immigrants.

Moreover, Otitoju and Seng (2014) surveyed the militating factors of waste segregation among the household in Malaysia. The result revealed that methods of collecting the waste and easy access to waste facilities are the key factors which prevent the segregation of waste among households. Similar findings were reported by Tadesse, Ruijs & Hagos (2008) in the context of Africa. They found that the household decision on waste disposal is significantly influenced by facilities. Inadequate supply and long distance of waste containers increase likelihood of dumping the waste in the roadside and other open area. Alike, Saxena, Srivastava & Samaddar (2010) found in their study that the approach of solid waste management is highly unprofessional and unscientific because of the improper collection, treatment and disposal of solid waste. Most of the waste dropped in an open area lead to air, water and land pollution.

On the other hand, Budhiarta, Siwar, & Basri (2012) in their survey of the current status of Malaysian solid waste generation found that government involvement in waste campaign programs has not yielded a fruitful result to the communities because the daily volume of waste generated has not declined. Thus, in order to ensure a healthy and clean nation, Behzad et al. (2011) argued that solid waste management practice needs to be improved to achieve environmental quality and socio-economic development of the nation. Similarly, Jereme et al. (2015) observed that an effective service of solid waste management is an essential device for ensuring environmental protection, both urban and rural communities' health, employment and revenue generation.

A study carried out by the Solid Waste And Public Cleansing Management Corporation (SWCorp) in 2009 found thatthe public perceived waste issues and concern as not that serious and a majority of the respondents believed the cause of the issues is due to lack of enforcement. Although 80% were satisfied with the collection service, 90% agreed that the federal or local government should enforce strict laws on waste management. A majority of the respondents agreed that there is a need for involvement of private sector and NGOs in waste management.

In a related study, Saat (2013) argued that for a sustainable solid waste management, there is a need for transformation in the environmental governance, specifically in solid waste management. This is because transformation is one of the main aspects that would ensure the sustainability of solid waste management in Malaysia. At the same time, Osmi et al. (2013) investigated how to enhance solid waste management in Malaysia. The study identified several action plans which include

Pay as You Throw, Refused Derived Fuel, Incinerator and Dual System as the best strategies for immediate and long-term improvement of solid waste management.

In the context of Africa, Peprah, Amoah & Achana (2015) assessed the effects of 3Rs model on solid waste management. Their investigation revealed that the model is effective and efficient for the management of solid waste. This is due to the fact that the model is in accordance with the tenets of achieving environmental sustainability. Nonetheless, Samah et al. (2013) in their study of trend and management of household solid waste in Malaysia discovered that the trend of solid waste management in Malaysia is like in other parts of the world, i.e. it depends mostly on changes in the pattern of consumption, seasonal variation and climate. Consequently, the management of solid waste must be improved to ensure the suitability of the technology used with the current development of solid waste.

Furthermore, Teyet al. (2013) focused on the current practice of waste management system in Malaysia. They noted that there is a lack of proper system of waste management among the construction practitioners because the predominant disposal technique used for dumping construction waste is landfill and the concept of 3Rs is adopted by only a few construction practitioners. Likewise, Visvanathan (2006), in their study of management of solid waste in Asia, argued that appropriate management of solid waste is a crucial aspect of reducing the environmental pollution. Hence, awareness on the impacts and problems of generating solid waste must be promoted among the public through enlightenment campaigns and education. In a related study, Lau (2004) found the policy on management of solid waste in Malaysia is inefficient due to improper guidance. In addition, most of the households in the country lack sufficient knowledge about waste management practices.

III. RESEARCH METHODS

This study conducted a questionnaire survey to seek households' awareness and satisfaction of the services provided by the case organization, FreshAir. In developing the questionnaire, we refer to the literature and a questionnaire developed by A-N-D Consultants. A pilot test was conducted before the questionnaires were distributed to the intended target respondents. Thirty individuals comprising of academic and administrative staff of a higher education institution in Malaysia became the respondents of the pilot test.

The questionnaire consists of three sections. The first section seeks the background information of the respondents, namely gender, race, tenancy, employment and education. The second section seeks respondents' level of awareness on solid waste management, perceptions on key waste issues, and concerns, as well as solutions to the issues and concerns. The last section deals with the household's satisfaction with customer services and garbage collection, as well as their perceptions on solutions to waste management and waste minimization.

The sampling frame of this study covers households residing in three big districts of one of the states in the region under study. The districts were identified as FSA1, FSA2, and FSA3. Within each of the districts, one housing area served by FreshAir was selected. A total of 400 households were finally sampled; 200 sets of questionnaires were distributed in FSA1, and 100 in each FSA2 and FSA3. The distribution was uneven because of the higher population density in FSA1. In order to ensure a high response, the questionnaires were distributed by hand, by eight enumerators. In most cases, a face-to-face approach was taken. This is important because some of the respondents need the assistance and clarification from the enumerators in answering the questions. In other cases, respondents were given a few days to answer the questionnaires, and the enumerators would later come back to collect the questionnaires. Out of 400 sets of questionnaires distributed, 398 were returned. This shows that almost all the sampled respondents (99.5%) responded.

IV. RESULTS AND FINDINGS

4.1 Respondent's Profile

Table 1 shows the distribution of respondents according to district, gender, race, highest education level, employment status, and tenancy. It is observed that 50.2% of the respondents are residents of district FSA 1, while 24.9% belong to each FSA 2 and FSA 3 districts. Male respondents (53.8%) are slightly more than the female respondents (46.2%). A majority of the respondents (52.8%) are Malays, followed by Chinese (35.2%), Indians (10.8%) and others (1.2%). With regards to the level of education, a majority of the respondents had at least a secondary school qualification (83.1%). Only 3.8% of the respondents had no formal education, while 13.1% attended primary school only.

A majority of the respondents are either employed or self-employed (64.3%). Students and housewives represent 18.3% and 16.1% of the sample, respectively. In terms of tenancy, 73.1% owned the house, while the remaining 26.9% rented the house.

TABLE 1
DEMOGRAPHIC BACKGROUND OF RESPONDENTS

		Frequency	Percentage (%)
District	FSA1	200	50.2
	FSA2	99	24.9
	FSA3	99	24.9
	Total	398	100.0
Gender	Male	214	53.8
	Female	184	46.2
	Total	398	100.0
Race	Malay	210	52.8
	Chinese	140	35.2
	Indian	43	10.8
	Others	5	1.2
	Total	398	100.0
Highest education	No Formal Education	15	3.8
	Primary	52	13.1
	Secondary/ Certificate	185	46.5
	Diploma/ Degree	130	32.6
	Postgraduate	16	4.0
	Total	398	100.0
Employment status	Employed	174	43.7
	Self-employed	82	20.6
	Student	73	18.3
	Housewife	64	16.1
	Unemployed	5	1.3
	Total	398	100
Tenancy	Self-owned	291	73.1
	Rented	107	26.9
	Total	398	100.0

4.2 Household Awareness, Knowledge and Understanding of Waste

Table 2 shows the level of awareness of the respondents about the company that collects and manages the waste, as well as their awareness in managing household waste. It is observed that a majority of the respondents (74.4%) are aware that waste management in their area has been privatized, with two-thirds of them are able to name the company. The remaining 25.6% of the respondents still believe that their local councils collect the waste. Considering that privatization of solid waste is quite new in Malaysia, the level of awareness is acceptable.

As far as we are concerned, the collection days for every housing-area are fixed, and there is a notice posted in the area. It is the practice of FreshAir to collect waste twice a week in residential areas. However, less than half of the respondents (43.2%) are aware that waste is collected twice a week. The responses from the remaining respondents are mixed between once, three times and four times a week. About 20% of the households could not guess the frequency of waste collection. The statistics may give two different interpretations. First, it may imply that a majority of the households are ignorant about the frequency of waste collection in a week. This may be because a majority of the respondents are working and students; thus, they are not at home when the garbage is collected. Second, it may imply that the schedule is not adhered to by the garbage collectors, causing confusion among households.

In respect of payments for waste collection, the table indicates that 40% of the respondents agree that house-owners pay the bill, while 7.0% believe that tenants are responsible for the payment of the bills. Another 19.8% believe that the government pays the bill while 33.2% have no idea about who makes the payment. This implies that a majority of the households are not aware that the home-owners are responsible for the payment of waste collection. This payment is actually included in their assessment tax paid to local councils.

The table also shows that a majority of the respondents are conscious about how their waste should be disposed, in which 94.0% believe that waste should not be thrown anywhere, such as in the sea or river. There are still some people (6.0%) who have the negative attitude by believing that waste can be thrown in the sea and river.

This study also shows that a majority of the respondents (84.7%) believe that rubbish can be useful for other things while 15.3% do not subscribe to this idea. Further, many of the respondents are aware of the health consequence of poor waste collection; 87.4% believe that poor waste collection can have a negative impact on residents' health, while 12.3% do not agree with the idea.

In addition, respondents were asked on what they can do with waste. Here, respondents were allowed to choose more than one answer. Table 2 reveals that a majority of the households (72.1%) believe that waste can be recycled. Many also believe that waste can be used for compost (56.3%). This is followed by beliefs that waste can be reused (42.0%), waste can be used for animal feed (30.9%) and that energy can be generated from waste (26.6%).

TABLE 2
HOUSEHOLD AWARENESS, KNOWLEDGE AND UNDERSTANDING OF WASTE

		Frequency	Percentage (%)
Company that collects waste	FreshAir	268	67.3
	Local council/ Authority	102	25.6
	Private Entity	27	7.1
	Total	398	100.0
Frequency of collection	Once a week	59	14.8
	Twice a week	172	43.2
	Three times a week	66	16.6
	Four times a week	21	5.3
	Don't know	80	20.1
	Total	398	100.0
Payee	House Owner	159	40.0
	Tenant	28	7.0
	Government	79	19.8
	Don't know	132	33.2
	Total	398	100.0
Rubbish can be thrown in the sea and river	Yes	24	6.0
	No	374	94.0
	Total	398	100.0
Rubbish can be useful	Yes	337	84.7
	No	61	15.3
	Total	398	100.0
Poor collection affects our health	Yes	348	87.4
	No	50	12.6
	Total	398	100.0
What we do with waste	Recycle	287	72.1
	Compost	224	56.3
	Reuse	167	42.0
	Animal feed	123	30.9
	Energy product	106	26.6

4.3 Waste Disposal and Collection Problems

With respect to waste disposal problems, respondents express mixed opinions. Table 3 shows different levels of responses for five identified problems. Generally, respondents have between slight and serious problems with all the issues. The most serious issue is that collection schedules are not adhered to by the collectors. Related to this issue is that rubbish collection is irregular.

The next problem is that the number of bins is not enough to accommodate the amount of waste to be disposed. When this happens, there is a disorderly disposal of rubbish. Respondents also face more than a slight problem of no specific rubbish disposal spot.

Respondents were also asked about the causes of the disorderly disposal of waste. A majority of the respondents (74.1%) reveal that lack of awareness and knowledge is the major cause of the problem. Another factor is that there is a lack of enforcement from the government (57.8%). This is in line with the findings by Solid Waste and Public Cleansing Management Corporation (SWCorp, 2009). In addition, a number of respondents (32.9%) believe that non-human factors such as cats, dogs and monkeys may create the problem. This shows that waste management operators should put more effort in educating and training their staff in dealing with their job and in handling the public. Measures should also be taken to deter animals from scavenging in rubbish and digging through the bins.

TABLE 3
SERIOUSNESS OF WASTE DISPOSAL AND COLLECTION PROBLEMS (n = 398)

			No problem (1)	Slight problem (2)	Serious problem (3)	Very serious problem (4)	Extremely serious problem (5)	Mean score
1.	**No specific rubbish disposal spot (n=390)**	Freq.	132	95	60	31	72	2.53
		%	(33.8)	(24.4)	(15.4)	(7.9)	(18.5)	
2.	**Number of bins not enough (n=388)**	Freq.	68	99	102	69	50	2.83
		%	(17.5)	(25.5)	(26.3)	(17.8)	(12.9)	
3.	**Irregular rubbish collection (389)**	Freq.	78	85	126	72	28	2.71
		%	(20.1)	(21.9)	(32.4)	(18.5)	(7.2)	
4.	**Collection schedule not adhered to (n=390)**	Freq.	72	82	98	68	70	2.95
		%	(18.5)	(21.0)	(25.1)	(17.4)	(17.9)	
5.	**Disorderly disposal of rubbish (389)**	Freq.	116	91	77	36	69	2.62
		%	(29.8)	(23.4)	(19.8)	(9.3)	(17.7)	

		Freq.	**%**
Causes of disorderly disposal	Lack awareness/ knowledge	295	74.1
	Lack of enforcement	230	57.8
	Other than human factors	131	32.9

4.4 Satisfaction with the Conduct of Waste Collectors

This study also seeks household satisfaction with respect to the conduct of waste collectors. The responses are depicted in Table 4. Overall, the mean scores show that respondents are moderately satisfied with waste collections in their area. Generally, respondents believe that the collection service at curbs and alleys is good.

As far as safe driving is concerned, more respondents feel that they are satisfied rather than dissatisfied with the drivers of garbage trucks. The respondents have mixed opinion on whether the workers are friendly and helpful. Their opinion of whether the service areas are left clean are also divided; however, more respondents say that they are satisfied than not satisfied. Further, respondents are moderately satisfied that garbage carts and containers are placed at their original location.

In addition, respondents were asked whether they had ever contacted their waste management company to pick up large, used items. Only 178 had used the service, and out of that, 100 (56.2%) are satisfied with the service. The remaining 43.8% are not satisfied with the service. However, this study did not further investigate the reasons for their dissatisfaction.

TABLE 4
HOUSEHOLD SATISFACTION - WASTE COLLECTORS

		Excellent (1)	Good (2)	Average (3)	Poor (4)		
Collection service at curbs and alleys (n=394)	Freq.	34	169	156	35		
	%	(8.6)	(42.9)	(39.6)	(8.9)		
Customer Satisfaction - Waste Collection		**Not at all satisfied (1)**	**Not satisfied (2)**	**Undecided (3)**	**Satisfied (4)**	**Extremely satisfied (5)**	**Mean Score**
1. Safe driver (n=390)	Freq.	45	68	122	86	69	3.17
	%	(11.5)	(17.4)	(31.3)	(22.1)	(17.7)	
2. Friendly and helpful workers (n=390)	Freq.	62	69	135	67	57	2.97
	%	(15.9)	(17.7)	(34.6)	(17.2)	(14.6)	
3. Service area left clean (n=388)	Freq.	44	61	138	84	61	3.15
	%	(11.3)	(15.7)	(35.6)	(21.6)	(15.7)	
4. Garbage carts placed at original location (n=387)	Freq.	40	68	130	91	58	3.15
	%	(10.3)	(17.6)	(33.6)	(23.5)	(15.0)	
5. Waste containers placed at original location (n=392)	Freq.	46	48	137	64	97	3.30
	%	(11.7)	(12.2)	(34.9)	(16.3)	(24.7)	
Satisfied with service performed in collecting large items (n=387)				**Freq.**		**%**	
		Yes		100		56.2	
		No		78		43.8	

4.5 Household Satisfaction - Customer Service

Customer service is very important in the management of waste. One of the major channels used for customer service is through telephones. In this study, respondents were asked whether they have ever contacted customer service through telephones for enquiries related to waste management in their area. Only 68 respondents (16.8%) had used the service, and a majority of them (54.4%) had made one to two phone calls as indicated in Table 5. Further, the respondents that had made phone calls are asked to rate their satisfaction with the over-the-phone service that they received. Generally, they are between undecided and unsatisfied with the service received on the phone, the friendliness of staff, the helpfulness of staff and the staff's ability to answer questions. This shows that the waste management company needs to give adequate training to their staff to be courteous in handling inquiries and complaints in ways that create improved relationships with clients.

TABLE 5
HOUSEHOLD SATISFACTION – CUSTOMER SERVICE

				Freq.	%			
Frequency calls made (n = 68)			**1-2 times**	37	54.4			
			3-5 times	25	36.8			
			More than 5 times	6	8.8			
			Not at all satisfied (1)	**Not satisfied (2)**	**Undecided (3)**	**Satisfied (4)**	**Extremely satisfied (5)**	**Mean Score**
1.	**Service received on the phone (n = 68)**	Freq.	16	15	23	12	2	2.54
		%	(23.5)	(22.1)	(33.8)	(17.6)	(2.9)	
2.	**Friendliness of staff (n = 68)**	Freq.	8	17	24	15	4	2.85
		%	(11.8)	(25.0)	(35.3)	(22.0)	(5.9)	
3.	**Helpfulness of staff (n = 68)**	Freq.	8	21	25	10	4	2.72
		%	(11.8)	(30.9)	(36.8)	(14.7)	(5.9)	
4.	**Ability to answer question (n = 68)**	Freq.	9	16	22	13	8	2.93
		%	(13.2)	(23.5)	(32.4)	(19.1)	(11.8)	
5.	**Overall satisfaction (n = 68)**	Freq.	13	14	21	13	7	2.81
		%	(19.1)	(20.6)	(30.9)	(19.1)	(10.3)	

4.6 Waste Management and Waste Minimization Solutions

In addition to the series of questions that were asked on solid waste management, the level of awareness and practice of households about waste minimization, that is reducing the amount of waste sent to landfill, were also measured. The results are reported in Table 6. Overall, a majority of the respondents (60.3%) believe that the facilities and services provided by the waste management company in managing waste are inadequate. Among the facilities and services that can improve waste management, in descending order of importance are regular collection (63.8%), enforcement (60.3%), creating awareness (44.2%), sanitary dumping (25.4%), and strict standards (24.4%).

TABLE 6
HOUSEHOLD AWARENESS –WASTE MINIMIZATION

		Frequency	Percentage (%)
Facilities and services are adequate	Yes	158	39.7
	No	240	60.3
Facilities and services to improve	Regular collection	272	63.8
	Enforcement	240	60.3
	Awareness	176	44.2
	Sanitary dumping	101	25.4
	Strict standards	97	24.4
How dispose-off household waste	Collection service	289	72.6
	Reuse/ recycle	136	34.2
	Compost	99	24.9
	Animal food	65	16.3
	Burn/bury/dump backyard	53	13.3
	Sell	49	12.3
	As fill material	43	10.8
	Return to shop	28	7.0
	Dump on beach/ sea/drain/river	19	4.8
Make compost at home	Yes	99	24.9
	No	299	75.1
Agree if compost demonstrated	Yes	322	80.9
	No	76	18.3
Support centralized composting	Yes	331	83.2
	No	67	16.1

In addition to the above, the ways in which households dispose-off their waste in addition to regular collections are solicited. Respondents are allowed to give more than one answer. Table 6 indicates that a majority of the respondents (72.6%) use the collection services provided by the concession company. Other acceptable ways of minimizing waste are by reusing or recycling the waste, composting, feeding the animals, selling, using as fill materials, and returning waste to shops. However, there are some respondents who dumped the waste on the beach or in the sea, drain and river. Burning, burying or dumping in the backyard is not commonly practiced by the respondents. The activity may not be appropriate, depending on the housing location and density of the population. It can be unhealthy and non-acceptable if practiced in housing areas in which houses are linked or close to each other with limited vacant land.

This study shows that about 34% of the respondents reuse or recycle the waste, in an effort to minimize the disposing of waste in landfills. The percentage is relatively small considering that various campaigns have been conducted to educate the society on the benefit of waste recycling, and the fact that recycling bins have been placed at various strategic locations. Our finding supports the study by Zainu & Songip (2017) who found that recycling campaigns receive low response from the public.

We also find that one-fourth of the respondents made compost at their respective homes. However, it appears that a high majority of the respondents (81%) welcome the idea of demonstrating composting and support centralized composting (83.2%). This shows that many respondents are interested but do not have the knowledge to compost. We believe that given proper and continuous demonstrations on composting, waste disposal can be minimized.

V. DISCUSSION AND CONCLUSION

This study surveys households on the issues pertaining to household waste management in one of the regions in Malaysia. By and large, households are aware that waste management in their region has been privatized even though some of them could name the concessionaire company. Considering that privatization of solid waste is quite new in Malaysia, the awareness is acceptable. What is more important is that the households or society understand their role in managing waste, not byjust passing the responsibility to the organization in charge of waste management. Instead, the society should work hand in hand with the government and concessionaires in realizing a sustainable and healthy environment.

Our study shows that households' knowledge and understanding of waste is reasonably good, namely in terms of health consequences and how waste can be of benefit to the society and environment. However, households are ignorant towards the frequency of garbage collection. Knowing the right schedule is very important because it helps the household to plan their disposing of rubbish in the bin. On the other hand, we find that the respondents are putting the blame on the garbage collectors; generally the household agree that not adhering to the collection schedule and irregular rubbish collection are giving them problems. It is not the intention of this study to put the blame on anyone. Both ignorance on the part of the society and irregular waste collection by the service provider can lead to serious consequences, such as overflow of bins, rubbish left uncollected for a long time, and smelly environment, which would subsequently attract flies and other insects and animals. Thus, it is the duty of the concessionaires to ensure that the waste is collected as being scheduled, and of the households not to be ignorant of the schedule. This will thus contribute to a clean and healthy environment. The respondents also agree that that there is a lack of awareness, knowledge and enforcement that causes disorderly waste disposal.

Generally, respondents are moderately satisfied with the attitude of waste collectors during waste collection. Improvements are recommended in terms of safe driving, friendliness, cleanliness of service areas, and proper placing of garbage carts and bins after collection. As far as customer service is concerned, more training should be given to the staffs who handle customer service. It appears that generally, the respondents are not satisfied with customer service.

Waste minimization is one of the issues surveyed in this study. Among the various ways by which waste can be minimized, reusing and recycling have been practiced by about one-third of the respondents. The figure is still not promising; more and continuous programs and campaigns, especially by the government and concessionaires, to educate and discipline the society are very much needed, in addition to providing recycling bins at various strategic locations. The campaigns should be able to instill the feeling of guilt among the society if they do not recycle their household waste. The social media, mass media, schools and colleges, as well as places of worship should act as channels in educating the public about the importance of recycling.

Another method of waste of minimization that can be practiced is composting. Although not many respondents compost their household waste, a majority of the respondents are keen in doing it if demonstrated, and are very supportive of the idea of centralized composting. Again, educating the public on the know-how is an important element of waste minimization.

Although this study is conducted in only one of the regions in Malaysia, it may be applicable and generalized to other parts of the country. We believe that the society would benefit most if all parties would be able to work hand in hand both on the technical and behavioral elements. Continuous campaigns, more in-depth knowledge sharing, motivation and training would be among the immediate initiatives required of the concessionaires, the local councils, the state, and the federal government for a cleaner, greener and environmentally safer Malaysia. Besides, enforcement mechanisms should be in place and authorities should enforce whatever rules and regulations that have been implemented.

At the time this survey was conducted, campaigns on household waste separation, another means of waste minimization, have not been widespread. Beginning September 2015, some states under the three concessionaires enforced waste separation, in pursuant to regulations under Solid Waste and Public Cleansing Management Act 2007 (Act 672). The process of separating solid waste involves separating solid waste according to waste composition such as recyclable waste, residual waste and bulky/garden waste. The separated waste will be collected every week based on fixed schedules. The success of this campaign has yet to be investigated, and this can be an avenue for future research in Malaysia.

REFERENCES

[1] Abdullah, S. J. H. (2013) Overview of municipal solid waste management practices and challenges in Sabah: A review paper.

[2] Agamuthu, P., Fauziah, S.H. and Khidzir, K. (2009). Evolution of Solid Waste Management in Malaysia: Impacts and Implications of the Solid Waste Bill, 2007. *Journal of Material Cycles and Waste Management*, 11, 96-103.

[3] Agamuthu, P., Santha Chenayah, Fauziah, S.H. and Dennis Victor, (2011). 3R Related Policies for Sustainable Waste Management in Malaysia in Coference Proceedings of 'Innovation and Sustainability Transitions in Asia', Kuala Lumpur, Malaysia, Paper 135 (*Non-ISI/Non-SCOPUS Cited Publication*).

[4] Baud, I. S. A., Grafakos, S., Hordijk, M., & Post, J. (2001). Quality of life and alliances in solid waste management: contributions to urban sustainable development. *Cities*, *18*(1), 3-12.

[5] Behzad, N., Ahmad, R., Saied, P., Elmira, S., & Bin, M. M. (2011). Challenges of solid waste management in Malaysia. *Research Journal of Chemistry and Environment*, *15*(2), 597-600.

[6] Brooks, E., Harris, C.R. & Clayton, P.H. (2010). Deepening applied learning: An enhanced case study approach using critical reflection. *Journal of Applied Learning in Higher Education*, 2, 55-76.

[7] Budhiarta, I., Siwar, C., & Basri, H. (2012). Current status of municipal solid waste generation in Malaysia. *International Journal on Advanced Science, Engineering and Information Technology*, *2*(2), 129-134.

[8] Dawda Badgie, Mohd Armi Abu Samah, Latifah Abd Manaf, Azizi Muda (2012). Assessment of Municipal Solid Waste Composition in Malaysia: Management, Practice, and Challenges. *Polish Journal of Environmental Studies*. 21(3), 539-547.

[9] Ekere, W., Mugisha, J., & Drake, L. (2009). Factors influencing waste separation and utilization among households in the Lake Victoria crescent, Uganda. *Waste management*, *29*(12), 3047-3051.

[10] Geng, Y., Zhu, Q., Doberstein, B., & Fujita, T. (2009). Implementing China's circular economy concept at the regional level: A review of progress in Dalian, China. *Waste Management*, *29*(2), 996-1002.

[11] Jereme, I. A., Begum, R. A., Talib, B., Siwar, C., & Alam, M. (2015). Assessing Problems and Prospects of Solid Waste Management in Malaysia.

[12] Kedah Hari Ini (3rd May 2012) accessed from Blog kedahan69ers.com

[13] Lau, V. L. (2004). Case study on the management of waste materials in Malaysia. *In Forum Geookol 15(2), 7-14.*

[14] Manaf, L. A., Samah, M. A. A., & Zukki, N. I. M. (2009). Municipal solid waste management in Malaysia: Practices and challenges. *Waste management*, *29*(11), 2902-2906.

[15] Mason, J. *Qualitative Researching*, 2nd Edition. SAGE Publications, 1996.

[16] Md. Abdul Jalil (2010). Sustainable Development in Malaysia: A Case Study on Household Waste Management. *Journal of Sustainable Development*, 3(3), 91-102.

[17] Memon, M.A. (2010). Integrated Solid Waste Management based on the 3R Approach. *Journal of Material Cycles and Waste Management*, 12, 30-40.

[18] Ministry of Housing and Local Government Malaysia Report (2006). The Study on National Waste Minimisation in Malaysia – Final Report.

[19] Ogbonna, D.N., Amangabara, G.T., and Ekere, T.O. (2007). Urban Solid Waste Generation in Port Harcourt Metropolis and Its Implications for Waste Management. *Management of Environmental Quality: An International Journal*. 18(1), 71-88.

[20] Osmi S. F. C., Mokhtar, N. Romali, N. S. & Osmi, S. K. C. (2013).Enhanced the municipal solid waste management in Kuala Lumpur by implementing technologies from European countries: Strategies and action plans. *International Journal of Civil Engineering & Geo-Environmental 4, 22-34.*

[21] Otitoju, T. A., & Seng, L. (2014). Municipal solid waste management: household waste segregation in Kuching South City, Sarawak, Malaysia. *American Journal of Engineering Research (AJER)*, *3*(6), 82.

[22] Papargyropoulou, E. (2010). For The Love of Food. Chartered Institution of Wastes Management, CIWM, October, 30-32.

[23] Peprah, K., Amoah, S. T., & Achana, G. T. W. (2015). Assessing '3Rs' model in relation to municipal solid waste management in wa, Ghana. *World Environment*, *5*(3), 112-120.

[24] Saat, S. A. (2013). Solid waste management in Malaysia and ecological modernization theory perspective. *Journal of Sustainability Science and Management*, *8*(2), 268-275.

[25] Samah, M. A. A., Manaf, L. A., Ahsan, A., Sulaiman, W. N. A., Agamuthu, P., & D'Silva, J. L. (2013). Household solid waste composition in Balakong City, Malaysia: Trend and Management. *Polish Journal of Environmental Studies*, *22*(6).

[26] Saxena, S., Srivastava, R.K. and Samaddar, A.B. (2010). Towards Sustainable Municipal Sold Waste Management in Allahabad City. *Management of Environmental Quality: An International Journal*. 21(3), 308-323.

[27] Selangor Runs out of Garbage Space, NST, November 2008

[28] Sharifah, A.S.A.K., Zainal Abidin, H., Sulaiman, M.R., Khoo, K.H. and Ali, H. (2008). Combustion Characteristics of Malaysian Municipal Solid Waste and Predictions of Air Flow In A Rotary Kiln Incinerator. *Journal of Material Cycles and Waste Management,* 10, 116-123.

[29] Shekdar, A. V. (2009). Sustainable solid waste management: an integrated approach for Asian countries. *Waste management*, *29*(4), 1438-1448.

[30] SMARTRanger (2009-2013). Accessed at http://www.smartranger.net/index.cfm?&menuid=69&parentid=3 on 5th December 2013 at 9.10pm.

[31] Sreenivasan, J., Govindan, M., Chinnasami, M., & Kadiresu, I. (2012). Solid Waste Management in Malaysia–A Move Towards Sustainability. In *Waste Management-An Integrated Vision*. Intech

[32] Subhan, Bashawir and Hasanur (2014) Urban Community Willingness to Pay for Improved Solid Waste Management in Malaysian Municipality: A Choice Modeling Approach. *Asian Social Science*, Vol. 10, No. 18, 122-136.

[33] Sujauddin, M., Huda, S. M. S., & Hoque, A. R. (2008). Household solid waste characteristics and management in Chittagong, Bangladesh. *Waste management*, *28*(9), 1688-1695.

[34] Tchobanoglous, G., & Kreith, F. (2002). Handbook of solid waste management (2nd ed., p. 950). McGraw-Hill.

[35] Tadesse, T., Ruijs, A., & Hagos, F. (2008). Household waste disposal in Mekelle city, Northern Ethiopia. *Waste Management*, *28*(10), 2003-2012.

[36] Tai, J., Zhang, W., Che, Y., & Feng, D. (2011). Municipal solid waste source-separated collection in China: A comparative analysis. *Waste management*, *31*(8), 1673-1682.

[37] Tarmiji M., Usman Y., Hassan N., K. (2011). Pertumbuhan Penduduk dan Pembandaran di Semenanjung Malaysia 1911 – 2000.

[38] Tchobanoglous, G. (1993). *Integrated solid waste management engineering principles and management issues* (No. 628 T3).

[39] Tey, J. S., Goh, K. C., Kek, S. L., & Goh, H. H. (2013). Current practice of waste management system in Malaysia: towards sustainable waste management.

[40] UNCRD, U. R. (2011). National 3R Strategy Development: a progress report on seven countries in Asia from 2005 to 2009. United Nations Centre for Regional Development. *Institute for Global Environmental Strategies, Nagoya*.

[41] UNDP, U. (2008). Malaysia Developing a Solid Waste Management Model for Penang.

[42] Visvanathan, C. (2006). Solid Waste Management in Asian Perspectives. *Environmental Management Tools*, 131.

[43] Zaini Sakawi (2011). Municipal Solid Waste Management in Malaysia: Solution for Sustainable Waste Management. *Journal of Applied Sciences in Environmental Sanitation*, 6(1), 29-38.

[44] Zainu, Z. A., & Songip, A. R. (2017). Policies, challenges and strategies for Municipal waste management in Malaysia. *Journal of Science, Technology and Innovation Policy*, *3*(1).

[45] Zeeda, F. M., Keng, J. (2014). Opportunities and Challenges in Sustainable Waste Management Transition in Malaysia: A Multi-Level Social Technical Perspective.

Probable health damage to dogs by intakeing the Bravecto™ Product

Sara Maria Chalfoun de Souza

Researcher, PhD - EPAMIG Sul, Brazil

***Abstract*—** *The product based on fluralaner, brand name Bravecto was launched worldwide in 2014 in the form of a chewable tablet whose action on fleas and ticks lasts around three months. Presented by MSD ANIMAL HEALTH as an innovative product, highly safe, and can be ingested by dogs, including puppies, breeders, pregnant and lactating bitches and Collie dogs. In addition, it is indicated as part of the strategy to control allergic dermatitis and reduce the risk of transmitting fatal diseases transmitted by ticks. However, after its launch, numerous cases of development of pathologies and deaths of dogs of various races and ages, coinciding with the intake of the product began to be reported. Later on, the owners whose dogs had adverse effects on the product were organizing and forwarding reports to the manufacturer and entities responsible for product regulation and marketing and consumer support worldwide such as the CVM's Adverse Drug Event (ADE) EMA (European Medicines Agency), various sites on the pathologies developed in different breeds and the signs presented by the animals in the period of product administration. All this data which have become a database available for consultation.*

The European Medicines Agency (EMA) in July 2017 had requested the company to investigate all relevant reports related to various disorders such as neurological, skin and appendage diseases, hypersensitivity or immune-mediated reactions and liver diseases, some of which were fatal. The present review aims to summarize the information and provide a scenario in which, despite the efficacy of the product in the control of ectoparasites, has coincided in the period after its application, with the incidence of several pathologies and even deaths of the canine species

Keywords*— *Animal safe, pathologies, drugs, civilservice, data base.

I. INTRODUCTION

Fluralaner is a novel, recently developed systemically distributed molecule of the isoxazoline class with a highly selective ectoparasiticidal activity achieved through blocking arthropod γ-aminobenzoic acid (GABA) and glutamate-ligand gated chloride channels (1,2).The mode of *action* of fluralaner is the antagonism of the ligand-gated chloride channels (gamma-aminobutyric acid (GABA)-receptor and glutamate-receptor).

American regulators, the US Food and Drug Administration (EMA), Canadian, VDD (Veterinary Drugs Directorate) and Brazilian, MAPA (Ministry of Agriculture, Livestock and Food Supply) have approved the registration of Bravecto™ chewable tablets, from 2014.

MSD Animal Health presented the active ingredient of Bravecto ™, fluralaner, as a novel, long-acting systemic insecticide and acaricide belonging to the isoxazoline class of parasiticides marketed in the form of a chewable tablet whose action on fleas and ticks lasts around three months.

Cited as an innovative, highly safe product, it could be ingested by dogs, including puppies, breeders, pregnant and lactating bitches and Collie dogs. In addition, it is indicated as part of the strategy to control allergic dermatitis and reduce the risk of transmitting fatal diseases transmitted by ticks.

After its launch, cases of development of pathologies and deaths of dogs of various races and ages coinciding with the intake of the product began to be reported.

The owners of dogs were organizing and forwarding the reports to the manufacturer or distributor and entities performed in the Civil Service People Survey worldwide, such as the CVM's Adverse Drug Event (ADE), EMA (European Medicines Agency), VMD: Civil Service People Survey, UK Gov and MAPA, Brazil, behong others.

The scientific literature concerned to the compound Fluralaner, the novel isoxazolineparasiticide, reports the effective results of the product in the treatment of dogs in the control of two pests, namely fleas and ticks (3,4,5,6).

All of them agree about the fact that Bravecto is a novel systemic insecticide and acaricide that provides long acting efficacy in dogs after a single oral treatment.

However, research becomes scarce when searching for scientific articles on the mechanisms of action of fluralaner on the non-target species as canine and consequent side effects from its application.

The present study sought, through a search for papers, reports based on the data on the harmful effects of the Bravecto product, based on the fluralaner compound, on animal health, to demonstrate that research is insufficient for the even if it remains in the market, due to the injuries suffered by the animals and their owners.

II. METHODOLOGY

We searched for scientific papers indexed in different databases, databases containing reports of possible side effects of the product in Brazil, the United States and Europe as well as on sites in the same countries.

III. RESULTS AND DISCUSSION

In the article about the Bravecto Technical Dossier (Wengenmayer et al., 2014), we found some of the publications used for product registration, available at: http://www.parasitesandvectors.com/series/bravecto(7). published, whose citations and additional information regarding them are presented in Table 1 below.

TABLE 1
PAPERS ABOUT ASPECTS OF SEGURANCE AND EFFICACY OF BRAVECTO ™

Paper citation	Interest conflites	Number of health dogs receiving Fluraner
Meadows et al.: A randomized, blinded, controlled USA field study to assess the use of fluralaner tablets in controlling canine flea infestations. **Parasites & Vectors** 2014 7:375.	Drs. Meadows, Guerino, e Sun are hired by pela Merck Animal Health.	118
Walther et al.: Safety of the concurrent treatment of dogs with Bravecto™ (fluralaner) and Scalibor™ protectorband (deltamethrin). **Parasites & Vectors** 2014 7:105.	FMW, PF, MJA, RKAR e MCN are hired by Merck/MSD Animal Health.	24
Kilp et al.: Pharmacokinetics of fluralaner in dogs following a single oral or intravenous administration. **Parasites & Vectors** 2014 7:85.	All the authors except DR, are hired by Merck / MSD Animal Health.	24
Rohdich et al.: A randomized, blinded, controlled and multi-centered field study comparing the efficacy and safety of Bravecto™ (fluralaner) against Frontline™ (fipronil) in flea- and tick-infested dogs. **Parasites & Vectors** 2014 7:83.	All the authors except DR, are hired by MSD Animal Health.	108
Walther et al.: Safety of fluralaner, a novel systemic antiparasitic drug, in MDR1(−/−) Collies after oral administration. Parasites & Vectors 2014 7:86.	FMW, MJA, RKAR e MCN are hired by Merck / MSD Animal Health. AJP is hired by IllinoisUniversity.	8
Walther et al.: The effect of food on the pharmacokinetics of oral fluralaner in dogs. **Parasites & Vectors** 2014 7:84.	FMW, MJA, RKAR e MCN are hired by Merck / MSD Animal Health.	12
Taenzler et al.: Onset of activity of fluralaner (BRAVECTO™) against Ctenocephalidesfelis on dogs. Parasites & Vectors 2014 7:567.	JF is haired by ClinVet and all the others authors are MSD Animal Health..	44
Walther et al.: Safety of concurrent treatment of dogs with fluralaner (Bravecto™) and milbemycin oxime - praziquantel. **Parasites & Vectors** 2014 7:481.	FMW, PF, MJA, RKAR e MCN are hired by Merck/MSD Animal Health.	20

As common points among the publications it is quoted that all of them are published in an the open access publication, all in the year of 2014.. Another point to be considered is that in all studies healthy dogs were used as quoted in the Technical Dossier, aiming the registration of the product. Being a free-marketing product offered by pet shop attendants, in the case of Brazil, it would be impossible to know if the dog that will receive the product is healthy or if it presents a genetic predisposition or of another nature that would restrict the use of the same one .

Regarding the form of commercialization, in Europe and the United States, the law restricts this drug to use by or on the order of a licensed veterinarian (8,9).

In Brazil, there is a free commercialization of the product, without the order of a veterinarian licensed, including by e-commerce although there are many cases of administration of the product under the guidance of veterinarians who seem to ignore or ignore the harmful effects of the drug.

On the website http://www.seubuldoguefrances.com.br/2016/03/a-polemica-do-bravecto.html founded by CamilliChamone (10), geneticist and molecular biologist, on 08/14/2008 that became the first and largest blog in Latin America on French bulldogs, a post was published in March 2016 whose comments on the product insert highlighting the fact that:

In the leaflet of the medication, the first sentence of Indications is already quite elucidative: For the treatment of infestations by ticks and fleas ..." That is, there is no indication of use of this product for prevention.

This is still reiterated and very well explained when the manufacturer himself says: "Fleas and ticks should be attached to the dog and already started feeding [dog's blood] to be exposed to the active principle."

"I may be mistaken, but logic tells me that if fleas and ticks are not prevented from stinging an animal, the risks of transmission or manifestation of disease (DAPP, erlichiosis, babesiosis) are not eliminated."

The author completes her comments regarding the systemic action of the product with little known effects on the animal organism.

Demonstrating a return to claims from organized civil society, the European Medicines Agency (EMA) after 11 February 2014; it has granted a marketing authorization valid throughout the European Union for the veterinary medicinal product Bravecto. The full EPAR for Bravecto can be found on the Agency's website at: ema.europa.eu/Find medicine / Veterinary medicines / European public assessment reports. Last updated in March 2016, it presented in July 2017 some questions and new requirements regarding the maintenance of the product in the market.

The European Medicines Agency (EMA) concluded in July 2017 that" Bravecto, a medicine that treats tick and flea infestations in dogs and cats, continues to have an acceptable safety profile. However, the company that markets the product will have to update the package leaflet and include convulsions as a new side effect that is reported very rarely, i.e., less than one animal out of 10,000 animals treated. Veterinarians and pet owners will also be advised to use Bravecto with caution in dogs with epilepsy.

EMA's Committee for Medicinal Products for Veterinary Use (CVMP), following a regular but inconclusive analysis of limited data on serious side effects, had requested the company to investigate all relevant reports related to various disorders such as neurological, skin and appendage diseases, hypersensitivity or immune-mediated reactions and liver diseases, some of which were fatal. The investigation had to take into account the age and breed of animals, the number of treatments, underlying disease conditions and concomitant treatments.

The company that markets the medicine is expected to implement the changes to the package leaflet within six months of the CVMP conclusion.

According to the company, about 41.6 million doses had been distributed worldwide, of which approximately 18 million were in the European Union (EU) between February 2014 and December 2016. By 15 August 2017, suspected side effects had been reported electronically for 5,326 dogs, of which 2,144 were in the EU. Between February 2014 and 15 August 2017, deaths had been reported in 1,265 dogs worldwide and 342 in the EU. Each report relates to dogs under different health conditions, often receiving multiple medicines, therefore these figures may or may not be related to the use of Bravecto in dogs"(11).

Another survey conducted by the European Medicines Agency (EMA) reports all races that suffered side effects coincident with the post-administration period and were reported to MSD and MERK. About 89 breeds were reported involving about 870 dogs. It was observed that many cases reported were of crossed and unlisted races (12).

Numerous petitions are under way and some have already been forwarded. The recent petition with 40,000 signatures from the Netherlands: the anti bravecto petition with 40,000 signers has been delivered at the Ministry of Agriculture, Nature and Food quality), released by social media around the world.

IV. CONCLUSIONS

Bravecto™ product, an insecticide acaracide, based on the fluralaner compound, has it administration coincided to the occurrence of serious pathologies leading to death of dogs in all the countries in which it is in commercialization.

Strong evidences that the product not only targets flea and tick, but compromises the health of the non target species like dogs, its recommendation as oral treatment in the form of chewable tablets, should be carefully reviewed.

CONFLICT OF INTEREST

The author has no interest involved besides contributing to the safety of animals, in the present case dogs.

ACKNOWLEDGEMENT

To Nix, wishing his sacrifice was not in vain.

REFERENCES

[1] OZOE Y, ASAHi M, OZOe F, NAKAHIRA K, MITA T. The antiparasiticisoxazoline A1443 is a potent blocker of insect ligand-gated chloride channels. BiochemBiophys Res Commun. 2010;391:744–749. doi: 10.1016/j.bbrc.2009.11.131.

[2] KILP S, RAMIREZ D, Allan MJ, Roepke RK, Nuernberger MC. Pharmacokinetics of fluralaner in dogs following a single oral or intravenous administration. Parasite& Vectors. 2014;7:85.

[3] MEADOWS et al.: A randomized, blinded, controlled USA field study to assess the use of fluralaner tablets in controlling canine flea infestations. Parasites & Vectors 2014 7:375.

[4] WALTHER et al.: Safety of the concurrent treatment of dogs with Bravecto™ (fluralaner) and Scalibor™ protectorband (deltamethrin). Parasites & Vectors 2014 7:105.

[5] GASSEL, M1; WOLF .C1; Noack, S; WILLIAMS, H; ILG,T.The novel isoxazolineectoparasiticidefluralaner: Selective inhibitionof arthropod g-aminobutyric acid- and L-glutamate-gated chloridechannels and insecticidal/acaricidalactivity.Insect Biochemistry and Molecular Biology 45 (2014) p.111-124

[6] ROHDICH, N; ROEPKE; R.K.A.; ZSCHIESCHE E. A randomized, blinded, controlled and multi-centered field study comparing the efficacy and safety of Bravecto™ (fluralaner) against Frontline™ (fipronil) in flea- and tick-infested dogs. Parasit&Vectors. 2014;7:83.

[7] WENGENMAYER et al. Dossiê Técnico Bravecto.(*licenciado* por *BioMed Central Ltd*. *Este* é um *artigo* de *acesso livre distribuído* sob os termos da Licença Atribuída a CreativeCommons(http://creativecommons.org/licenses/by/4.0)2014. Disponível em:https://www.bravecto.com.br/sou_vet/DOSSIE_TECNICO_BRAVECTO. Acesso em 06/12/2017.

[8] Flavoured chews for dogs.Caution:FEDERAL (USA)- Bracecto. Disponível em:https://us.bravecto.com/pdfs/bravecto_pi_mah.pdf .Acesso em 07/12/2017.

[9] Bravecto, INN- Fluralaner- Europa EU Disponível em:https://ec.europa.eu/health/documents/community-register/.../anx_127740_pt.pdf.Acesso em 07/12/2017.

[10] Camilli Chamone. Disponível em:http://www.seubuldoguefrances.com.br/2016/03/a-polemica-do-bravecto.html. Acessoem 07/12/2017.

[11] EUROPEAN MEDICINE AGENCY.Tick and flea control agent Bravecto continues to be acceptably safe to use.**New adverse reaction to be included in** package leaflet.Disponívelem:http://www.ema.europa.eu/ema/acesso em 07/12/2017.

[12] FDA-CVM FOIA. Response 2016-96 January 2016A. Disponível em :yourpetsneedthis.com/.../Bravecto-fluralaner-ADE-report-Jul201. Acesso em 11/12/2017.

Analysis of Marketing Efficiency of Beef Cattle on "Tirto Sari" Livestock at Sub District of Samboja, District of Kutai Kartanegara, East Kalimantan

Riansyah Comdeca Surya Pratama[1], Koesnoto Supranianondo[2]

[1]Student, Veterinary Agribusiness (Postgraduate Program), Universitas Airlangga, INDONESIA
[2]Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga

Abstract*— *The purpose of this study is to find out the marketing efficiency of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara, East Kalimantan. The result of this study indicates that there are four varieties in terms of beef cattle marketing on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara, East Kalimantan: direct marketing of marketing type I, intermediate marketing of marketing type II and III and long-processed marketing of marketing type IV. The marketing agencies involved in the marketing process of beef cattle are livestock, small-sized enterprises, wholesalers, final consumers or slaughterhouse. The result of marketing efficiency calculation shows that each marketing type of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara is considered to be efficient, ranging from 0 – 33%. After the calculation of marketing margin and farmer's share, the most efficient marketing type is marketing type I (direct marketing), showing lowest value of marketing margin and highest value of farmer's share. It is, then, followed by marketing type II (intermediate marketing), marketing type III (intermediate marketing), and marketing type IV (long-processed marketing).

Keywords*— *Marketing Efficiency, Beef Cattle, Kutai Kartanegara.

I. INTRODUCTION

An increasing demand in terms of beef cattle in East Kalimantan as a source of meat occurs every year; however, it is not supported by the increase in cattle population meaning that the need for meat and cattle in East Kalimantan is hardly met. In 2014, East Kalimantan supplied 59,216 cows from East Java, South Sulawesi, Central Sulawesi, Bali, and West Nusa Tenggara (East Kalimantan Livestock Service, 2015).

Establishing an efficient marketing system is most likely to benefit farmers and consumers; one of which is through establishing a direct marketing. The sequence of marketing determines the value of marketing margin, the profit of farmer's share and the profit to be obtained; starting there it is possible to analyze the marketing efficiency (Soekartawi, 1997). The purpose of livestock development is neither to increase the number demand by expanding the market nor to increase the purchasing power, but the main purpose is to increase the income of farmers (Saragih, 2000).

District of Kutai Kartanegara constitutes the district with most cattle population in East Kalimantan of 26,198 cows and contributes to supply the meat for the community; at the same time, it is also required to provide beef cattle for cattle farmers outside the district, so that establishing efficient marketing system is considered to be imperative (East Kalimantan Livestock Service, 2015).

By 2016, the number of livestock farming groups of beef cattle in the district of Kutai Kertanegara is 211 groups, spread across the district and divided in 7 areas of livestock. There has been no study in terms of analyzing the marketing efficiency of beef cattle in the District of Kutai Kartanegara (District Kutai Kartanegara Livestock and Animal Health Service, 2016). Of the description above, this study is essential in order to determine and analyze the marketing efficiency of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara, East Kalimantan.

II. MATERIAL AND METHOD

2.1 Research Design

The study was conducted in the District of Kutai Kartanegara, East Kalimantan. The District of Kutai Kartanegara was selected due to the fact that Kutai Kartanegara is the district with the highest population of cattle livestock in East Kalimantan (East Kalimantan Livestock Service, 2015).

The study on the marketing of beef cattle in the district of Kutai Kartanegara is the form of case study. Winartha (2006) states that case study is an approach aiming at maintaining the needs or wholeness of an object. The instruments used in the study were survey and interview in order to obtain information from the respondents. The selection of beef cattle livestock used purposive sampling under the criteria of employing group maintenance system and complete administration. The sample of marketing agency was determined by accidental sampling following the distribution of beef cattle from livestock to consumers. Data obtained consists of primary and secondary data. Primary data were obtained directly from beef cattle livestock and marketing agency through interview (questionnaire). Secondary data were obtained from agencies related to the topic of the study. The data were processed systematically and presented in the form of tables and figures.

2.2 Sampling

The sample of this study is on Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara, East Kalimantan developed by UPTD PUSKESWAN, Sub District of Samboja, District of Kutai Kartanegara. The sample size is 20 members of beef cattle livestock with the ownership of 2 – 5 cows.

The sampling technique used purposive sampling due to the fact that the Sub District of Samboja is the central of beef cattle livestock in the district of Kutai Kartanegara and "Tirto Sari" livestock develops well with well-conduct administration.

2.3 Data Analysis

The analysis used in this study is descriptive analysis, marketing margin analysis, profit analysis, farmer's share analysis and marketing efficiency analysis.

III. MARKETING STRATEGIES

3.1 Marketing Margin

The marketing margin on marketing type I (direct marketing) is 0 IDR meaning that price difference between producers and consumers is a non-existent. On marketing type II (intermediate marketing), the value of marketing margin is 1,000,000 IDR/head. On marketing type III (intermediate marketing), the value of marketing margin is 2,000,000 IDR/head. On marketing IV (long-processed marketing), there are two marketing agencies involved: small-sized enterprises (inter-village/sub district) and wholesalers (interdistrict/cities/provinces). The highest marketing margin is obtained by wholesalers of 2,000,000 IDR/head, while the lowest marketing margin is obtained by small-sized enterprises of 1,500,000 IDR/head.

3.2 Profit

On marketing type I (direct marketing), the profit obtained is 2,532,500 IDR/head. On marketing type II (intermediate marketing), the profit earned is 600,000 IDR/head. On marketing type III (intermediate marketing), the profit is 1,235,000 IDR/head. Marketing type IV obtains the highest profit from wholesalers (interdistrict/cities/provinces) of 1,235,000 IDR/head, while the lowest profit comes from the small-sized enterprises (inter-village/sub district) of 1,100,000/head.

3.3 Farmer's Share

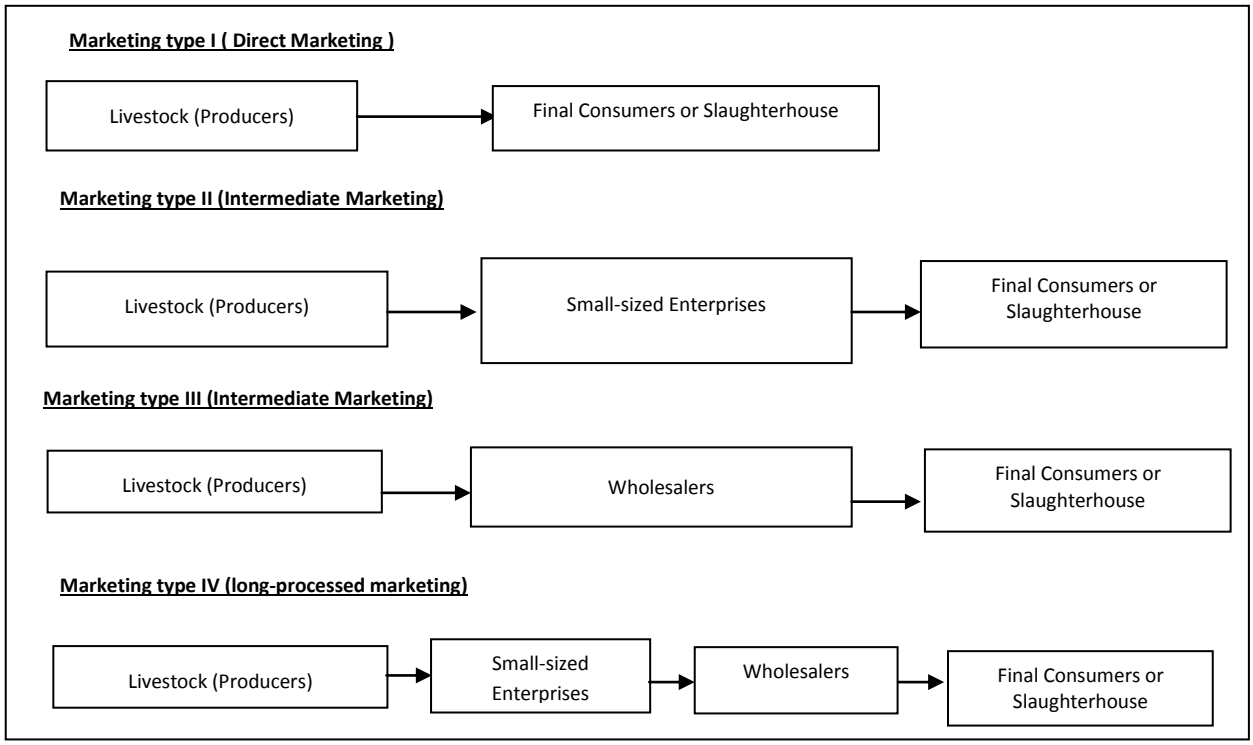
The highest farmer's share is on marketing type I (direct marketing) of 100% which means that the producers receives 100% of the profit paid by the final consumers of 14,500,000 IDR/head, while the lowest farmer's share is on marketing type IV (long-processed marketing) of 79.4% meaning that the farmers receive 79.4% of price paid by the final consumers of 17,000,000 IDR/head.

Based on the calculation of marketing type I, the value of marketing efficiency is 0%. On marketing type II, the value of marketing efficiency is 2.67%; meanwhile the value of marketing efficiency on marketing type III is 4.78%. On marketing type IV, the value of marketing efficiency is 6.85%. The result of marketing efficiency on "Tirto Sari" livestock at Sub district of Samboja, District of Kutai Kartanegara indicates that each marketing type is considered to be efficient ranging from 0 – 33%.

IV. RESULT AND DISCUSSION

The result indicates that there are four marketing varieties of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara: direct marketing of marketing type I, intermediate marketing of marketing type II and III and long-processed marketing of marketing type IV. The marketing varieties can be seen in Table 1. The marketing agencies in the marketing process of beef cattle are livestock, small-sized enterprises, wholesalers, final consumers or slaughterhouse.

TABLE 1
THE MARKETING VARIETIES OF BEEF CATTLE ON "TIRTO SARI" LIVESTOCK AT SUB DISTRICT OF SAMBOJA, DISTRICT OF KUTAI KARTANEGARA



The value of marketing margin at the level of producers, consumers and farmer's share obtained on each marketing type of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara can be seen in Table 2. The The analysis of marketing efficiency on each marketing type can be seen in Table 3.

TABLE 2
MARKETING MARGIN AND FARMER'S SHARE OF BEEF CATTLE ON "TIRTO SARI" LIVESTOCK AT SUB DISTRICT OF SAMBOJA, DISTRICT OF KUTAI KARTANEGARA, EAST KALIMANTAN.

Marketing	Type I	Type II	Type III	Type IV
Marketing Margin (%)	0	6,67	12,5	20,58
Farmer's share (%)	100	93,3	87,5	79,4

TABLE 3
ANALYSIS OF MARKETING EFFICIENCY OF BEEF CATTLE MARKETING ON "TIRTO SARI" LIVESTOCK AT SUB DISTRICT OF SAMBOJA, DISTRICT OF KUTAI KARTANEGARA, EAST KALIMANTAN

Marketing	Type I	Type II	Type III	Type IV
Marketing Efficiency (%)	0	2,67	4,78	6,85

V. CONCLUSION

The result of marketing efficiency calculation shows that each marketing type of beef cattle on "Tirto Sari" livestock at Sub District of Samboja, District of Kutai Kartanegara is considered to be efficient, ranging from 0 – 33%. After the calculation of marketing margin and farmer's share, the most efficient marketing type is marketing type I (direct marketing), showing

lowest value of marketing margin and highest value of farmer's share. It is, then, followed by marketing type II (intermediate marketing), marketing type III (intermediate marketing), and marketing type IV (long-processed marketing). The research of analysis of marketing efficiency of beef cattle is useful for farmer as additional information through which marketing type the most efficient.

REFERENCES

[1] Department of Agriculture and Animal Husbandry of Kutai Kartanegara Regency. 2017. List of Animal Health Service Report of UPT Animal Health Center of Samboja Sub-district 2017.

[2] Animal Husbandry and Animal Health Office of Kutai Kartanegara Regency. 2016. Data of Cattle Farms Region in Kutai Kartanegara Regency Year 2016.

[3] East Kalimantan Provincial Livestock Service Office. 2015. Livestock Population by Regency / City in 2010-2014.

[4] Saragih, B. 2000. Agribisnis Berbasis Peternakan. Uses Foundation and Center for Development Studies Bogor Agricultural University. Bogor.

[5] Soekartawi. 1997. Analisis Fungsi Produksi, Rajawali Pers, PT. Raja Grafindo Persada. Jakarta

[6] Winartha, I.M. 2006. Metode Penelitian Sosial Ekonomi. CV. Andi Offset. Yogyakarta.

[7] Dahl D.C and J.W. Hammond. 2010. Market and Price Analysis The Agricultural Industry. Mc. Grawhill Book Company. New York.

Effects of Fermentation of Cashew Kernel on the Nutrient Value of Cassava Semolina Flour (Attieke)

Salimata KONE[1], Doudjo SORO[2], Soronikpoho SORO[3], Ernest Kouadio KOFFI[4]

[1,3,4]Laboratory of Biochemistry and Sciences of Food, Training and Research Unit Biosciences, Felix Houphouët-Boigny University of Abidjan, UFHB 01 BP V 34 Abidjan, Ivory Coast.

[2]Sciences of the Food and Environmental Chemical Processes, Polytechnic National Institute, Felix Houphouët-Boigny (INP-HB), BP 1313 Yamoussoukro, Ivory Coast.

Abstract— *Protein-energy malnutrition in children is a public health problem. This nutrition problem is attributed to inappropriate complementary feeding. Indeed, the cost of high-quality food supplements is high and traditional food supplements have a low nutritional quality related to the presence of antinutritional factors. The objective of this study is to determine acceptability and antinutritional factors in attiéké / cashew kernel composite flours. The cashew kernel flour is produced after various technological treatments to obtain two types of flour (unfermented flour and fermented flour). Physico-chemical and sensory analyzes are performed. The results showed that fermentation has an influence on the parameters studied. The protein contents of the unfermented formulations range from 7.53% to 10.62% while those of the fermented formulations range from 8.23% to 11.53%. Both formulations contain antinutritional factors.*

Keywords— cashew kernel, attiéké, antinutritional factors, nutritional quality.

I. INTRODUCTION

Breast milk is considered the best food for the newborn. It provides children from zero to four months with all the essential elements in balanced proportions. It also protects them against infections. Beyond this age, breast milk is no longer sufficient to fully cover energy and protein requirements (WHO, 1988). For example, the WHO recommends that mothers feed their babies exclusively for up to 4-6 months and continue breastfeeding as much as possible for up to two years, while gradually introducing other foods including those from the family dish. It is at this time that infant malnutrition takes place, which is due to the different complementary foods given to infants. These foods are usually made from cereal flour, tubers and roots, which are high in carbohydrates and low in protein.

It is clear that in the countries of the third world, there are crops that produce foods with high calorific value and protein. Thus several attempts are made to improve the nutritional quality of flours used as complementary foods. Enrichment is defined as the addition to a food of one or more essential nutrients, normally contained or not in the food, with the objective of preventing or correcting a deficiency of one or more nutrients, within a population or specifically vulnerable population groups (FAO / WHO, 1994). Fermentation also reduces antinutritional factors that affect the bioavailability of minerals (Yao, et al., 2009). It improves the organoleptic qualities of food and limits the development of pathogenic microorganisms and / or degradation (Caplice and Fitzgerald, 1999), through the acidification of the environment. This study aims to determine the effect of enrichment and fermentation of cashew kernel meal on attiéké flour

II. MATERIAL AND METHODS

2.1 Biological Material

The plant material used in this study consists of cassava semolina (attiéké) purchased in the village of Djahakro (Yamoussoukro, Ivory Coast) from three producers, and cashew kernel flour obtained after various treatments of cashew nuts. Two (2) commercial flours (Blédine corn and FARINOR potato) are used as controls**.**

2.2 Attiékéflour production

The attieke collected from the producers is dried in an oven at 60°C for 24 hours and crushed using a grinder. The flour obtained was kept in polyethylene bags.

2.3 Production of deoiled and unfermented cashew kernel flour

The cashew kernels are obtained after dehulling, drying and pruning cashews. The cashew kernel flour is produced by the method described by Sze-Tao and Sathe, 2004 modified. The dried almonds were crushed using a semi-artisanal grinder and placed in a stainless steel tank. Hexane was added 1: 1 (w / v) to the flakes for oil extraction. The mixture was macerated for 30 minutes before being heated at 130 ° for 50 minutes and allowed to stand for 24 hours at room temperature. Then the pellet is separated from the supernatant (oil and hexane). The operation is performed twice. The cakes are pressed for 24 hours to extract the rest of the oil. The deoiled cake was oven dried at 70°C for 12 hours. They are milled in a mill and the flour obtained was stored in polyethylene bags.

2.4 Production of fermented deoiled cashew kernel flour

The cashew kernels were fermented according to the method of Ijarotimi et al., (2012) modified. The almonds are boiled at 100 ° C for 1 hour. The boiled almonds are wrapped in the plantain leaf for 72 hours for fermentation. The fermented seeds are oven dried at 60 ° C for 48 hours. The fermented almonds are crushed and the hexane added (confers the production of unfermented flour). The cakes are ground in a grinder and packaged in polyethylene bags

2.5 Formulation of infant flours: attiéké / cashew kernel

Attiéké / cashew kernel composite flours were obtained by incorporating respective proportions of 0, 10, 15 and 20% of cashew kernel meal (fermented and unfermented) into attiéké flour. Each formulation was thoroughly mixed in a blender, then divided into 250 g fractions in polyethylene plastic bags and stored for analysis.

2.6 Physicochemical composition

About 0.1 g of attiéké-almond cashew meal is used to determine crude protein levels from the total nitrogen assay using the Kjeldhal method (AOAC, 1990). The protein level was obtained by multiplying the total nitrogen content by a factor of 6.25 convention.

The total phenols are extracted according to the AOAC Method (Christensen, 1974) using the FolinCiocalteu reagent. For extraction, 1 g of flour is placed in a beaker into which are added 100 ml of oxalic acid at 0.3% (g / v). The mixture is stirred with a magnetic stirrer for 30 minutes the whole is centrifuged at 3000trs for 15 minutes. Then the extract is filtered on Whatman paper No. 41. For the assay, 1 ml of the extract diluted in 8 ml of distilled water is added to a tube, plus 0.5 ml of diluted FolinCiocalteu reagent (1 ml). / 10), and 1.5 ml of sodium carbonate solution (7.5%). The mixture is left in the dark for 1 hour at room temperature. The reading is made at an absorption of 765 nm at the spectrophotometer. A standard range is made with gallic acid at different concentrations (0 to 1 mg / ml).

The tannins are determined according to the method described by Ba et al., 2010). One (1) mL of methanolic extract is introduced into a test tube. To the contents of the tube are added 5 ml of vanillin reagent at 1% (w / v). The tube is left standing for 30 min in the dark and the optical density (OD) is read at 500 nm against a blank. The amount of tannin in the samples is determined using a standard range established from a stock solution of tannic acid (2 mg / mL) carried out under the same conditions as the test.

Phytates were quantified according to the method of Latta and Eskin (1980) based on the discoloration of the Wade reagent by phytates. The oxalate assay is performed according to the method described by Day and Underwood (1986) using potassium permanganate. One (1) gram of oven-dried and ground sample is homogenized in 75 mL of 3M sulfuric acid. The mixture is magnetically stirred for 1 hour and then filtered. Twenty-five (25) mL of the filtrate is heat-titrated with 0.05M potassium permanganate solution until a steady pink turn. Hydrocyanicacid contents were determined by the AOAC method (1990). A quantity of 10 g of sample was milled in a distillation flask and 200 ml of distilled water added. Distil the mixture for 3 hours by trapping the distillate in a 250 mL receiver flask containing 50 mL of 2.5% NaOH and make up the distillate with distilled water to the mark. 100 ml of the mixture were taken and 2 ml of 5% KI are added and titrated with a solution of 0.02M $AgNO_3$, until cloudiness appears.

2.7 Swelling

The swelling was performed by the method of Okezie and Bello, 1988. A well-defined volume (10 mL) of each flour sample was introduced into a graduated cylinder (initial volume). 50 ml of distilled water were introduced into the test tube. The volume occupied by the swollen flour (final volume) was read after 30 min. The rate of swelling is given by the following formula:

$$Tg = \frac{\text{final volume}}{\text{initial volume}} X\ 100$$

2.8 Sensory Analysis

2.8.1 Preparation of the Porridges

The porridges were cooked by introducing 50 g of flour into 300 ml of drinking water. The cooking lasted 6 minutes over a low heat. Then 6% sugar was added at the end of cooking. The slurries were cooled to room temperature in the preparation room before being served.

2.8.2 Acceptability Test

The panel was formed with 60 untrained people recruited on the basis of their availability. The coded (three-digit) coded porridge samples were presented to each panelist in a randomized order. The pleasure perceived by each panelist was scored on a nine-point hedonic scale. Notes ranging from (9) nine (extremely pleasant) to (1) one (extremely unpleasant) were assigned to the different modalities of the scale (Meilgaard et al., 1999).

2.8.3 Descriptive Analysis

The method consists of evaluating and quantifying the appropriate descriptors (odor, taste, color and texture) according to a category scale. A panel of 15 people was recruited and trained in the analysis methodology. Then, he became familiar with the groceries produced by the commercial infant flours (BLEDINE Corn and FARINOR Potato). These porridges were coded (with three digits). The various slurries of attiéké flour enriched at 0%, 10%, 15% and 20% of the fermented cashew kernel flour were presented simultaneously in a random order and then the attiéké meal mixes enriched at 10% 15%, 20% of the unfermented cashew kernel meal was presented simultaneously in a randomized order.

2.8.4 Statistical Analysis

Results are expressed as mean ± standard deviation from triplicate measurements. The non-parametric test of Duncan is used to analyze the difference between the means at 5% risk, using Statistica 7.1 software ANOVA analysis of variance method.

III. RESULT

3.1 Physicochemical and functional composition

Table 1 show the results in protein, swelling and antinutrients. Protein content, flour FCNF and FCF respectively have the highest values (24.57%, 33.77%). The lowest value is observed with FA flour (2.97%). An increase in the level of protein was observed with the rate of incorporation of the different cashew kernel flours but these contents are higher with the incorporation of fermented cashew kernel flour.

Polyphenol values range from 104.66 mg GAE / 100 g DM for FA flour to 631 mg GAE / 100 g DM for FCF flour. The values of the different formulations are higher than that of the commercial meal BM (104, 26 mg GAE / 100g MS) and those of the fermented formulations are the highest.

Incorporation of unfermented cashew flour increases the rate of swelling (192.33 ± 2.51% for FAFCNF10 to 214.33 ± 4.04% for FAFCNF20) significantly ($P < 0.05$). However, the opposite is observed with fermented cashew flour where the rate of swelling decreases (217.33 ± 2.51% for FAFCF10 to 158.00 ± 2.64% for FAFCF20) with the rate of incorporation. BM shows the highest value (433.66 ± 3.21%) compared to the different formulations. All the values of the different formulations are lower than that of attiéké flour (298.33 ± 2.88%) thanks to its high carbohydrate content.

3.2 Antinutritional Factors

Tannin values range from 10.07 mg / 100g (FCF) to 28.83 mg / 100g (FCNF). The values of the formulations with the fermented cashew meal are lower than those of the unfermented cashew flour formulations. All these values are lower than that of commercial flour BM (39.29 mg / 100g).

Fermentation decreases the content of oxalates in flours. The contents of the fermented formulations are between 73.99 mg / 100g for the flour FAFCF10 and 140mg / 100g for the flour FCF. As for unfermented formulations, the values vary from 198 mg / 100g for FCNF flour to 76mg / 100g for FAFCNF10 flour. These are superior to that of commercial flour.

Phytate results ranged from 72.72 mg / 100g (FA) to 23.74 mg / 100g (FAFCF20). These levels decrease with the incorporation rate of different cashew kernels. Sample values with fermented cashew kernel meal are significantly ($P < 0.05$) lower than samples with unfermented cashew kernel meal.

The different formulations have a low content of hydrocyanic acid. Hydrocyanicacidvalues range from 0.27 mg / 100g to 1.08 mg / 100g. All show significant differences ($P < 0.05$).

3.3 Sensory Analysis

3.3.1 Acceptability of the porridges

The overall acceptability of the porridge presented in Table 2 shows that the porridge were variously appreciated. unfermented attiéké-almond composite meal mixes (score greater than 6) are more popular than the attiéké-fermented cashew almond meal mixes (note 5 except FAFCF15 with a score of 6). The porridge of attiéké flour enriched in 10% of unfermented cashew kernel flour (7.35) is the most popular of the unfermented formulations, commercially flours (BLEDINE Mais and FARINOR) and attiéké flour. The attiéké flour mixture enriched with 15% fermented cashew kernel flour is the most popular fermented formulation.

3.3.2 Sensory profiles

The sensory profiles of the unfermented -attiéké- cashews kernel flour (FAFCNF10, FAFCNF15 and FAFCNF20) and commercial flours are presented in Figure 1. The porridge based on fermented attiéké- cashews kernel meal (FAFCF10, FAFCF15 and FAFCF20) and commercial flours are shown in Figure 2. Figures 1 and 2 show that the different descriptors taste, texture, creamy appearance influenced the acceptability of the most popular porridge (FAFCNF10 and FAFCF15).

TABLE 1
PHYSICOCHEMICAL AND FUNCTIONAL COMPOSITION

Samples	Protein%	Swelling%	Polyphenolsmg EAG / 100g	Tannins mg/100g	Oxalates mg/100g	Hydrocyanic acidmg/100g	Phytates mg/100g
FA	2,97 ± 0.17^{a}	298,33±2.88^{e}	104, 66±0,7^{a}	27,65±00^{h}	88,00±0^{d}	1,08±0^{J}	72,72±0,48^{i}
FCNF	24,57 ± 0.3^{i}	-	521, 33±0,7^{h}	21,17±0,08^{d}	198,66±1,15^{i}	0,31±0,02^{b}	56,50±0^{h}
FAFCNF10	7,53 ± 0.10^{c}	192,33±2.51^{c}	210,42±0,14^{b}	20,85±0,08^{c}	76,66±0,57^{c}	0,86±0,02^{i}	49,50±0,15^{g}
FAFCNF15	9,16 ± 0.09^{e}	197,33±2.51^{c}	250,57±0,7^{c}	25,53±0,16^{g}	99,33±0,57^{e}	0,68±03^{g}	35,92±0,47^{d}
FAFCNF20	10,62 ± 0.32^{g}	23,42 ± 0,46^{a}	312,55±0,5^{d}	28,83±0,16^{i}	110,33±0,57^{g}	0,45±0,01^{d}	31,25±0,9^{C}
FCF	33,77 ± 0.17^{j}	-	631,52±0,7^{i}	10,74±0,19^{a}	140,00±0^{h}	0,27±0^{a}	45,50±0,61^{e}
FAFCF10	8,23 ± 0.20^{d}	217,33±2.51^{d}	319,28±0,6^{e}	19,29±0^{b}	73,66±0,22^{b}	0,80±0,04^{h}	25,24±0,42^{b}
FAFCF15	9,98 ± 0.10^{f}	167,66±2.51^{b}	416,71±0,1^{f}	22,54±0,11^{e}	88,66±0,19^{d}	0,58± 0,01^{f}	24,49±0,72^{ab}
FAFCF20	11,53 ± 0.28^{h}	158,00±2.64^{a}	421, 10±0,7^{g}	24,74±0,39^{f}	104,66±0,5^{f}	0,41±0^{c}	23,74±0,70^{a}
BM	5,40 ± 0.09^{b}	433,66±3.21^{f}	104,26±0,14^{a}	39,29±0,12^{j}	33,33±0,47^{a}	0,54±0^{e}	47,39±0,31^{f}

Values are mean ± standard deviation of three measurements (n = 3). The same letter in the same row index indicates that there is no significant difference between samples for the parameter concerned (p<0.05).). According to Duncan's test.
FA :** Attiéké Flour ; **FAFCNF10 :**Attiéké Flour enriched with 10% unfermented cashew kernel flour ; **FAFCNF15 :** Attiéké Flour enriched with 15% unfermented cashew kernel flour ; **FAFCNF20 :** Attiéké Flour enriched with 20% unfermented cashew kernel flour ; **FAFCF10 :** Attiéké Flour enriched with 10% fermented cashew kernel flour ; **FAFCF15 :**Attiéké Flour enriched with 15% fermented cashew kernel flour ; **FAFCF20 :** Attiéké Flour enriched with 20% fermented cashew kernel flour ; **BM: Commercial infant flour BLEDINE corn

TABLE 2
OVERALL ACCEPTABILITY OF DEHYDRATED SEMOLINA CASSAVA / CASHEW KERNEL FLOUR AND CONTROL FLOURS

Flours	ACCEPTABILITY
Unfermented flour	
FA	6,63±1,79[c]
FAFCNF10	7,35±1,4[d]
FAFCNF15	6,64±1,54[c]
FAFCNF20	6,22±1,84[b]
BM	5,64±1,70[b]
FP	5,00±1,52[a]
Fermented flour	
FA	6,63±1,79[b]
FAFCF10	5,67±1,74[b]
FAFCF15	6,53±0,95[c]
FAFCF20	5,89±1,35[b]
BM	5,64±1,70[b]
FP	5,00±1,52[a]

Values are mean ± standard deviation of three measurements (n = 3). The same letter in the same row index indicates that there is no significant difference between samples for the parameter concerned (p<0.05).). According to Duncan's test.
FA :** Attiéké Flour ; **FAFCNF10 :**Attiéké Flour enriched with 10% unfermented cashew kernel flour; **FAFCNF15 :** Attiéké Flour enriched with 15% unfermented cashew kernel flour; **FAFCNF20 :** Attiéké Flour enriched with 20% unfermented cashew kernel flour; **FAFCF10 :** Attiéké Flour enriched with 10% fermented cashew kernel flour ; **FAFCF15 :**Attiéké Flour enriched with 15% fermented cashew kernel flour ; **FAFCF20 :** Attiéké Flour enriched with 20% fermented cashew kernel flour; **BM: Commercial infant flour BLEDINE corn;

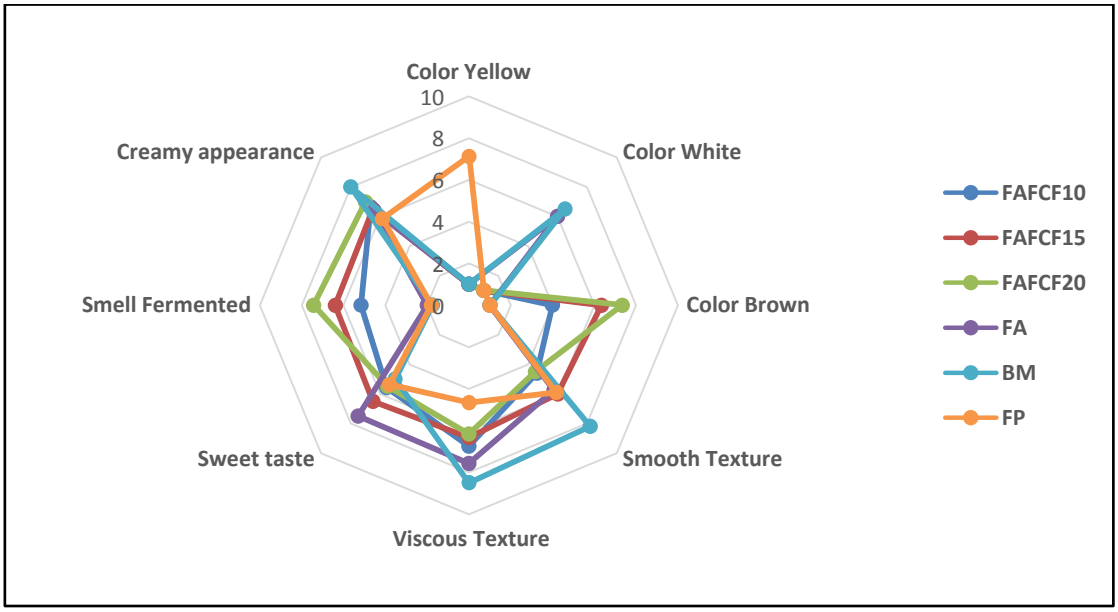


FIGURE 1: SENSORY PROFILES OF COMPOSITE MEAL MIXES OF ATTIÉKÉ / FERMENTED CASHEW KERNEL AND COMMERCIAL FLOURS

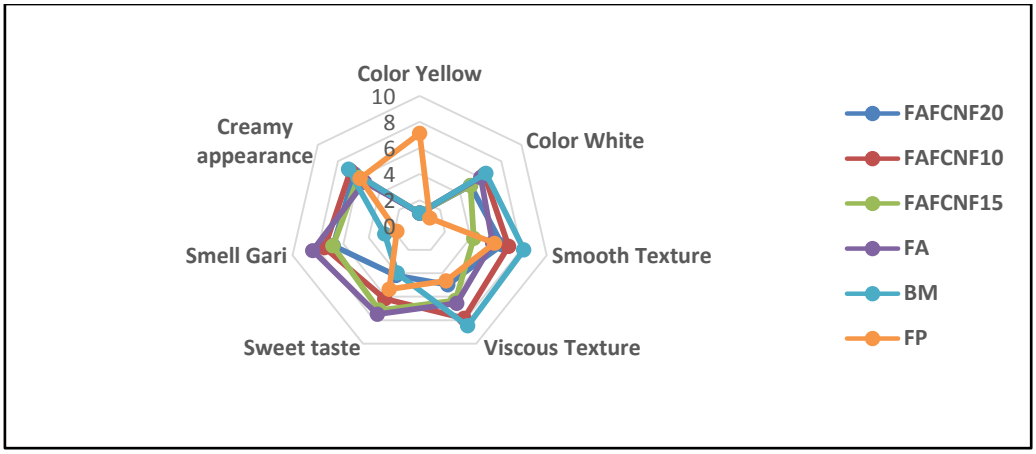


FIGURE 2: SENSORY PROFILES OF ATTIÉKÉ / UNFERMENTED CASHEW KERNELS AND COMMERCIAL FLOURS

IV. DISCUSSION

An increase in protein level was observed with the incorporation rate of fermented and unfermented cashew kernel meal. These results are in agreement with those found by Fofana et al. (2017) for the fortification of plantain flour with cashew kernel meal. However, fermented cashew kernel flour has the highest rate. This may be due to the proteolytic activities of the enzymes produced by the microorganisms during fermentation (Amankwah et al., 2009, Ojokoh et al, 2013, Ojokoh et al, 2014). This increase has been shown by (Amankwah et al., 2009) for fermented maize meal and fermented millet meal (Sade, 2009).

When cashew kernel flour is added to attiéké flour, the swelling rate decreases. The swelling rate of attiéké flour with incorporation of fermented cashew kernel meal is lower than that of flour with incorporation of unfermented cashew flour. This is due to fermentation which allows the hydrolysis of starch into simpler compounds such as glucose, fructose, sucrose, maltose and dextrins. Thus, the reduced viscosity increases the energy and nutritional density of the pores (Nguyen et al., 2007b, Songré-Ouattara et al., 2009).

The polyphenol contents increase with the rate of incorporation of cashew kernel flour; with fermented formulations with higher values. These results are consistent with several studies that show that fermentation increases polyphenol levels (Dajanta et al., 2013, Guzmán-Uriarte et al., 2013, Plaitho et al., 2013). In fact, the phenolic compounds in the natural form are combined or linked to sugar; which reduces their availability in the body. During fermentation, the proteolytic enzymes in the body hydrolyze phenolic complexes to yield free and simple soluble phenols that are biologically more active and easily absorbed (Shrestha et al., 2010, Ademiluyi and Oboh, 2011). These high levels of polyphenol make it possible as an antioxidant to combat free radicals, cancer, cardiovascular diseases and the aging process (Saluanke, 2006).

The presence of antinutritional factors in flours limits the nutritional quality and digestibility of vitamins, minerals, proteins and carbohydrates by preventing their proteolytic and amylolytic activities. The operations used for the production of flours make it possible to reduce these antinutritional factors, notably fermentation, which reduces them to more than 60%. The values obtained in tannin in this study are higher than that of Magdi, (2010) for the fermentation of millet. The decrease in tannin content was observed by Abdel-Haleem et al., 2008 for the fermentation of sorghum. This decrease may be due to the action of microorganisms on phenoloxidase. The low total oxalate content obtained after fermentation is due to the activity of microorganisms. During fermentation, the reduction in pH results in the separation of oxalate salts to give calcium ions and soluble oxalates (Simpson et al., 2009). These are used by bacteria as sources of energy. This decrease in oxalate content is shown by Wadamori, et al (2014) for fermented kimchi. The reduction in the phytate content of the various flours is due to the operations used for the production of flours (drying, cooking, fermentation). Fermentation reduces phytates by 90%. During fermentation, the decrease in pH is likely to increase the enzymatic activity of phytases for phytate degradation. PH is the influencing factor of phytate reduction (Buddrick et al., 2013). Thus the reduction of phytates could increase the mineral content and their bioavailability. The fermentation of the cassava paste for the production of attiéké causes a decrease in the content of hydrocyanic acid. In fact, linamarin is converted into hydrocyanic acid by linamarase. This has an optimal activity at pH 6 (Askurrahman 2010, Diallo et al., 2013). During fermentation microorganisms break down toxins into organic acids, leading to a decrease in pH and inactivation of linamarase. Fermentation therefore reduces the level of hydrocyanic acid in flours (Gunawan et al., 2015). Fermentation is an effective process for reducing antinutritional factors in flours.

The porridge of Attiéké-cashew kernels flour fermented are less acceptable than unfermented cashew kernelflour. This result is due to the aroma developed during fermentation. The attiéké flour mixture enriched with 15% fermented cashew kernel flour is the most popular fermented formulation with a score of 6.53. This result is consistent with that of (Ijarotimi et al., 2012) for porn corn enriched with fermented cashew kernel flour (6,50). The attiéké flour mixture enriched with 10% unfermented cashew kernel flour is more popular than attiéké flour. This result agrees with those of Achi (1999) which shows enrichment as a method of improving the nutritional and organoleptic quality of infant foods. These results are influenced by smell, taste, viscosity and texture.

V. CONCLUSION

Antinutritional factors in the fermented formulations are lower than those of the unfermented formulations. This could increase the digestibility of the nutrients in these flours. However, the boils of fermented flours are less appreciated than the boiled flours of unfermented flours because of the very strong fermented odor. So enrichment and fermentation are strategies for improving the quality of food. These attiéké / cashew almond composite flours can be used in children's diets.

REFERENCES

[1] Abdel-Haleem, W.H., El Tinay, A.H., Mustafa, A.I., Babiker, E.E., 2008. Effect of fermentation, malt-pretreatment and cooking on antinutritional factors and protein digestibility of sorghum cultivars. Pakistan J. Nutr. 7, 335–341.

[2] Achi O K., 1999. Quality attributes of fermented yam flour supplemented with processed soy flour. Plant food for Human Nutrition.54: 151-158.

[3] Ademiluyi, A.O.et Oboh, G.,2011. Antioxidant properties of condiment produced from fermented bambara groundnut*(Vignasubterranean*L. Verdc). *Journal of FoodBiochemistry*35: 1145 –1160.

[4] AOAC, 1990. The Official Methods of Analysis of the Association of Official Analytical Chemists (15th ed.). Washington DC, 1-230p.

[5] Amankwah E, Barimah J, Acheampong R, Addai L, Nnaji C.,2009. Effect of fermentation and malting on the viscosity of maize-soyabean weaning blends. Pak. J. Nutr. 8:1671-1675.

[6] Askurrahman. 2010. Isolation dan characterization of linamarase produced from cassava roots (Manihotesculentacrantz). Agrointek4 : 138 – 145.

[7] Ba K., Tine E., Destain J., Cissé N., Thonart P., 2010. Etude comparative des composés polyphénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt. *Biotecnology, Agronomy, Social and envioronmental Sciences*, vol. 14, pp. 131-139.

[8] Buddrick Oliver, Jones Oliver A.H., Hugh J. Cornell, Darryl M. Small, 2013. The influence of fermentation processes and cereal grains in whole grain bread on reducing phytate content. Journal of Cereal Science, 59, 3-8.

[9] Caplice E., Fitzgerald G.F., 1999**.** Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology 50, 131-149.

[10] Dajanta, K., Janpum, P. and Leksing, W., 2013. Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by Bacillus subtilis: A comparative study of Thaifermented soybeans (thuanao). International Food Research Journal 20(6): 3125-3132.

[11] Day RA and AL Underwood, 1986Qualitative Analysis, 5th Ed. Prentice-Hall Publications, New Delhi, India, p.701.de Lumen, B.O., Becker, R., Reyes, P.S., 1986. Legumes and a cereal with high methionine/cystine contents. *Journal of Agric. Food Chem.* **34**:361-364.

[12] Diallo Younoussa, GueyeMomarTalla, Sakho Mama, DarbouxPraxèdeGbaguidi, Kane Amadou, Barthelemy Jean-Paul, Lognay Georges, 2013. Importance nutritionnelle du manioc et perspectives pour l'alimentation de base au Sénégal (synthèse bibliographique). Biotechnol. Agron. Soc. Environ. 17(4), 634-643.

[13] Guzmán-Uriarte, M.L., Sánchez-Magaña, L.M., Angulo-Meza,G.Y., Cuevas-Rodríguez, E.O., GutiérrezDorado, R., Mora-Rochín, S., Milán-Carrillo, J., Valdez-Ortiz, A. and Reyes-Moreno, C., 2013. Solid state bioconversion for producing common bean (Phaseolusvulgaris L.) Functional flour with high antioxidant activity and antihypertensive potential. Food and Nutrition Sciences 4: 480 – 490.

[14] FOFANA Ibrahim, SORO Doudjo, YEO Mohamed Anderson, KOFFI Kouadio Ernest, 2017.Influence de la fermentation sur les caractéristiques physicochimiques et sensorielles de la farine composite à base de banane plantain et d'amande de cajou

[15] Gunawan, S., Widjaja, T., Zullaikah, S., Ernawati, L., Istianah, N., Aparamarta, H.W. Prasetyoko, D., 2015. Effect of fermenting cassava with Lactobacillus plantarum, Saccharomyces cereviseae, and Rhizopusoryzae on the chemical composition of their flour. International Food Research Journal 22(3): 1280-1287.

[16] Ijarotimi OS, Oluwalana IB, MO Ogunedojutimi, 2012. Nutrient composition, functional, sensory and microbial status of popcorn-based (zea may everta) complementary foods enriched with cashew nut (Anacardiumoccidentale L.) flour. AJFAND, volume 12, N°5, 6424-6446.

[17] Latta, M., Eskin, M., 1980. A colorimetric method, for phytate determination. *J. Agric. Food. Chem.***28**, 1313–1315.

[18] Magdi A. Osman, 2010. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. Journal of the Saudi Society of Agricultural Sciences, 1-6.

[19] Meilgaard, Civille GV et BT Carr. 1999. Sensory Evaluation Techniques. *3rd Edn., CRC Press*, New York.

[20] Nguyen T.T.T., Loiseau G., Icard-Verniere C., Rochette I., Treche S., Guyot J.P., 2007b. Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries : A new method for preparing high energy density complementary foods for young children. Food Chemistry 100, 623-631

[21] Ojokoh AO, Daramola MK, Oluoti O.J., 2013. Effect of fermentation on nutrient and anti-nutrient composition of breadfruit (Treculiaafricana) and cowpea (Vignaunguiculata) blend flours. Afr. J. Agric. Res. 8:3566-3570.

[22] Ojokoh AO, Fayemi EO, Ocloo FCK, Alakija O., 2014. Proximate composition, antinutritional contents and physicochemical properties of breadfruit (Treculiaafricana) and cowpea (Vignaunguiculata) flour blends fermented with Lactobacillus plantarum. Afr. J. Microbiol. Res. 8:1352-1359.

[23] Okezie O. B.et Bello A. B. 1988. Physicochemical and functional properties of winged beans flour and isolate compared with soy isolate. Journal of Food Science, **53**(**2**): 450-454.

[24] OMS/UNICEF. 2003. Alimentation complémentaire des jeunesenfantsdansles pays endéveloppement. OMS: Genève; 130-131.

[25] Plaitho, Y., Kangsadalampai, K. and Sukprasansap, M., 2013. The protective effect of Thai fermented pigmented rice on urethane induced somatic mutation and recombination in Drosophila melanogaster. Journal of Medicinal Plants Research 7(2): 91 – 98.

[26] Sade FO (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (Pennisetumglaucum). J. Food Technol. 7:92-97.

[27] Salunke, B.K., 2006. Anti-nutritional constituents of different grain legumes grown in North Maharastra. J. Food Sci. 43(5): 519-521.

[28] Shrestha, A.K., Dahal, N.R. and Ndungustse, V., 2010. Bacillusfermentation of soybean: A review. Journal of Food Science andTechnologyNepal 6: 1 – 9.

[29] Simpson, T.S.; Savage, G.P.; Sherlock, R.; Vanhanen, L.P., 2009. Oxalate content of silver beet leaves (Beta vulgaris var. cicla) at different stages of maturation and the effect of cooking with different milk sources. J. Agric. Food Chem., 57, 10804–10808.

[30] Songré-Ouattara L., Mouquet-Rivier C., Icard-Vernière C., Rochette I., Diawara B., Guyot J.P., 2009. Potential of amylolytic lactic acid bacteria to replace the use of malt for partial starch hydrolysis to produce African fermented pearl millet gruel fortified with groundnut. International Journal of Food Microbiology 130, 258-264.

[31] Swain T and WE Hillis The phenolic constituents of Prunus.domestica. I The quantitative analysis of phenolic constituents. J. Sci. Fd. Agric. 1959; 10: 63 – 68.

[32] Sze-Tao KWC and SK Sathe.(2004). Functional properties and in-vitro digestibility of Almond (Prunusdulcis L) protein isolate. *Food chemistry***69**: 153- 160.

[33] Wadamori Yukiko, Vanhanen Leo et Savage Geoffrey P., 2014. Effect of Kimchi Fermentation on Oxalate Levels in Silver Beet (Beta vulgaris var. cicla). Foods, 3, 269-278

[34] WHO. Codex Alimentarius. 2nd edition. 1994;4.

[35] Yao A.A., Egounlety M., Kouamé L.P. etThonartPh.,2009.Lesbactéries lactiques dans les aliments ou boissons amylacés et fermentés del'Afrique de l'Ouest : leur utilisation actuelle. Ann. Méd. Vét., 153, 54-65.

Treatment of piggery wastewater through struvite precipitation and nitrogen removal bacteria and poly-phosphate bacteria (in-pots experiment)

Nguyen Thi Xuan My[1], Le Van Hien[2], Dao Thi Minh Chau[3], Cao Ngoc Diep[4]

Department of Microbiology Biotechnology, Can Tho University, VIET NAM

***Abstract*—** *Piggery wastewater is a type of wastewater which contains large amounts of nitrogen and phosphorus, therefore it needed to be treated before releasing to directly to the environment. The combination between struvite precipitation and nitrogen removal and poly-P bacteria into wastewater for piggery wastewater treatment has been found to be a cost-effective practice, a iable technology in terms of environmental protection and sustainability, especially in the developing-countries. For optimum struvite crystallization from piggery wastewater, the Mg:PO4 molar ratio as (1.2:1) was used, the pH of reaction was adjusted to 9 and the sample was stirred continuously during 40 minutes. The supernatant sample was then added 1% nitrogen removal bacteria (Pseudomonas stutzeri D3b strain) and 1% poly-P bacteria (Kurthia sp. TGT1013L strain), 5 g glucose/L and aeration 12/24h during 3 days, ammonium concentration reduced significantly from 1271 mg/L to 1.2 mg/L and orthophosphate concentration decreased noticeably from 24.91 mg/L to 16.1 mg/L.*

***Keywords*— ammonium, bacteria, orthophosphate, pH, piggery wastewater, struvite precipitation.**

I. INTRODUCTION

Wastewaters contain a high amount of organic matter, nitrogen and phosphorus [1], a considerable amount of Mg [2], different macro and micro elements [3], and heavy metals [4,5,6] due to which it is considered as one of the major polluting agents discharged into the environment. The livestock waste stream is therefore, very rich in phosphorus. A significant amount of nitrogen comes out through excreta as a residue of protein supplement as well as dead animals. Improper management of livestock waste creates a nuisance and obnoxious environment which also greatly affect on public health. Some forms of nitrogen (ammonia, nitrite, and nitrate) and phosphorus (orthophosphate and monophosphate) produce toxicity in the water and affect on aquatic life. It is obligatory to remove nitrogen and phosphorus from wastewaters before discharging it into the water stream to create an eco-friendly, pollution-free environment. So, many countries are paying attention to water pollution resulted from wastewaters, and have tightened legislation and discharging standards.

Great efforts have been done by researchers for the removal of nitrogen from wastewater through biological nitrification and denitrification [7], ammonia-stripping [8], electrochemical conversion [9], ion exchange [6][10], microwave irradiation 11] and struvite precipitation [12].

Wastewater that contains high concentration of N, and P is an effective source of struvite recovery. In recent years, struvite has been recovered from different types of wastewaters, such as swine wastewater [1][2][13][14], calf manure wastewater 15], leather tanning wastewater [16], sewage sludge [17], dairy wastewater [18], wasted sludge [19], digester supernatant [20][21], industrial wastewater [22], municipal land fill leachate [23], lagoon wastewater [24], poultry manure wastewater [25], agro-industrial wastes [26], slaughterhouse wastewater [27], anaerobic digester effluents [28], synthetic wastewater [29], slurry type swine wastewater [23], animal manure [30], urine [31] and fertilizer plant wastewater [32]. Recently, struvite has been produced from wastewaters using microbial fuel cells by Ichihashi and Hirooka [33]. Struvite is a crystalline substance consisting of magnesium, ammonium and phosphorus in equal molar concentrations ($MgNH_4PO_4$-$6H_2O$). The crystals form in an alkaline condition according to the reaction shown below [34].

$$Mg_2^+ + NH_4^+ + H_2PO_4 + 6H_2O \longrightarrow MgNH_4PO_4^- + 6H_2O + 2H_2$$

This study investigates the following steps: (1) struvite pretreatment of raw piggery wastewater (after biogas), (2) applying nitrogen removal bacteria and poly-P bacteria to remove N and P out wastewater. The objectives of this study were: (1) to investigate the effects of pH and molar ratios for magnesium, and phosphate ions on ammonia N and phosphate P removal from raw piggery wastewater, (2) to apply nitrogen removal bacteria and poly-P bacteria to enhance the waste disposal process.

II. MATERIAL AND METHOD

The composition of raw piggery wastewater used in this study (collected from Pig farm, Binh Minh dist., Vinh Long province, Vietnam) was presented in Table 1.

TABLE 1
THE MAJOR COMPOSITION OF RAW PIGGERY WASTEWATER

Content	Concentration
pH	7.8
TSS (mg/L)	332
NH_4^+ (mg/L)	1271
PO_4^{3-} (mg/L) BOD_5 (mg/L)	50 640

2.1 Chemicals

Magnesium sulphate octahydrate ($MgSO_4.7H_2O$) and Calcium dihydrogen phosphate $Ca(HPO_4)2.H_2O$ were employed as magnesium and phosphate sources, respectively. pH was adjusted using 10 and 6 M sodium hydroxide, as well as concentrated sulfuric acid (98%). All chemicals used in the study are analytical grade.

2.2 Struvite precipitation

The initial precipitation experiments for optimization were carried out in 1-L beakers. Precipitation was initiated with the addition of the desired amount of $MgSO_4.7H_2O$ and $Ca(HPO_4)2.H_2O$ at stoichiometric ratios with pH adjusted by using NaOH. Mixing with the use of magnetic stirrer was continued until a steady pH. During MAP reaction, the pH of samples was adjusted to desired values by adding gradually 6N or 10N NaOH. Subsequent to this, the mixtures were allowed to settle for 30 min and the supernatant samples were collected for ammonium and orthophosphates analyses. Chemicals were added into piggery wastewater to receive struvite, applying to equation as follows:

$$m = (n - a) \times M$$

with

- m is weigh of chemicals adding into landfill leachate ($MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$)
- n (nmol) of mol NH_4^+ in 1 litre landfill leachate
- a (nmol) of mol Mg^{2+} (or PO_4^{3-}) in 1 litre landfill leachate
- M is block molecule of $MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$

2.3 Effects of pH, ratio of mol Mg and PO_4 and stirring time on struvite crystallization

Exp1. The pH treatments were estimated at 8, 9, 10 while control was not adjusted pH. The Mg^{2+}:NH_4^+:PO_4^{3-} molar ratio was controlled at 1:1:1 . Each treatment replicated 3 times and the volume of reaction was 0.5 L piggery wastewater.

Exp.2. Based on the result of preliminary test (Exp. 1), the subsequence was then carried out at the optimum pH. Five ratios of mol (Mg^{2+}:NH_4^+:PO_4^{3-}) ratio of 1:1:1, 1.2:1:1, 1.5:1:1, 1:1:1.2 and 1:1:1.5 were estimated with three replications, each replication was 1 beaker 1-L containing 0.5 L piggery wastewater.

Exp. 3. Optimal stiring time (0, 10, 20, 40, 60, 80, 100 and 120 min) was conducted with three replications, and each replication was a beaker 1-L containing 0.05 L piggery wastewater.

The objective of these three experiments was not only the highest struvite precipitation but also removed ammonium and orthophosphate out of piggery wastewater.

Piggery wastewater samples after precipitation process above were filtered through filter paper (Ø11 cm, Hangzhou Special Paper Industry Co. Ltd, Zhejiand, China) and the supernatant samples were analysed ammonium and orthophosphate concentration at Advanced Lab., Can Tho University, Vietnam.

2.4 Application of nitrogen removal bacteria and poly-P bacteria in piggery wastewater treatment

After experiment 3, the supernatants (of piggery wastewater) were analysed ammonia and orthophosphate concentration, and they were then added nitrogen removal bacteria (*Pseudomonas stutzeri* D3b strain) [35] and poly-P bacteria (*Kurthia* sp. TGT013L strain) [36] (1.0%), glucose (5 g/L), aeration with different times (6, 12, 18 and 24/24 h). The experiment was completely randomized design with 3 replications, the experiment consisted of 6 treatments as follows:

1. T1: control [without bacteria and aeration],
2. T2: piggery wastewater [after withdrawal struvite] without bacteria and aeration,
3. T3: T2 applied D3b + TGT13L + aeration 6/24h
4. T4: T2 applied D3b + TGT13L + aeration 12/24h
5. T5: T2 applied D3b + TGT13L + aeration 18/24h
6. T6: T2 applied D3b + TGT13L + aeration 24/24h

Each treatment was one 2-L plastic container containing 1L piggery wastewater

The result from the above experiment was done with 10-L bigger plastic container containing 5 litres piggery wastewater and the experiment with only two treatments as control and optimal treatment were conducted with 3 replications.

2.5 Analytical methods

NH_4^+-N (Colometric method or Phenol nitroprusside method) [37], COD, BOD, Orthophosphate (Colormetric method) and pH (pH meter) were determined by Advanced Analyses Laboratory, Can Tho University, Viet Nam.

III. RESULTS AND DISCUSSIONS

In Table 2 showed that the optimization of struvite precipitation for pretreatment of the raw piggerry wastewater was pH 9 (21.17 g/L) and at pH 9 obtained at the theoretical stoichiometric ratio of Mg^{2+}:NH_4^+:PO_4^{3-} ratio of 1.2:1:1 the highest struvite precititation (26.34 g/L).

TABLE 2
EFFECTS OF PH AND MG:PO_4 ON AMOUNT OF STRUVITE CRYSTALLIZATION

Treatment	g/L	Treatment	g/L
Control*	19.57	1 mol Mg : 1 mol PO_4	32.71
pH=8	31.57	1.2 mol Mg : 1 mol PO_4	34.47
pH=9	**32.03**	1.5 mol Mg : 1 mol PO_4	35.20
pH=10	29.97	1 mol Mg : 1.2 mol PO_4	37.47
LSD.01	**1.70**	1 mol Mg : 1.5 mol PO_4	**44.37**
C.V (%)	**2.20**	**LSD.01**	**0.88**
		C.V (%)	**0.89**

After 20 minute stiring, 1.773 g struvite was formed from 50 ml piggery wastewater and this stirring time was the best in comparison with others or the optimal stirring time for struvie formation (Figure 1)

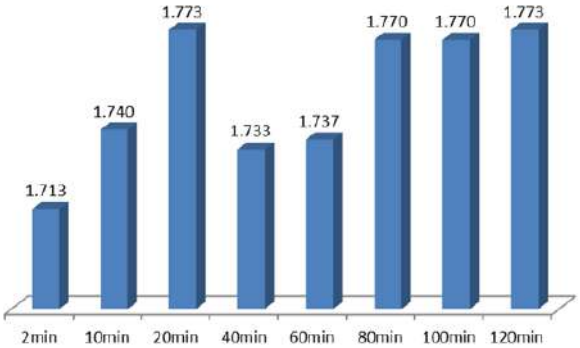


FIGURE 1. EFFECTS OF STIRRING TIME (min) ON STRUVITE FORMATION (g/50 mL PIGGERY WASTEWATER)

Over 80.0% of ammonium (509 mg/L NH_4-N) and orthophosphate (169 mg/L) were removed at pH 9, with a residual concentration of 126.2 mg/L NH_4-N and 42.1 mg/L PO_4_P as depicted in Figure 2, thus indicated that pH 9 was the most suitable for struvite formation for the raw piggery wastewater under investigation. Similarly, molar ratio Mg:PO4 (1:1.2) was the best molar molecule to remove ammonium and orthophosphate in piggery wastewater (Figure 3).

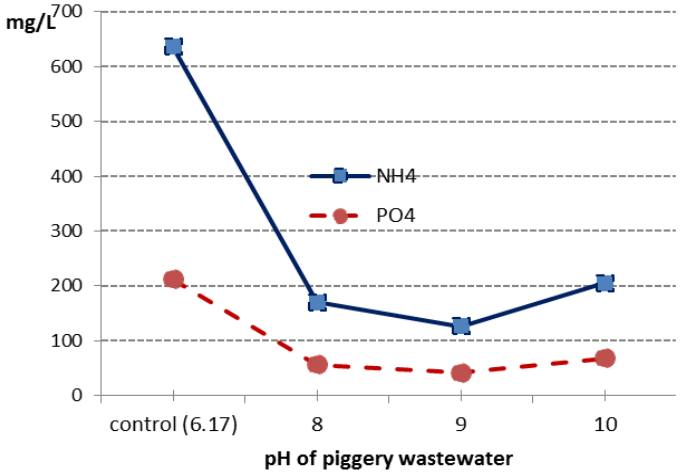


FIGURE 2. EFFECT OF pH ON NH_4^+-N and PO_4^{3-} REMOVAL DURING STRUVITE FORMATION

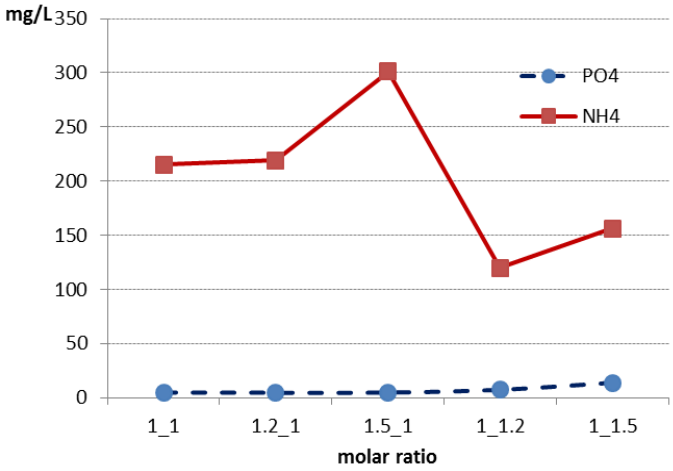


FIGURE 3. EFFECT OF MOL Mg:PO_4 on NH_4^+-N and PO_4^{3-} REMOVAL DURING STRUVITE FORMATION

From the above results (Table 2, Figure 2, Figure 3), pH 9, mol Mg:PO_4 (1:1.2:1) were chosen the optimal conditions for sturvite formation and the low ammonium and orthophosphate in piggery wastewater.

In experiment 3, the piggery wastewater was adjusted at pH 9 and mol Mg:PO_4 (1:1.2:1), the stiring time or aeration began, the result from Figure 4 showed that ammonium concentration reduced to 10-min and ammonium concentration increased to 70 mg/L at 40-min, after the this time ammonium reduced to the time (to 120-min) but orthophosphate concentration in piggery wastewater enhanced from 7.93 mg/L to 9.96 mg/L at 40-min and orthophosphate concentration reduced slowly to 120-min. At 100-min and 120-min, ammonium concentration in piggery wastewater reduced the lowest (3.93 and 1.41 mg/L NH_4-N) perhaps ammonium in wastewater was escaped to the air by the stirrer however struvite formation at all the times was not difference (Figure 1). To save energy for electricity, 10-min was choose in this experiment because at this time low ammonium and orthophosphate concentration in wastewater.

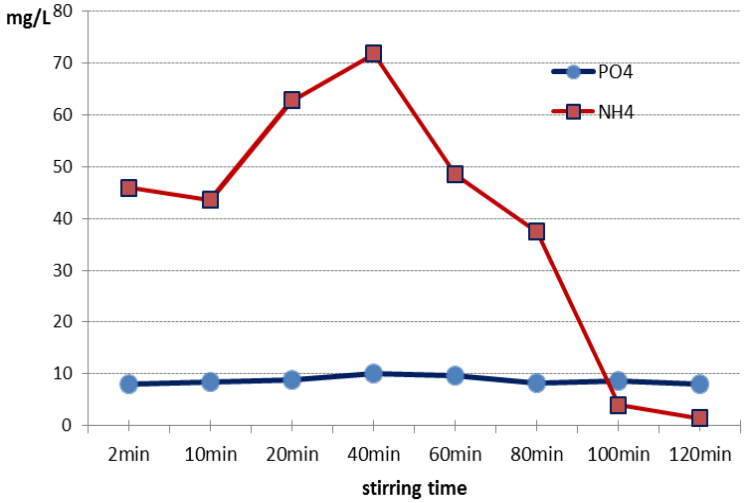
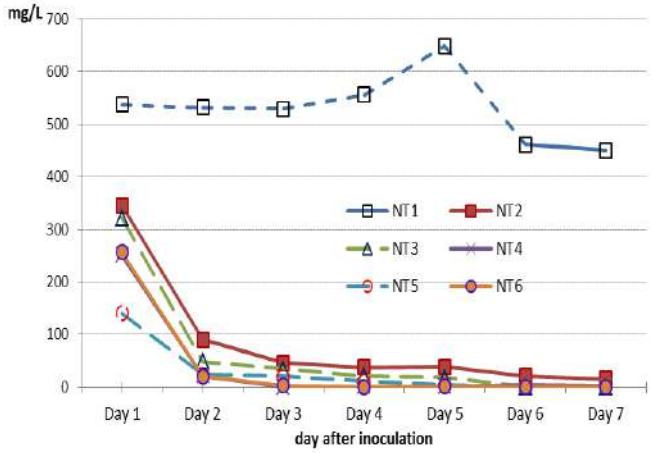


FIGURE 4. EFFECT OF STIRRING TIME ON NH_4^+-N and PO_4^{3-} REMOVAL CONCENTRATION

3.1 Effects of nitrogen removal bacteria and poly-P bacteria in piggery wastewater treatment

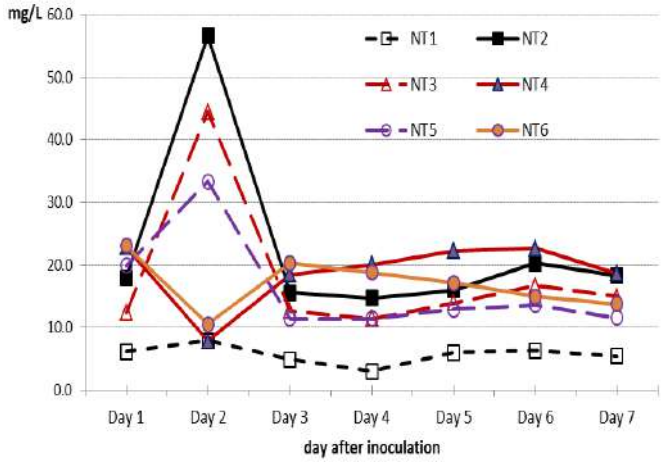
In experiment 1-L, application of nitrogen removal bacteria (D3b strain) and poly-P bacteria (TGT013L strain) into piggery wastewater reduced ammonium concentration to the time however aeration 12/24 h (NT4) reduced ammonium concentration at day 3 and saved energy (only aeration 12/24 h compared to 18/24 or 24/24 h) (Figure 5).



NT1: control
NT2: piggery wastewater (evicted struvite)
NT3: NT2 + N removed and poly-P bacteria + 6 g glucose/l + aeration 6/24 h
NT4: NT3 but aeration 12/24 h
NT5: NT3 but aeration 18/24 h
NT6: NT3 but aeration 24/24 h

FIGURE 5. EFFECTS OF NITROGEN REMOVAL BACTERIA AND POLY-P BACTERIA ON AMMONIUM CONCENTRATION IN PIGGERY WASTEWATER

However, orthophosphate concentration in piggery wastewater of treatments (NT4 and NT6) reduced from day 1 to day 3 after that PO_4 concentration induced to 18 and 20 mg/L PO_4/L perhaps aeration increased PO_4 level in piggery wastewater, and PO_4 concentration of treatment 2 (NT2), NT3, and NT4 increased in day 2 after that decreased from day 3 to day 6, especially control treatment had orthophosphate concentration lower during 6 days, this demonstrated that aeration supported to increasing PO_4 in wastewater. Herewith day 3 reduced ammonium concentration (Figure 5), day 3 was chosen to low PO_4 concentration (Figure 6) for experiment 5L.



NT1: control
NT2: piggery wastewater (evicted struvite)
NT3: NT2 + N removed and poly-P bacteria + 6 g glucose/l + aeration 6/24 h
NT4: NT3 but aeration 12/24 h
NT5: NT3 but aeration 18/24 h
NT6: NT3 but aeration 24/24 h

FIGURE 6. EFFECTS OF NITROGEN REMOVAL BACTERIA AND POLY-P BACTERIA ON ORTHOPHOSPHATE CONCENTRATION IN PIGGERY WASTEWATER

Struvite crystallization process is an effective eco-friendly process that removes and recovers P and N from wastewaters. The hazardous elements in wastewaters (mainly NH_4 and PO_4) might be converted to a valuable resource through this process. So, the optimization of Mg molar ratio, pH level, aeration rate, reaction time and temperature would enhance the quality as well as production.

The pH plays an important role during the struvite precipitation process. Struvite or MAP can be precipitated at a wide range of pH (7.0–11.5), but the suitable pH ranges between 7.5 to 9.0 [38]. Efficiency of MAP precipitation depends on the concentration and molar ratios of $Mg_2^{+,}$ NH_+^4 , & PO_4^{3-} , pH, aeration rate, temperature, and presence of Ca^{2+} in the reacting media [25][38][39][40].

It is found that a wide range of PO_4 and Mg ratio was applied for struvite precipitation, but in most cases, the effective ratio was 1:1 or 1:1.2 (Rahman *et al*., 2011)[12]. The addition of chemicals to the wastewaters would be needed to provide an equimolecular condition of PO_4 and Mg. Yetilmezsoy and Zengin [25] conducted a series of experiments to see the effect of Mg, NH_4 and PO_4 ratio on struvite precipitation and nitrogen removal efficiency.

Air flow plays an important role in the removal of NH_4-N from the solution. Air flow agitates the solution and creates a removal pathway for dissolved ammonia to volatilize from the solution. Moreover, air flow dilutes the concentration of gas-

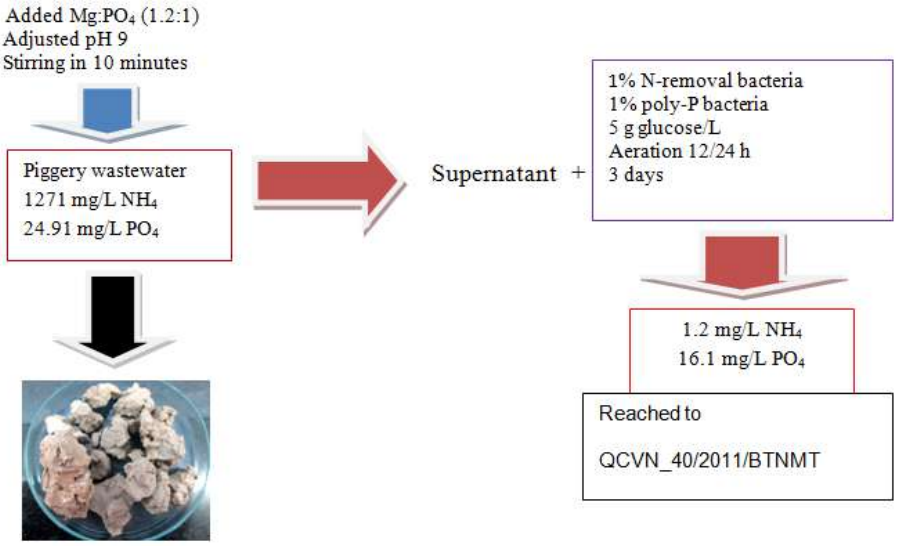
phase NH_4-N and increase the driving force for dissolving NH_4-N to separate the gaseous phase. So, there is an increasing tendency for ammonia volatilization with increasing airflow rate. Yetilmezsoy and Zengin [25] stated that a sufficient aeration time should be provided to achieve high removal efficiencies. They obtained about 93.4% NH_4-N removal with an aeration rate of 0.6 L min^{-1} within a period of 24 h. They also found the highest NH_4-N removal (95.3%) in 12 h reaction time with an aeration rate of 10 L min^{-1}. Lei *et al.* [41]found about 60.2% ammonia removal with an aeration rate of 0.6 L min 1 in a reaction time of four hours. On the contrary, they achieved the same removal efficiency without aeration in a period of 24 h. Liu *et al.* [5][6]found that struvite formation is proportional to the aeration rate and reached a plateau at around 0.73 L min^{-1}.

Pseudomonas stutzeri strain D3b was isolated from in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatment effectively [35]. Application of *Pseudomonas stutzeri* D3b strain and *Acinetobacter lwoffii* TN7 strain to remove ammonia in wastewater of biowaste was carried out to evaluate their ability of ammonia removal at different concentrations with and without aeration condition in laboratory; The results showed that these species had ammonia removal ability effectively at both 50 mg/l and 100 mg/l ammonia. *Pseudomonas stutzeri* strain D3b and *Acinetobacter lwoffii* strain TN7 are the best bacterial species to remove ammonia. Besides that, both of species removed ammonia in aerobic condition better than anaerobic condition. In three days, the ammonia removal efficiency of *Pseudomonas stutzeri* D3b were 97.2% and 98.57% and *Acinetobacter lwoffii* TN7 were 96.32% and 98.31% in 50 mg/l and 100 mg/l ammonia concentrations in wastewater of biowaste, respectively [42]. Polyphosphate accumulating organisms (PAOs) is known as the microorganisms to absorb free phosphate in the environment and assimilate them as intracellular polyphosphate (poly-P) particles. This process was viewed as enhanced biological phosphorus removal (EBPR) in wastewater treatment systems [43][44][45]. Khoi *et al.* [36] applied *Kurthia* sp. TGT013L to remove orthophosphate in wastewater effectively.

IV. CONCLUSION

Production of struvite from wastewaters will reduce the hazard of eutrophication in the water bodies by removing N and P. Production of struvite from wastewater and its utilization as fertilizer would partially help to reduce global warming and thus, it would be an effective eco-friendly fertilizer.

Treatment of piggery wastewater consisting of struvite eviction and removal of nitrogen and phosphate using nitrogen removal bacteria and poly-P bacteria were high effectiveness and low cost with process as follows:



ACKNOWLEDGEMENT

This study was sponsored by Can Tho University, Viet nam.

REFERENCES

[1] L.W. Deng, P. Zheng, and Z.A. Chen, "Anaerobic digestion and post-treatment of swine wastewater using IC–SBR process with bypass of raw wastewater." Process Biochem. Vol. 41, 2006, pp. 965–969.

[2] K. Suzuki, Y. Tanaka, K., Kuroda, D. Hanajima, Y. Fukumoto, T. Yasuda, and M. Waki, "Removal and recovery of phosphorous from swine wastewater by demonstration crystallization reactor and struvite accumulation device". Bioresour. Technol. Vol. 98, 2007, pp.1573–1578.

[3] M.I. Ali, "Struvite crystallization from nutrient rich wastewater." Ph. D thesis. School of Engineering, James Cook University, Townsville, Australia., 2005

[4] Y.H. Liu, J..H., Kwag, J.H., Kim, and C.S. Ra, "Recovery of nitrogen and phosphorus by struvite crystallization from swine wastewater". Desalination vol. 277, 2011a, pp.364–369.

[5] Y.H. Liu, M.M, Rahman, J.H., Kwag, J.H., Kim, and C.S. Ra, "Eco-friendly production of maize using struvite recovered from swine wastewater as a sustainable fertilizer source." Asian-Aust. J. Anim. Sci. vol. 24, 2011b. pp.1699–1705.

[6] Y.H. Liu, S. Kumar, J.H. Kwag, J.H., Kim, J.D.Kim, and C.S Ra, "Recycle of electrolytically dissolved struvite as an alternative to enhance phosphate and nitrogen recovery from swine wastewater." J. Hazard. Mater. Vol. 195, 2011c, pp.175–181.

[7] U. Welander, T. Henrysson, and T., Welander, "Biological nitrogen removal from municipal landfill leachate in a pilot scale suspended carrier biofilm process." Water Res. Vol. 32, 1998, pp. 1564–1570.

[8] A. Bonmati, and X. Flotats, "Air stripping of ammonia from pig slurry: characterization and feasibility as a pre- of post-treatment to mesophilic anaerobic digestion. Waste Manage. (Oxford) vol. 23, 2003, pp.261–272.

[9] K.W. Kim, Y.J. Kim, I.T. Kim, G.I. Park, and E.H.Lee, "Electrochemical conversion characteristics of ammonia to nitrogen". Water Res. Vol. 40, 2006, pp.1431–1441.

[10] B.Y. Jeong, S.H. Song, B.K..Baek, I.H. Cho, and T.S. Hwang, "Preparation and properties of heterogeneous cation change membrane for recovery of ammonium ion from waste water." Polymer (Korea) vol. 30, 2006, pp.486–491.

[11] J.H. Cho, J.E, Lee, and C.S Ra, "Microwave irradiation as a way to reutilize the recovered struvite slurry and to enhance system performance". J. Anim. Sci. Technol. Vol. 51, 2009, pp.337–342.

[12] M.M. Rahman, Y.H. Liu, J.H. Kwag, and C.S. Ra, "Recovery of struvite from animal wastewater and its nutrient leaching loss in soil". J. Hazard. Mater. Vol. 186, 2011, pp.2026–2030.

[13] N.O. Nelson, R.L. Mikkelsen, and D.L. Hesterberg, "Struvite precipitation in anaerobic swine lagoon liquid: effect of pH and Mg:P ratio and determination of rate constant." Bioresour. Technol. Vol. 89, 2003, pp.229–236.

[14] K. Suzuki, Y. Tanaka, K. Kuroda, D. Hanajima, and Y. Fukumoto, "Recovery of phosphorous from swine wastewater through crystallization." Bioresour. Technol. Vol. 96, 2005, pp.1544–1550.

[15] R.D. Schuiling, and A. Andrade, "Recovery of struvite from calf-manure". Environ. Technol. Vol. 20, 1999, pp.765–768.

[16] O. Tunay, I, Kabdasli, D. Orhon, and S. Kolcak, "Ammonia removal by magnesium ammonium phosphate precipitation in industrial wastewaters". Water Sci. Technol. Vol. 36, 1997, pp.225–228.

[17] E.V. Munch, and K. Barr, "Controlled struvite crystallisation for removing phosphorus from anaerobic digester sidestreams." Water Res. Vol. 35, 2001, pp.151–159.

[18] M.S. Massey, J.G. Davis, R.E. Sheffield, and J.A. Ippolito, 'Struvite production from dairy wastewater and its potential as a fertilizer for organic production in calcareous soils'. In: International Symposium on Air Quality and Waste Management for Agriculture. CD-Rom Proceedings of the 16–19 September 2007, Conference (Broomfield, Colorado), USA. ASABE Publication Number 701P0907cd.

[19] Y. Jaffer, T.A. Clark, P. Pearce, and S.A. Parsons, "Potential phosphorus recovery by struvite formation" Water Res. Vol. 36, 2002, pp. 1834–1842.

[20] P. Battistoni, M.P., Pavan, M. Prisciandaro, and F. Cecchi, "Struvite crystallization: a feasible and reliable way to fix phosphorous in anaerobic supernatants." Water Res. Vol. 34, 2000, pp.3033–3041.

[21] L. Pastor, D. Mangin, J. Ferrer, and A. Seco, "Struvite formation from the supernatants of an anaerobic digestion pilot plant." Bioresour. Technol. Vol. 101, 2010, pp.118–125.

[22] G.E. Diwani, S.E. Rafie, N.N.E.Ibiari, and H.I. El-Aila, "Recovery of ammonia nitrogen from industrial wastewater treatment as struvite slow releasing fertilizer". Desalination vol. 214, 2007, pp.200–214.

[23] B.U. Kim, W.H. Lee, H.J. Lee, and J.M. Rim, "Ammonium nitrogen removal from slurry-type swine wastewater by pretreatment using struvite crystallization for nitrogen control of anaerobic digestion". Water Sci. Technol. Vol. 49, 2004, pp.215–222.

[24] P.W. Westerman, K.D. Zering, and Rashash, "Struvite crystallizer for recovering phosphorous from lagoon and digester liquid." Unpublished article. NC State University collaborative Extension program, USA. The article is available in: http://www.bae.ncsu.edu/programs/extension/manure/lagoon/ag-724w_struvite_crystallizer.pdf., 2009

[25] K. Yetilmezsoy, and Z.S., Zengin, "Recovery of ammonium nitrogen from the effluent of UASB treating poultry manure wastewater by MAP precipitation as a slow release fertilizer." J. Hazard. Mater. Vol. 166, 2009, pp. 260–269.

[26] W. Moerman, M. Carballa, A. Wandekerckhove, D. Derycke, and W. Werstraete, "Phosphate removal in agro-industry: pilot-and full-scale operational considerations of struvite crystallization". Water Res. Vol. 43, 2009, pp.1887–1892.

[27] I. Kabdasli, P. Ozcan, and O. Tunay, "Nitrogen removal by magnesium ammonium phosphate precipitation in slaugtheryhouse wastewater". Su Kirlenmesi Kontrolu Dergisi. Vol, 13, 2003, pp.13–18.

[28] I. Celen, and M. Turker, "Recovery of ammonia as struvite from anaerobic digester effluents" Environ. Technol. Vol. 22, 2001, pp.1263–1272.

[29] A. Adnan, F.A. Koch, and D.S. Mavinic, "Pilot-scale study of phosphorus recovery through struvite crystallisation-II: applying in-reactor supersaturation ratio as a process control parameter". J. Environ. Eng. Sci. vol. 2, 2003, pp. 473–483.

[30] R.T. Burns, and L.B. Moody, "Phosphorus recovery from animal manures using optimized struvite precipitation". In: Proceedings of Coagulants and Flocculants: Global Market and Technical Opportunities forWater Treatment Chemicals, Chicago, Illinois.May, 2002, pp. 22–24.

[31] B. Etter, E. Tilley, R. Khadka, and K.M. Udert, "Low-cost struvite production using source-separated urine in Nepal. Water Res. Vol, 45, 2011, pp.852–862.

[32] R. Yu, J. Geng, H. Ren, Y. Wang, and K. Xu, "Struvite pyrolysate recycling combined with dry pyrolysis for ammonium removal from wastewater". Bioresour. Technol. Vol. 132, 2013, pp.154–159.

[33] O. Ichihashi, and K. Hirooka, "Removal and recovery of phosphorus as struvite from swine wastewater using microbial fuel cell". Bioresour. Technol. Vol. 114, 2012, pp.303–307.

[34] N.C. Bouropoulos, and P.G. Koutsoukos, "Spontaneous precipitation of struvite from aqueous solutions". J. Cryst. Growth. Vol. 213, 2000, pp. 381–388.

[35] C.N. Diep, C. N.**,** P. M. Cam, N. H. Vung, T T. Lai and N. T. Xuan My, "Isolation of *Pseudomonas stutzeri* in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatment." Bioresource Technology. Vol. 100, 2009, pp. 3787-3791.

[36] Khoi, L. Q., T. T. Ngon và C. N. Điệp, 'Ứng dụng vi khuẩn tích luỹ poly-Phosphate để loại bỏ phosphate hoà tan trong nước thải." Tuyển tập Hội nghị khoa học công nghệ sinh học toàn quốc tổ chức ngày 27 tháng 9 năm 2013 tại Hà Nội. Nhà xuất bản Khoa học Tự nhiên và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt nam, Hà Nội, Quyển 2, 2013, pp.284-288. (Vietnamese)

[37] D.R. Keeney, and D. W. Nelson. "Nitrogen-inorganic forms. In: Page, A.L.R.H. Miller and D.R. Keeney (eds.) Methods of soil analysis". Part 2-Chemical and microbiological properties. (2nd Ed.). Agronomy 9, 1982 pp.643-698.

[38] X.D. Hao, C.C. Wang, L. Lan, and M.C.M. Von Loosdrecht, "Struvite formation, analytical methods and effects of pH and Ca^{2+}." Water Sci. Technol. Vol. 58, 2008, pp.1687–1692.

[39] I. Stratful, M, Scrimshaw, and J. Lester, "Conditions influencing the precipitation of magnesium ammonium phosphate". Water Res. Vol. 35, 2001, pp.4191–4199.

[40] K.S. Le Corre, E. Valsami-Jones, P. Hobbs, and S.A. Parsons, "Impact of calcium on struvite crystal size, shape and purity". J. Cryst. Growth. Vol. 283, 2005, pp. 514–522.

[41] X. Lei, S. Shimada, K. Intabon, and T. Maekawa, "Pretreatment of methane fermentation effluent by physico-chemical processes before applied to soil trench system." Agric. Eng. Int.: CIGR E J. vol. 8, 2006, 1–15.

[42] C.N. Diep, C. N. và N. T H. Nam, "Ứng dụng vi khuẩn *Pseudomonas stutzeri* và *Acinetobacter lwoffii* loại bỏ amoni trong nước thải từ rác hữu cơ. Tạp chí Khoa học Trường Đại học Cần Thơ, vol. 22b, 2012, 1-8. (Vietnamese)

[43] P.L. Bond, R. Erhart, M. Wagner, J. Keller, and L. L. Blackall, "Identification of some of the the major groups of bacteria in efficient and non-efficient biolical phosphorus removal activated sludge systems" Appl. Environ. Microbiol., vol. 65(9), 1999, pp. 4077-4088.

[44] T. Mino, M.C.M.Van Loosdrecht and J. J. Heijnen, "Microbiology and biochemistry of the enhanced biological phosphate removal process." Water Res. Vol. 32, 1996, pp.3193–3207.

[45] A.P.C. Oehmen, G. Lemos, Z. Carvalho, J. Yuan, L. Keller, L. Backall, and M.A.M.Reis, "Advances in enhanced biological phosphorus removal: from micro to macro scale. Water Research. Vol. 41, 2007, pp. 2272-2300.

A Preliminary Investigation of Bird Diversity in Nanjing

Jie Wang[1], QuanmengXia[2], Chenling Zhang[3*], Jian Wang[4]

[1,4]Jiangsu Agri-animal Husbandry Vocational College, China
[2,3]Jiangsu Second Normal University, China

Abstract**—** *To find out the classification information and distribution information of common birds in Nanjing, China, and provide reference for the government to formulate the protection policy of bird, this paper analyzes the bird observation data from 2007 to 2016 in the Bird Observation Center (Nanjing area), and analyzes the ecological changes of the observation points in combination with the Nanjing land using situation. the results show that Nanjing is an important bird habitat, with many species and national protected species, but in recent years, the acceleration of urbanization and human activities have seriously affected these originally good bird distribution areas, government departments should strengthen the response and management.*

Keywords**—** ***Nanjing, Bird diversity, Protected species.***

I. INTRODUCTION

From May 2012 to April 2013, Peng lifeng selected four habitats in Suzhou industrial park, set up 17 sample lines and 24 sample points, and studied the bird community monthly. A total of 130 species of birds were recorded, belonging to 41 families of 12 orders. Among them, the resident birds, summer birds, winter birds and passing migrant birds accounted for 26.15 %, 25.38 %, 27.69 % and 20.77 % of the total species, respectively. The cosmopolitan species and palaearctic realm species accounted main part of them, and has both oriental species and obvious characteristics of north-south transition. In terms of seasonal dynamics, the number of bird species in the park showed that, spring > winter > autumn > summer, and the density of the birds showed that, winter > autumn > spring > summer [1]. The wetland habitat has the largest number of birds and uniform distribution; Road habitat bird species are the least, uneven distribution, but the dominant species, mainly common companion birds. The clustering results show that environmental conditions are important factors affecting bird composition.

II. RESEARCH AREAS AND METHODS

2.1 Study of regional profiles

Nanjing is located in the eastern part of China, is the capital city of Jiangsu province, located in the southwest of Jiangsu province, geographical coordinates for 31 14 " to 32 37" north latitude, longitude 118 22" to 119 14", the terrain is given priority to with low hills, there are ZiJin mountain, Mufu mountain, Qixia mountain, Tang mountain, etc., accounting for about 60 % of the total area of the city, and the water system is developed, there are the Yangtze river, Qinhuai river, The city 's forest coverage rate is 26.4 %, 45 %, good environmental air quality, urban environment is beautiful. Nanjing is a subtropical monsoon climate, four distinct seasons, annual average temperature is 15.4^{o}C, and rainfall is abundant, annual average rainfall reached 1200 mm [2].

2.2 Research methods

2.2.1 Material

According to the records of the bird watching record center of the network, the bird watching records from 2007 to 2016 in Nanjing area were collected, and the data were sorted and dealt with.

2.2.2 Research methods

The records of bird watching center were sorted out by office 2007 suite, and the bird diversity was statistically analyzed in Nanjing from 2007 to 2016.Using the statistical function of Excel, the distribution characteristics of birds were analyzed and Shannon - Weiner index was calculated.

$$H' = -\sum_{i=1}^{S} P_i \log_2 P_i$$

III. RESEARCH RESULTS AND DATA ANALYSIS

3.1 Distribution and land use change of 15 bird species observation sites in Nanjing

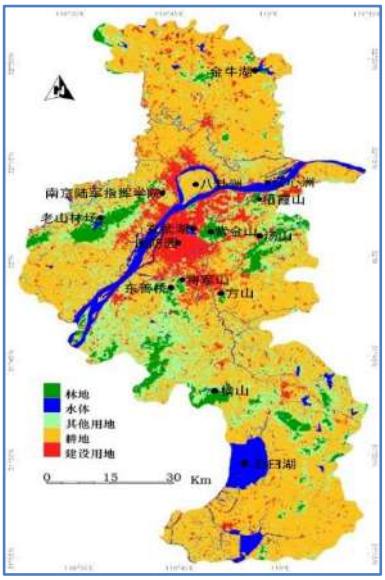


FIG.1: DISTRIBUTION AND LAND USE CHANGE OF 15BIRD SPECIES OBSERVATION SITES IN NANJING OF 2007

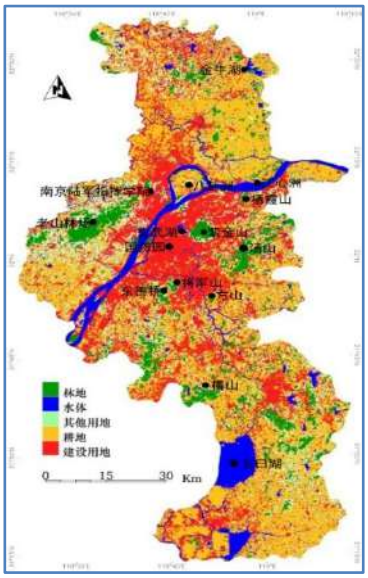


FIG.2: DISTRIBUTION AND LAND USE CHANGE OF 15 BIRD SPECIES OBSERVATION SITES IN NANJING OF 2012

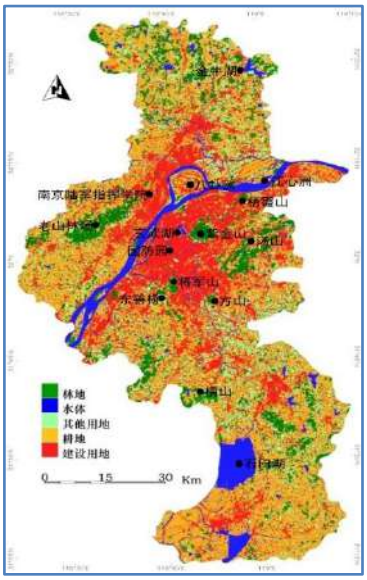


FIG.3: DISTRIBUTION AND LAND USE CHANGE OF 15 BIRD SPECIES OBSERVATION SITES IN NANJING OF 2016

Comparative analysis of the distribution and land use of 15 bird observation points in Nanjing from 2007 to 2016 shows that the construction land in Nanjing is increasing, while the forest land and cultivated land on which birds live are decreasing gradually. The land use of 15 bird observation points also conforms to this law. A total of 45 families and 269 species of birds have been recorded in Nanjing during the past decade, including 4 species of primary protected birds and 18 species of two protected birds. In 2007, 37 families and 129 species of birds were recorded; Thirty - nine families and 177 species of birds were recorded in 2008; In 2009, 42 families and 225 species of birds were recorded; In 2010, 43 families and 237 species of birds were recorded; In 2011, 38 families and 184 species of birds were recorded; Forty families and 179 species of birds were recorded in 2012; In 2013, 39 families and 149 species of birds were recorded; Thirty nine families and 160 species of birds were recorded in 2014; In 2015, 41 families and 201 species of birds were recorded; In 2016, 43 families and 198 species of birds were recorded.

3.2 Shannon - wil index of birds in Nanjing city from 2007 to 2016

Due to the differences in the ecological environment of each observation point, the number of birds is also different, and Shannon - wil index is generally used in the area with many species. Therefore, the bird data of each observation point is integrated, and the whole city of Nanjing is taken as a large observation point for calculation. The Shannon - wil index of birds in Nanjing from 2007 to 2016 was 3.968, 3.933, 4, 166, 4.248, 3.868, 3.290, 3.770, 3.635, 3.403 and 3.936, respectively. The data show that the diversity of birds in Nanjing fluctuates steadily in a relatively small atmosphere, with little change around ten years, and the diversity index of birds is high and species are abundant.

3.3 Correlation analysis between two observation points of Mount Zijin and Xuanwu Lake and the birds in Nanjing City

Mount Zijin and Xuanwu Lake, as two typical bird observation points in Nanjing, located in the city center of Nanjing, mainly in forest land and water area, surrounded by construction land, greatly influenced by human activities.

The number of birds in Nanjing city fluctuates within a stable range (figure 4), and Zijin mountain and Xuanwu Lake both the number of birds and the number of species compared with Nanjing city, especially in recent years, with the increase of construction land around the two places, the vigorous development of tourism, free open to tourists, resulting in the number of birds' fluctuations. Originally is an important habitat of birds in Nanjing Xuanwu lake has been greatly affected, it is difficult to reproduce the original birds chirping, competing to fly.

3.3.1 Correlation analysis between the number of birds and species

A linear correlation analysis was conducted between the number of families and species of birds in Nanjing for ten years and Mount Zijin and Xuanwu Lake. In terms of the number of families, the correlation coefficient r was 0.345 and 0.292, respectively. Therefore, there was only a very low linear correlation between the number of families of Mount Zijin birds and Nanjing, while there was no linear correlation between the number of families of Xuanwu Lake birds and Nanjing. In terms of species number, correlation coefficients were 0.669 and 0.511, respectively. The results showed that there was a significant linear correlation between the species numbers of birds in Mount Zijin and Xuanwu Lake, and Mount Zijin was higher than Xuanwu Lake. ($0< r < 1$ indicates that there is linear correlation in different degrees: $r \leq 0.3$ indicates that there is no linear correlation; $0.3 < r \leq 0.5$ is low linear correlation; $0.5 < r \leq 0.8$ was significant linear correlation; $R > 0.8$ is highly linear correlation).

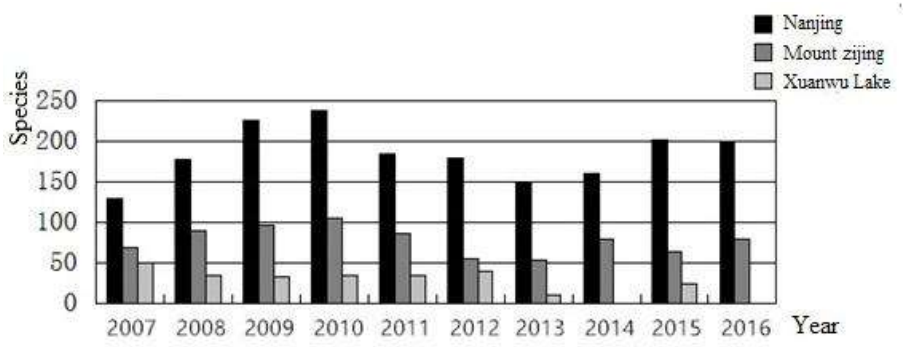


FIG. 4: TEN YEARS' BIRD SPECIES IN THE CITY OF NANJING AND MOUNT ZIJIN AND XUANWU LAKE

3.3.2 Correlation analysis of diversity index

Using the data of fig. 5, we analyzed the bird diversity index of Nanjing in the past ten years with the linear correlation coefficient r of 0.472 and 0.262, respectively. the results showed that the bird diversity of Mount Zijin had significant linear correlation with the bird diversity of Nanjing, while the bird diversity of Xuanwu lake had no linear correlation with the bird diversity of Nanjing.

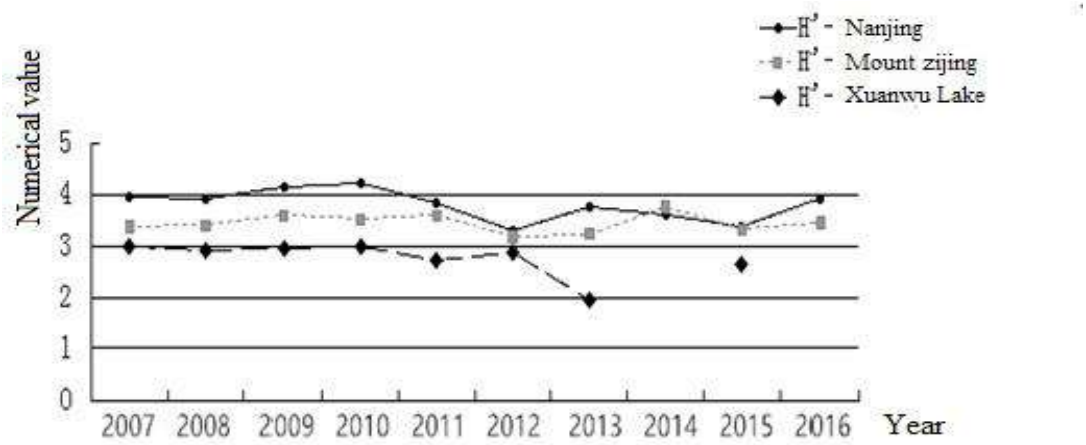


FIG. 5: NANJING CITY AND MOUNT ZIJIN, XUANWU LAKE TEN YEARS BIRD SHANNON - WILL INDEX LINE STATISTICAL CHART

On the whole, with high abundance and diversity of birds in Nanjing city as a reference, Zijin mountain because of the terrain as a mountainous area, there are large areas of undeveloped forest land, ecological environment preservation is good, moderate human disturbance and construction and development, so that the bird habitat is rich and diverse, eventually make the Zijin mountain bird richness and species diversity in ten years to remain stable, Zijin mountain is an important habitat of Nanjing birds Basaltic lake is different, the surrounding construction in a decade a large number of buildings, the interior has gradually been artificial, the number of tourists surge, resulting in the loss of bird original diversity habitats, bird richness and species diversity significantly reduced, the national protection of birds also slowly disappear (Table 1), Nanjing birds gradually lost basaltic lake, an important habitat.

TABLE 1
NANJING CITY AND MOUNT ZIJIN, XUANWU LAKE TEN YEARS' NATIONAL BIRD PROTECTION STATISTICS

Level	National first level protection			National second level protection		
Year	Nanjing	Mount Zijin	Xuanwu Lake	Nanjing	Mount Zijin	Xuanwu Lake
2007	4	0	0	7	2	3
2008	2	0	0	5	1	0
2009	2	0	0	11	3	0
2010	4	0	0	10	4	1
2011	1	0	0	10	4	0
2012	1	0	0	8	3	1
2013	1	0	0	5	3	1
2014	0	0		7	3	
2015	2	0	0	11	4	0
2016	2	0		9	2	

IV. DISCUSSION

Long - term investigation and study of species composition and dynamics in a certain area can provide reference for environmental monitoring, evaluation and protection, and birds as indicators of urban ecosystems, in the evaluation of urban ecological environment quality plays an indicator role [3]. With the aggravation of urbanization, not only the urban landscape changes its appearance, but also threatens the survival of urban birds. Different natural habitats, urban bird habitats have high heterogeneity, frequent human disturbance and other unique characteristics, and compared with natural habitat bird diversity research; urban and suburban bird diversity is obviously insufficient attention.

4.1 Impact of urbanization on urban birds

4.1.1 Urban birds and their characteristics

Urban birds are those living in the urban environment, they together with humans in the city without relying on human feeding, their own foraging [4]. Has the following characteristics: is the construction of urban bird community, living in a relatively dense population, productivity and economic development, has a certain degree of cultural development, artificial environment is given priority to with the comprehensive area (i.e., the city), is an important part of the natural factors of human survival, is one of the wild animals in the urban environment [5]. Mainly including the original area before the urbanization of the remaining birds; Birds migrating from outside into cities; Semi - domesticated birds escaping from domesticated places or markets; And birds that migrate and stay for a period.

Compared with non-urban birds, urban birds have their own unique characteristics, mainly in the following aspects: in the urban ecosystem, human activities are frequent, bird communities are under serious human activities, urban birds due to the lack of natural enemies and accustomed to the existence of human beings, smells and activities, they often lack of vigilance. Human production and living activities are often an important factor leading to the death of urban birds; Urban bird habitats range from almost natural ecological communities to completely artificial habitats, so the diversity of habitats is rich. Urbanization will further lead to the natural landscape gradually replaced by artificial landscape, especially the building and artificial trees gradually replace the trend of natural forest land is difficult to reverse in the short term, and urban environmental pollution is serious. Urban birds suitable for habitat is highly fragmented, is a typical isolated island habitat; The various habitat types, small relative area and enhanced manual management make the urban environment have mosaic characteristics, numerous boundaries and high heterogeneity. Urban bird habitats often undergo drastic changes, such as soil erosion, herbicide and pesticide use, drainage and the disappearance of shrub layers and other factors can cause habitat loss and isolation; Overall, urban bird species diversity is relatively low, but the number of species is sometimes quite large. Species that are sensitive to human disturbance and birds that have strict habitat requirements are generally difficult to adapt to urban ecosystems. The impact of urbanization on bird communities is twofold: higher heterogeneity provides abundant available resources for birds while human disturbance and habitat loss result in decreased diversity and density.

4.1.2 Impact of urbanization on bird communities

A. Effects of urbanization on bird community composition

Habitat landscape conditions, such as topography, presence or absence of water and vegetation community structure, directly affect the composition of animal communities. Urbanization leads to significant changes in urban landscape, which leads to the development of urban bird community in the direction of bad succession. Insect - eating birds, ground nesting or tree-hole nesting birds, etc., which are common in natural forest land, have a decreasing trend in species and quantity in cities [6], because these birds, especially some " area sensitive" birds, do not like to appear in smaller forest land.

Many non-urban habitats are often successfully adapted to urban habitats. For example, parakeet's native to Papua New Guinea rain forests appear in some highland towns in the country, while coastal birds, white kingfisher, extend to parks in Singapore and so on. There are some exotic species, the population increases with urbanization, and gradually become dominant species, such as sparrows, pigeons, floor Yan, etc. But the species that inhabit the artificial green space are generally far fewer than those that inhabit the native forest land. The smaller the area of the primary ecosystem remains, the greater the likelihood of species composition changes and extinction and the invasion of exotic birds [7].

B. Effects of urbanization on bird community structure

Studies have shown that the island of habitats has caused urban birds to suffer the same adverse effects as other fragmented landscapes. It is generally believed that with the increasing degree of urbanization and the resulting changes in urban landscape, the species of birds in the city will be reduced and species diversity will be reduced.

Some researchers argue that moderate urbanization helps to increase the richness and species diversity of bird communities, which validates the so-called moderate interference theory with urban bird communities [8]. Research shows that the diversity of birds in urban parks is significantly higher than other urban habitats. There is also a phenomenon that although there are few species of birds in cities, the number is much higher than that in neighboring rural areas, such as 21 species in Helsinki, Finland, and 54 species in neighboring virgin forest areas, but the number and weight of birds per km2 in cities are 3 and 10 times higher than those in forests, respectively.

Other studies have found that winter urban parks have more species and larger numbers of individuals than surrounding rural or forested areas, and this difference is particularly pronounced in northern Europe, which may be associated with winter feeding in urban parks. Winter feeding can help birds adapt to the urban environment, not only affect bird density and species richness, but also change their species composition. Birds in areas with higher levels of urbanization make greater use of food tables than in areas with lower levels of urbanization.

C. Effects of urbanization on bird reproduction

With the acceleration of urbanization and the change of urban landscape, many birds lose their habitat in the city. Ground nesting birds are reported to be rare in urban parks, and lack of hidden plants, lack of suitable nesting sites or competitive pressures in nesting areas may limit the number of ground nesting birds [9]. Studies conducted in Finland suggest that nest birds and deciduous forest birds prefer urban parks, which may be related to the characteristics of individual bird species, resource availability, high diversity of habitats in large parks, nest occupancy and intra-species competition. Some urban parks benefit from the large number of artificial nests in the park, and studies have shown that birds have a higher success rate in nesting than in natural caves. Another feature is that birds in isolated habitats tend to nest high.

Some studies have found that human activities may affect the success rate of urban birds breeding, among which the entertainment of urban parks is the main factor affecting the habitat and breeding of birds in urban forest land. However, some scholars found in the study, surrounded by high-density buildings in the park of human activities did not affect the breeding of birds, this may be because the park 's recreational use frequency is not big, or the impact of human activities compared with other variables is relatively small. For example, many species of crow birds around the world have spread from their original distribution area to urban environment nest breeding, and have become very accustomed to human activities. Perhaps less harm from humans is the main reason for these changes. In addition, waste and feeding in cities may also be one of the reasons for the high population density of crows.

V. CONCLUSION

Urbanization is the process of globalization's artificial environment change, and the effect of urbanization on birds is explored on a multi-scale basis. The results have a lot of enlightenment for us to study the relationship between human,

wildlife and environment. Because urbanization has a dual impact on urban bird diversity, we should take different measures to protect urban birds for different habitats. To strengthen the protection of Zijin mountain, Mufu mountain, Qixia mountain, Tang mountain, Fangmountain, Lao mountain, to minimize artificial interference and blind development and utilization; To adopt effective measures to restore these degraded ecosystems in the wetlands such as Xuanwu lake, Mochou lake and the wetlands along the river; For urban park green space and residential areas these birds indispensable important habitat, can increase plant species, the establishment of natural tree species of islands, make artificial landscape diversity. Through these measures to protect urban birds, promote the formation of a good bird - man - environment three-in-one symbiotic circle.

ACKNOWLEDGEMENTS

This work is supported by Jiangsu Agri-animal Husbandry Vocational College (Grant No. NSFPT201602, Grant No.00010115015), It is also supported by Jiangsu Second Normal University and “333 projects of Jiangsu province”; “Jiangsu Province Blue Project ([2016] No. 15)”.

REFERENCES

[1] Zhang X.L. Investigation of bird diversity in different habitats of Sichuan nature reserve, Sichuan animal, 2007,03, pp. 57-59.,

[2] Ou Yang. Bird community structure and seasonal dynamics in Suzhou Industrial Park. M.S. Thesis, Nanjing: Nanjing Forestry University., 2013.

[3] SunHanru. Study on land use change based on CCA. M.S. Thesis, Beijing: China University of Geosciences., 2014.

[4] Chen Shuihua, Ding Ping, Zheng Guangmei. Urban bird community ecology research prospect.Chinese Zoology, 2000,21 (02), pp.165-169.

[5] Cheng Kun, Ma Jianzhang. Urban bird management.Chinese Journal of Nature, 2008, 30 (02): pp.90-93.

[6] Chen Yuan. Study on the relationship between urban birds and garden plants. M.S. Thesis, Chongqing: Southwestern University; 2010.

[7] Chen Shuihua, Wang Yanping, Lan Sisi.The influence of urbanization on birds: from the community to the individual. [C]// Symposium on birds on both sides of the Taiwan Straits. China Zhejiang Hangzhou., 2013: 1.

[8] Lu Weiwei.Study on the influence of urbanization on the bird community and the adaptability of birds. M.S. Thesis, East China Normal University, 2007.

[9] Blair R B. Birds and Butterflies along an Urban Gradient: Surrogate Taxa for Assessing Biodiversity. Ecological Applications, 1999, 9(1), pp.164-170.

[10] Jokimäki J. Occurrence of breeding bird species in urban parks: Effects of park structure and broad-scale variables[J]. Urban Ecosystems, 1999, 3(1), pp.21-34.

The Effects of Grape Seed Powder and Extract on Antimicrobial of Fermented Turkish Sausage

Zeynep Koç

Department of Horticulture, Süleyman Demirel University, Isparta

Abstract— *In this study, the effects of the grape seed powders and extracts of from two different grape cultivars (Razakı and Siyah Gemre) on the quality characteristics of Turkish sausage were investigated during the ripening period. Some characteristics of Turkish sausage including phenolic content, radical scavenging, hydrogen peroxide scavenging, Fe^{+2} chelating activity, Inhibition of Escherichia coli, Staphylococcusaureus, Candida albicans were studied. In fermented sausage, grape seed powders and extracts demonstrated the greatest inhibitory activity against Staphylococcus aureus. Furthermore, the results showed that Siyah Gemre grape seed extract was able to reduce Staphylococcusaureus populations by 42 CFU/g, while the population of Escherichia Coli was reduced by 590 CFU/g. Siyah Gemre grape seed extract was able to reduce Candida albicans populations by 880 CFU/g. Also this study demonstrated that grape seed extracts were more effective than grape seed powders. Our results suggest that the use of grape seed extract is a feasible alternative as antibacterial and antioxidant agents to prevent the deterioration of foods by bacteria and oxidation.*

Keywords— Grape seed powder and extract, Razakı and Siyah Gemre antimicrobial, antioxidant, natural preservative.

I. INTRODUCTION

The main changes (microbial, chemical and sensory) affecting the quality and durability of sausage, which is one of the important meat products of the food sector, occur during the maturation process of sausages [1].Not paying attention to technological and hygienic conditions during the maturation stage of the sausages affects human health negatively. Many synthetic preservatives are currently being used to reduce microbial growth and thereby extend the shelf-life of sausage. However, synthetic preservatives have restricted use in foods as these agents are known to be carcinogenic [2].The medicinal and nutritional value of grapes (*Vitis vinifera*) has been know for thousands of years. Among other beneficial effects of parts of a grape, grape seeds are believed to have a powerful antioxidant property due to its rich source of polyphenol compounds. The polyphenol compounds are as much as 60-70 % in grape seeds compared only 10 % in the fruit and 28 - 35 % in the peels [3,4].The aim of this study was to determine the effect of two different grape seeds powder and extracts on antimicrobial of Fermented Turkish sausages.

II. MATERIAL AND METHOD

2.1 Material

The day after slaughter, three-year-olds beef, beef subcutaneous fat and sheep's tail fat were obtained from Biçici sausage ltd., Sti., in Afyonkarahisar (Turkey). Spices used in sausage making was obtained from Soylu spices ltd., Sti., in Afyonkarahisar (Turkey). Razakı and Siyah Gemre grapes cultivars was obtained from public market in Isparta (Turkey). In the study, different concentrations of grape seed powder and extract obtained from Razakı and Black Gemre varieties were added to sausage during the fermentation process.In research was used*Escherichia coli* ATCC Kodu:25922, *Staphylococcus aureus* ATCC kodu:25923 and *Candida albicans* for microbiological analyzes.

2.1.1 Preparation of Grape Seed Powder and Grape Extract

A grape seed obtained from bunches of grape varieties mature Razakı and Black Gemre were washed and cleaned, then dried in the shade. Dried grape seeds were triturated in a laboratory-type mill to obtain powder. The resulting grape seed powder was extracted with petroleum ether at 60 ° C for 6 hours in a Soxhelet extraction apparatus, and then the oil was separated. The remaining oil-free powder was again extruded in a Soxhelet extraction apparatus for 8 hours at 60 ° C with a solution of acetone: water: acetic acid (90: 9.5: 0.5) [5]. The extracts were passed through Whatman 1 filter paper and completely freed from solvent in a rotary evaporator, then freeze-dried in a lyophilizer.

2.1.2 Sausage Production

The meats are first made into the meat cubes in hygienic conditions and spices are added on them. Raw materials and additives used in sausage production are given in Table 1. The mixture was then passed through a mincemeat machine with frozen fat. The dough passed through the mincing machine was allowed to mature at 4°C for 8 hours. Then, grape seed, grape seed extract and bacterium were added into the sausage dough and mixed at a speed of 10-12 rpm for 1 hour and then filled into natural sheaths. Sausages stuffed in natural sheaths are attached to hanging carts by cotton threads. Samples of sausages were taken for microbiological and other analyzes at this stage. The sausages that were filled and put in carts were kept in equilibrium rooms (70-75 % relative humidity at 10-15°C) for 12 hours. Sausages kept in balancing rooms have been subjected to a certain period of submergence. The sausage samples were subjected to drying and maturation for 12 days in the incubation chamber. Sausage samples remaining in the incubation chamber were stored at 25°C and 90 % relative humidity for the first 2 days, 22°C and 80 % relative humidity for 3-4 days, 20°C and 70% relative humidity for 5-7 days, 18°C and 65% relative humidity for 8-10 days, it was maintained at 18°C and 60% relative humidity until the 12th day of maturation. During maturation, the air flow in the environment was set at 0.5 m / s and kept constant. Sausage production was done twice in the same quantity and conditions.

TABLE 1
RAW MATERIALS AND ADDITIVES USED IN FERMENTED TURKISH SAUSAGE

Materials	K	N	RT1/ SGT1	RT2/ SGT2	RT3/ SGT3	RT4/ SGT4	RE1/ SGE1	RE2/ SGE2	RE3/ SGE3	RE4/ SGE4
Meat cubes (kg)	5	5	5	5	5	5	5	5	5	5
Tail fat (g)	500	500	500	500	500	500	500	500	500	500
Salt (g)	5	5	5	5	5	5	5	5	5	5
Garlic (g)	75	75	75	75	75	75	75	75	75	75
Chili pepper (g)	35	35	35	35	35	35	35	35	35	35
Red pepper (g)	20	20	20	20	20	20	20	20	20	20
Black pepper (g)	35	35	35	35	35	35	35	35	35	35
Cumin	35	35	35	35	35	35	35	35	35	35
Pimento (g)	10	10	10	10	10	10	10	10	10	10
Grape seed powder (ppm)			250	500	1000	2000	250	500	1000	2000
Grape seed extract (ppm)			250	500	1000	2000	250	500	1000	2000
NaNO2 (g)		0.5								

RT1/SGT1: Razakı grape seed powder 250 ppm, RT2/SGT2: Razakı grape seed powder 500 ppm, RT3/SGT3: Razakı grape seed powder 1000 ppm, RT4/SGT4: Razakı grape seed powder 2000 ppm, RE1/SGE1: Siyah Gemre grape seed extract 250 ppm, RE2/SGE2: Siyah Gemre grape seed extract 500 ppm, RE3/SGE3: Siyah Gemre grape seed extract 1000 ppm, RE4/SGE4: Siyah Gemre grape seed extract 2000 ppm

2.2 Method

2.2.1 Detection of antioxidant activity values of grape seed extracts

2.2.1.1 Determination of the effect of free radical scavenging

2.5 g of GSE was weighed and extruded by homogenizing in a total of 50 ml of 50% aqueous methanol on an agitator (Ultra Turrax). Extracts from the coarse filter paper were centrifuged at 4000 x g for 3 minutes in a refrigerated centrifuge at 4 ° C and the blue band (No 589) was filtered through filter paper once the filtrate was complete to 50 ml.To determine the effect of extracts on DPPH added to the medium, Brand-Williams [6] method has been modified and used. For this purpose, dilutions from methanol to 0.4-4 mg / ml were prepared from the obtained extracts. Take 0.1 ml of each dilution and add 3.9

ml of DPPH solution prepared with $6x10^{-5}$ M methanol and mix well. Samples were left in the dark and at room temperature for 60 minutes. Then the absorbance at 515 nm was measured against methanol. The absorbance of DPPH radical is taken as a control.

The linear regression equation of the absorbances against concentrations was established by reading the absorbances of the serial solutions prepared at seven different concentrations from the DPPH (6×10^{-5} M) stock solution at 515 nm:

A (515 nm)=14,346 (C DPPH)-0:0188 (R^2=0,981)

The residual DPPH concentrations corresponding to the absorbance readings of the sample series prepared at different concentrations were calculated,

DPPH percentage:

$$\% \text{ residual DPPH}^{\cdot} = [\text{DPPH}^{\cdot}]_{sample} / [\text{DPPH}^{\cdot}]_{control}$$

A regression equation was established between the percentage of residual DPPH and the amount of sample in the test medium to the amount of DPPH (mg sample / mg DPPH), and sample concentration values were determined which reduced the initial DPPH concentration by 50% for the grape seed extract using this equation. The EC50 values were divided one to one and the antiradical activity (AE) was calculated to be 1 / EC50 value.

2.2.1.2 Detection of chelating activity with Fe^{+2}

The methods used to determine the chelating activity of Fe^{+2} of extracts were modified by Rival et al. [7]. Extracts prepared at different concentrations ranging from 6-45 mg / ml were taken 1 ml of sample and mixed with 3.7 ml of deionized water. Then 0.1 ml of 2 mM $FeCl_2$ solution was added, followed by thorough mixing and incubation for 70 minutes in the dark and at room temperature. Subsequently, 0.2 ml of 5 mM ferrozine was added again, and the absorbance of the Fe^{+2} - ferrosin complex formed after 10 minutes was measured at 562 nm. 1 ml of water was used instead of the sample in the control reading. The chelating capacity of the samples with Fe^{+2} was calculated by the following equation [8]:

% chelating activity = [1- (sample absorbance / control absorbance)] x 100

2.2.1.3 Determination of H_2O_2 disintegration effect

H_2O_2 remove ability of the plant extract can be determined spectrophotometrically [9.] For this, 1 ml (2, 6 and 10 mg / ml) sample was mixed with 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.6 ml of the same buffer prepared with 43 mM H_2O_2 and incubated for 60 min. Then the absorbance of the mixture was measured at 230 nm. A control solution without H_2O_2 was prepared for each sample concentration. The linear regression equation was used to determine the concentration of unreacted H_2O_2 (mM) in the medium. For this, 0.6 ml of 10, 15, 25, 43 and 50 mM H_2O_2 solution was added to 3.4 ml of phosphate buffer and the absorbance at 230 nm was measured, and a linear regression equation was drawn by plotting the absorbance versus concentration:

(+) - catechin was used as reference antioxidant. The H_2O_2 removal capacity of the samples was calculated by the following equation:

H_2O_2 removal capacity (%) = [1- (In the example H_2O_2conc./Control H_2O_2 conc.)] x 100

2.2.1.4 Determination of Total Phenolic Substance Content

The total phenolic substance contents of the samples were analyzed using the Folin Ciocalteu colorimetric method [10]. Reading was carried out on a spectrophotometer using a wavelength of 765 nm. Values are calculated as mg GA / g. The total phenolic substance content was given as gallic acid (GA) equivalent.

2.2.2 Detection of the effects of the applications on the bacteria Escherichia coli and Staphylococcus aureus on Turkish Fermente Sausage

A sample of 25 g sausage was homogenized with 225 ml dilution fluid (sample volume x 9 diluent fluid). *Escherichia coli* and *Staphylococcus* aureus kit were used to read the nutrient bar code and the broth was opened with 3 ml sterile pure water dispenser. One ml of the sample from the stomacher bag was added to the bottle and the vortex was placed in the filling stand

for a few seconds after mixing. The sample was filled in the card and then removed from the device after being sealed. Cards placed in the incubation stand were left to incubate at 37 ºC for 24-27 hours. Cards filled with the last incubation period were placed in the device and read [11].

2.2.3 Detection of the effects of applications Turkish Fermented Sausageon *Candida albicans* yeast

A sample of 25 g sucuk was homogenized with 225 ml dilution fluid (sample volume x 9 diluent fluid). To the Tempo device, a nutrient bar code was read from the Candida albicans kit and placed in a 3 ml sterile pure water dispenser. One ml of the sample from the stomacher bag was added to the bottle and the vortex was placed in the filling stand for a few seconds after mixing. The sample was filled in the card and then removed from the device after being sealed. Cards placed in the incubation stand were left to incubate for 72-76 hours at 25 ºC. Cards filled with the last incubation period were placed in the device and read [11].

2.2.4 Statistical analyzes

The data obtained were evaluated in the SAS statistical program [12]. Variance analysis was used in the General Linear Modeling implementation, and the Tukey test was used when the differences between the groups were determined [13].

III. RESULT AND DISCUSSION

3.1 Antioxidant Activity Values of Grape Varieties

The antioxidant capacities of grape varieties were evaluated in terms of antiradical activity, H_2O_2 inhibition and the total amount of phenolic compounds. Table 2 shows the results of the experiments. DPPH is a free radical compound widely used to determine the free radical scavenging ability of various extracts [3]. When the antiradical activities of the grape samples were compared, the highest value was found in the extracts obtained from Black Gemre grape, which is a black colored variety with 0.88%. The antiradical activity of the extract, which was obtained from Razakı grape seeds (a white variant), was determined to be 0.456%. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant. 64.15% of the Fe^{+2} chelating activity of the extract obtained from black Gemre seeds and 54.21% of the Fe^{+2} chelating activity of the extract obtained from the Razakı seeds were determined. When the H_2O_2 inhibitory activities of the grape varieties were compared, the inhibition value of the extract obtained from Black Gemre seeds was 78.67% and the inhibition value of the extract obtained from Razakı seeds was 57.71%. Phenolic compounds are extremely important in terms of antioxidant activity value. The total amount of phenolic compounds of the grape varieties in the study was determined as 139,16 mg gallic acid / g in Black Gemre and 123,54 mg gallic acid / g in Razakı variety

TABLE 2
ANTIOXIDANT ACTIVITY VALUES OF THE VARIETIES OF GRAPES

Group	Antiradical activity (%)	Fe^{+2} chelating activity (%)	H_2O_2inhibition (%)	Total amount of phenolic substance (mg gallic acid / g)
RE	0.456 [b*]	54.21 [b]	57.71 [b]	123.54 [b]
SGE	0.88 [a]	64.15 [a]	78.67 [a]	139.16 [a]

**** The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p> 0,05)***

3.2 Effects of *Staphylococcus aureus* applications on Turkish Fermented of Sausage

Staphylococcus aureus values at the fermentation stage of the sausages are shown in Table 3. It was observed that the difference between the values of *Staphylococcus aureus* was significant (p> 0.05) during the fermentation process of the samples. *Staphylococcus aureus* according to applications throughout the ripening period in general values. This decrease occurs when N, RE4, SGT2, SGE2, SGE3 and SGE4 administration continued until the 12th day, but 6th day it is not statistically significant after the day. According to control decrease in *Staphylococcus aureus* values in all treatments has arrived. Grape seed extract and powder have an important effect on *Staphylococcus aureus* bacteria. In many studies, the results obtained from different applications of grape seed powder and extract are similar to our study. The reason for this is that grape seed extract is rich in polyphenols and therefore has antibacterial activity as a powerful antioxidant[15,16].

TABLE 3
EFFECT OF APPLICATIONS ON SAUSAGE *STAPHYLOCOCCUS AUREUS* (CFU/G)

	Maturation time (days)				
Sample	**0. 1 d**	**3 d**	**6 d**	**9 d**	**12 d**
N	17916 ^{a*A**}	4150 ^{cbB}	2300 ^{bcdeBC}	1700 ^{cC}	250 ^{cC}
K	18833 ^{aA}	8200 ^{aB}	7150 ^{aB}	7700 ^{aB}	6800 ^{aB}
RT1	18000 ^{aA}	3450 ^{bcdeB}	2500 ^{bcdeB}	1400 ^{cB}	940 ^{bB}
RT2	9600 ^{Ba}	3700 ^{bcdB}	2700 ^{bcdC}	1301 ^{aD}	910 ^{bD}
RT3	9400 ^{bA}	4350 ^{bB}	3350 ^{bBC}	3000 ^{bC}	925 ^{bD}
RT4	9050 ^{bA}	1850 ^{fC}	1550 ^{edC}	3000 ^{bB}	930 ^{bC}
RE1	9250 ^{bA}	2250 ^{defB}	2100 ^{cdeB}	1300 ^{cBC}	820 ^{cbC}
RE2	9000 ^{bA}	4100 ^{bcB}	2250 ^{bcdeC}	1800 ^{cCD}	945 ^{bD}
RE3	8500 ^{bA}	2700 ^{cdefB}	2200 ^{bcdeBC}	1650 ^{cCD}	1100 ^{bD}
RE4	9050 ^{bA}	1850 ^{fB}	1550 ^{deBC}	1250 ^{cBC}	935 ^{bC}
SGT1	9050 ^{bA}	4250 ^{bB}	2400 ^{bcdeC}	1350 ^{cCD}	940 ^{bD}
SGT2	8450 ^{bA}	2300 ^{defB}	1800 ^{deBC}	1500 ^{cBC}	835 ^{bcC}
SGT3	8250 ^{bA}	4050 ^{bcB}	1950 ^{deC}	1650 ^{cDC}	975 ^{bD}
SGT4	8700 ^{bA}	4450 ^{bB}	3150 ^{bcC}	1250 ^{cD}	840 ^{bcD}
SGE1	8600 ^{bA}	3050 ^{bcdefB}	2150 ^{cdeBC}	1450 ^{cC}	920 ^{bD}
SGE2	8300 ^{bA}	2150 ^{efB}	1750 ^{deBC}	1250 ^{cBC}	830 ^{bcC}
SGE3	8200 ^{bA}	2400 ^{defB}	1500 ^{eBC}	880 ^{Cc}	420 ^{cC}
SGE4	8800 ^{bA}	2200 ^{defB}	1400 ^{eBC}	840 ^{Cbc}	455 ^{cC}

**** The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p> 0,05)***
***** The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0,05)***

3.3 Effects of Escherichia coli applications on Turkish Fermented of Sausage

Escherichia coli values in the pipening stages of the sausages are shown in Table 4. It was observed that the applications were significant (p> 0.05) in terms of *Escherichia coli* values during the fermentation process. During the maturation period, Escherichia coli values decreased. It was observed that the measurements made on different days were statistically significant, and during the 12th day of application, the values of *Escherichia coli* except K application decreased. Studies conducted to investigate the effect of grape seed extract on *Escherichia coli* have shown that grape seed extract reduces the number of *Escherichia coli*. This is due to the anti-bacterial properties of grape seeds. Similar results were obtained in our study [17,18,19].

TABLE 4
EFFECTS OF THE APPLICATIONS ON *SAUSAGE ESCHERICHIA COLI* (CFU/G)

	Maturation time (days)				
Sample	**1. 1 d**	**3 d**	**6 d**	**9 d**	**12 d**
N	13500 ^{abcd*A**}	9500 ^{bB}	3550 ^{bC}	1400 ^{bC}	675 ^{Bc}
K	16500 ^{aC}	73500 ^{aBC}	130000 ^{aB}	135000 ^{aB}	675000 ^{aA}
RT1	13500 ^{abcdA}	9250 ^{bB}	7500 ^{bB}	4700 ^{bC}	1900 ^{bD}
RT2	12000 ^{abcdeA}	9450 ^{bB}	7500 ^{bB}	4750 ^{bC}	1750 ^{bD}
RT3	11500 ^{bcdefA}	9550 ^{bAB}	7250 ^{bB}	4550 ^{bC}	1650 ^{bD}
RT4	10500 ^{cdefgA}	9650 ^{bAB}	7700 ^{bB}	5000 ^{bC}	1600 ^{bD}
RE1	14500 ^{abcA}	9800 ^{bB}	7200 ^{bC}	4500 ^{bCD}	2100 ^{bD}
RE2	12000 ^{abcdeA}	9667 ^{bB}	7250 ^{bC}	4200 ^{bCD}	2250 ^{bD}
RE3	16000 ^{abA}	9400 ^{bB}	6850 ^{bC}	3800 ^{bCD}	2000 ^{bD}
RE4	7700 ^{efgA}	5650 ^{bB}	3550 ^{bC}	1900 ^{bD}	1750 ^{bD}
SGT1	9400 ^{defgA}	7200 ^{bB}	3750 ^{bC}	1600 ^{bD}	1100 ^{bD}
SGT2	9200 ^{defgA}	7300 ^{bB}	4250 ^{bC}	1450 ^{bD}	970 ^{bD}
SGT3	6900 ^{fgA}	4600 ^{bB}	3200 ^{bC}	1500 ^{bD}	1400 ^{bD}
SGT4	6400 ^{gA}	4200 ^{bB}	3050 ^{bC}	1300 ^{bD}	1000 ^{bD}
SGE1	7900 ^{efgA}	6350 ^{bB}	4200 ^{bC}	1450 ^{bD}	955 ^{bD}
SGE2	6950 ^{fgA}	5250 ^{bB}	2850 ^{bC}	1050 ^{bD}	705 ^{bD}
SGE3	6450 ^{gA}	4100 ^{bB}	2400 ^{bC}	1100 ^{bD}	890 ^{bD}
SGE4	6850 ^{fgA}	4650 ^{bB}	2250 ^{bC}	955 ^{bD}	590 ^{bD}

**** The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p> 0,05)***
***** The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0,05)***

3.4 Effects of *Candida albicans* applications on Turkish Fermented of Sausage

Candida albicans values in the stages of ripening of sausages are shown in Table 5. It was observed that the difference between *Candida albicans* values was significant (p> 0.05) during the fermentation process of the applications. The decrease in *Candida albicans* values during the maturation period was observed to be statistically significant. When compared to two grape varieties, Black Gemre grape varieties became more effective. Sincerely [20], showed compliance with our study by reporting that Grape seed extract inhibited yeast growth in a variety of yeast investigations.

TABLE 5
EFFECTS OF APPLICATIONS ON SAUSAGE *CANDIDA ALBICANS*(CFU/G)

	Maturation time (days)				
Sample	**2. 1 d**	**3 d**	**6 d**	**9 d**	**12 d**
N	3500 ^{ef*A**}	3050 ^{fghB}	2750 ^{efgC}	2200 ^{cdeD}	1900 ^{cD}
K	3300 ^{efC}	9200^{Bc}	29500 ^{aBC}	68500 ^{aB}	470000 ^{aA}
RT1	14000^{Ab}	13500 ^{aB}	10517 ^{bB}	9600 ^{bB}	94500 ^{bA}
RT2	6900 ^{bcdeA}	6900 ^{bcA}	6300 ^{cA}	5100 ^{cB}	4800 ^{cB}
RT3	3950 ^{defB}	4700 ^{cdefgA}	3950 ^{cdefB}	3250 ^{cdeC}	2900^{cC}
RT4	6400 ^{bcdefA}	4850 ^{cdefgB}	4250^{cdefC}	2850 ^{cdeD}	2950 ^{cCD}
RE1	10000 ^{bA}	5900 ^{cdB}	5250 ^{cdBC}	4250 ^{cdC}	4050 ^{cC}
RE2	14000 ^{aA}	4550 ^{cdefgB}	3850 ^{cdefB}	3200 ^{cdeB}	2850 ^{cB}
RE3	4200 ^{cdefA}	3750 ^{defghB}	3400 ^{defgB}	2850 ^{cdeBC}	2450 ^{cC}
RE4	2800 ^{fC}	5150 ^{cdefA}	4600 ^{cdeAB}	3950 ^{cdeB}	3400 ^{cB}
SGT1	7650 ^{bcdA}	4850 ^{cdefgB}	4050 ^{cdefBC}	3050 ^{cdeC}	2700 ^{cD}
SGT2	9300 ^{bA}	5600 ^{cdeB}	5150 ^{cdeB}	4800 ^{cB}	4250 ^{cB}
SGT3	3900 ^{defBC}	5200 ^{cdefA}	4750 ^{cdeB}	4050 ^{cdeBC}	3550 ^{cC}
SGT4	14000 ^{aA}	12000 ^{aA}	3900 ^{cdefB}	2950 ^{cdeB}	2200 ^{cB}
SGE1	9300 ^{bA}	3400 ^{efghB}	3000 ^{defgB}	1650 ^{deC}	1200 ^{cC}
SGE2	4500 ^{cdefA}	2100 ^{hB}	1350 ^{gC}	985^{Ec}	880 ^{eC}
SGE3	8100 ^{bcA}	2550 ^{ghB}	2000 ^{fgBC}	1450 ^{edC}	1050 ^{eD}
SGE4	14000 ^{aA}	3750 ^{defghB}	3000 ^{defgB}	2050 ^{cdeB}	1650 ^{cB}

**** The difference between the averages with the same lowercase letters in the vertical column is not statistically significant (p> 0,05)***

***** The difference between the averages with the same uppercase letters on the horizontal line is not statistically significant (p>0,05)***

IV. CONCLUSIONS

Antioxidant and antibacterial influences of Black Gemre varieties are more common than Razakı varieties. If the grape kernel is evaluated as antimicrobial and antioxidant, a new alternative product to the food industry will be gained a waste material, which may be a negative effect on the environment, will be evaluated economically. Due to the high antimicrobial and antioxidant properties of grapes, the benefits of heart disease and cancer risk reduction should be exploited on human health. The use of grape seed as a food additive in sausage production is promising.

ACKNOWLEDGEMENTS

The author is grateful to the Süleyman Demirel University Research Fund (3039-D 11) for its financial support of this research work.

REFERENCES

[1] H.Y, Gökalp, M., Kaya, Ö., Zorba. Meat products processing engineering. 3th ed. Atatürk University, Faculty ofAgriculture; Erzurum. Publication No: 786 Shi etal.,potential of grape seed and bearberry extracts in raw andcooked pork. Meat Science, 76(4), 604-610, 1995.

[2] N.G.,Baydar,G., Özkan, S., Yasar.Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control 18: 1131-1136, 2007.

[3] M., Garcia-Marino, J.C., Rivas-Gonzalo, E., Ibanez, C., Garcia-Moreno.Recovery of catechins and proanthocyanidins from winery by-products usingsubcritical water extraction. Anal Chim Acta 563: 44-50, 2006.

[4] H., Nawaz, J., Sh, G.S., Mittal, Y., Kakuda. Extraction of polyphenols from grape seeds and concentration by ultrafiltration. Separation and Purification Technology 48: 176-181, 2006.

[5] G.K., Jayaprakasha, T., Selvi, K.K.,Sakariah. Antibacterial and antioxidant activities of grape (Vitis Vinifera) seed extracts. Food Research International, 36, 117-122, 2003.

[6] W., Brand-Williams, M. E., Cuvelier, C., Berset, Use of Free Radical Method to Evaluate Antioxidant Activity. Lebensm. Wiss.Technology, 28, 25–30, 1995.

[7] S.G., Rival, C.G., Boeriu, H.J., Wichers. Antioxidative Properties andRelevance to Lipoxygenase İnhibition. Journal Agricultural. Food Chemical, 295–302, 2001.

[8] G., Yen and J., Wu. Antioxidant and Radical Scavenging Properties ofExtracts from Ganoderma Tsugae. Food Chemistry 65, 375-379, 1999.

[9] R.J., Ruch, S.J., Cheng, , J.F., Klaunig. Prevention of Cytotoxicity andİnhibition of İntracellular Communication by Antioxidant Catechinsİsolated from Chinese Green Tea. Carcinogenesis 10, 1003–1008,1989.

[10] V. L., Singleton, J. R.,Rossi.. "Colorimetry of Total Phenolics with Phosphomolibdic-Phosphothungstic Acid". American Journal of Enology Viticulturel, 16:144-158, 1965.

[11] Biomerieux TEMPO® EC, 80 004. Automated test for use with TEMPO, for the enumeration of Escherichia coli in 22-27 hours in food, 2012.

[12] SAS Institute. SAS/STAT User's Guide. Version 9.2 Cary, North Carolina, 2009.

[13] Tukey, J. W., Comparing Individual Means in The Analyses of Variance.Biometrics, 5, 99- 114, 1949.

[14] R., Amarowicz, R.B., Pegg, P., Rahimi-Moghaddam, B.,Barl, J.A., Weil.Free radical scavenging capacity and antioxidant activity of selected plantspecies from the Canadian prairies, Food Chem. 84 (4) 551–562, 2004.

[15] P.L., Rhodes, J.W., Mitchell, M.W., Wilson, L.D., Melton. Antilisterial Activity of Grape Juice and Grape Extracts Derived from Vitis Vinifera Variety Ribier. International Journal of Food Microbiology, 107(3), 281–286, 2006.

[16] A., Al-Habib, E., Al-Saleh, AM., Safer, M.,Afzal.Bactericidal Effect of GrapeSeed Extract on Methicillin-Resistant Staphylococcus aureus (MRSA).Journal Toxicol Sci., 35(3), 357-64, 2010.

[17] A.T., Serra, A.A., Matias, A.V.M., Nunes, M.C., Leitão, D., Brito, R., Bronze, S., Silva, A., Pires, M.T., Crespo, M.V., San Romão, C.M., Duarte. In Vitro Evaluation of Olive- and Grape-Based Natural Extracts as Potential Preservatives for Food. Innovative Food Science & Emerging Technologies, 9(3), 311–319,2008.

[18] KF., Over, N., Hettiarachchy, M.G., Johnson, B., Davis. Effect of Organic Acids and Plant Extracts on Escherichia Coli O157:H7, ListeriaMonocytogenes, and Salmonella Typhimurium in Broth Culture Model and Chicken Meat Systems. Journal of Food Science, 74(9), 515-521, 2009.

[19] A.V.S., Perumalla, N.S., Hettiarachchy. Green Tea and Grape Seed Extracts-Potential Applications In Food Safety and Quality, Food Research International, 44 (4), 827–839,2011.

[20] O., Sağdıç, I., Ozturk, M. T., Yilmaz, H., Yetim. Effect of Grape Pomace Extracts Obtained from Different Grape Varieties on Microbial Quality ofBeef Patty. Journal of Food Science, 76(7), 515-521, 2011.

Ensuring Food Security by Reduction of Post-Harvest Fish Losses in Small-Scale Fisheries, Nigeria

Adelaja, Olusumbo Adeolu[1*], Kamaruddin, Roslina. Binti[2], Chiat, Lee Wen[3]

[1,2,3]School of Economics, Finance and Banking, College of Business, Universiti Utara Malaysia, 06010 Sintok

***Abstract*—** *This paper focus on types, causes and how post-harvest fish losses can be reduced in the artisanal fisheries sector to ensure food security and provision of adequate protein for the increasing population. Post-harvest fish loss is a serious threat to the artisanal fisheries sector which occurs from the capture to the final stage of marketing the product to the consumers. When fish undergoes microbiological decay, it leads to quality loss which results to depreciation in the market value. Consumption of such fish has adverse effect on human health; as a result of these, method of assessing post-harvest fish loss and ways of reducing it should be discovered in order to have good quality fish in abundance for the ever growing population and also improved livelihood of fisher folks.*

Keywords*— *Food security, Post-harvest fish losses, Artisanal fisheries sector, Livelihood.

I. INTRODUCTION

Major challenges faced globally are on how to improve food security to meet up with the world rising population and ensure sustainability. Over the years, studies have shown that food security can be improved if fish produced are properly used and this can be done through reduction of post-harvest losses in different distribution stages involved (FAO, 2010, Kumolou-Johnson & Ndimele, 2011).

Food security takes into consideration availability, accessibility, sustainability and utilization of food to ensure people have access to the basic food required (Kumolu-Johnson & Ndimele, 2011). The role of fisheries towards supply of animal protein in many industrialised and developing countries cannot be under estimated (Adewolu & Adeoti, 2010). Some communities in Low-Income Food-Deficit Countries (LIFDCs) rely on fish not just for protein source but likewise as micronutrients source which include essential fatty acids and minerals. Other alternatives for these sources are expensive and fish was considered as a better option (FAO, 2010; Getu *et. al.*, 2015). This was in agreement with Domingo *et. al.* (2007) stating that fish has high-quality of protein and provides 17% of total animal protein, 6% protein of human consumption, vitamins and other vital nutrients.

II. ARTISANAL FISHERIES SECTOR IN NIGERIA

In Nigeria, fisheries sector is sub-divided into artisanal, commercial and aquaculture fisheries (Oladimeji *et. al.*, 2013; Okeowo *et. al.*, 2015). Another name for artisanal fisheries is called small-scale fisheries due to the use of out-dated traditional fishing equipment such as small boat, low cost of operation, low capital expenses and low innovation (Adedokun *et. al.*, 2006; Oladimeji *et. al.*, 2013). Fish generate income which serves as source of livelihood for millions of people (Nowsad, 2010). Population of Nigeria which is over 180 million currently has been estimated by United Nations that there is a tendency for increase in demand of fish protein by additional 700,000 metric tonnes by 2020 due to increase in population which is likely to surpass 210 million by then.

According to Food and Agricultural Organization (FAO), fish consumption per person in Nigeria at the moment is 7.5 kilos which is opposing to 18.7 kilos global fish consumption per person. This shows a large deficit of 11.2 kilos of fish consumption per person in the country. Gbolagunte *et. al.* (2012) stated that in adult, total protein intake is over 60% which fish serves as the main factor supplying balanced vitamins, minerals and protein mostly in the rural areas. Fish is a highly perishable food which needs to be suitably handled, processed and marketed to reduce post-harvest losses (Nowsad, 2010). In fisheries sector, quantity and quality losses are very high which leads to nutritional loss of fish. Review of case studies of post-harvest losses in several countries in Africa shows high level of losses both quality and quantity (physical losses) of fishery products Reduction of post-harvest losses started after the mid-1970s food crisis and ever since then the problem still persists. This issue led the United Nations in 1975 to notify the attention of worldwide towards post-harvest losses reduction in developing countries that needs immediate intervention. Studies have shown in underdeveloped and developed countries that losses both quantity and quality takes place at all phases along the production chain, from harvesting, processing, storage, transportation, marketing till it gets to the final consumers (Kabahenda *et. al.*, 2009; Olusegun & Matthew, 2016).

Post-harvest losses reduction has commenced since the food crisis issue in mid-1970s and subsequently the problem continues. As a result of this, United Nations in 1975 seek immediate intervention globally towards reduction of post-harvest losses in developing countries (FAO, 1992; Patience & Campus, 2014). In 1992, meeting was held by Strategy for International Fisheries Research (SIFR) which concluded that developing a systematic approach for fish loss assessment should be the major concern of researchers (Ward, 1996, Mungai, 2014).Fig. 1 shows the three fisheries sub-sector where artisanal fisheries sub-sector account for over 80% of total fish production in Nigeria. But despite this, it is challenged with 30 – 50% post-harvest fish losses of landed weight (Adesehinwa *et. al.*, 2005; Emere & Dibal, 2013; Olusegun & Matthew, 2016). These losses as caused a serious threat to the artisanal fisheries sector in Nigeria. It occurs along the fish distribution chain from capture to marketing stage (Diei-Ouadi &Mgawe, 2011; Olusegun & Matthew, 2016). This issue has been a great concern especially to development practitioners whose main focus is to improve fisher's livelihood, processors, and marketers and ensure food security. FAO code of Conduct for Responsible Fisheries (CCRF) under Article 11.1 recognises the problem which fish loss poses and importance on loss reduction. In other to ensure that the supply shortage of 2.04 million metric tonnes which is as a result of wide gap between demand and supply is met, post-harvest fish losses needs to be reduced (Ward, 1998; Ibengwe & Kristofersson, 2012; CBN, 2014)

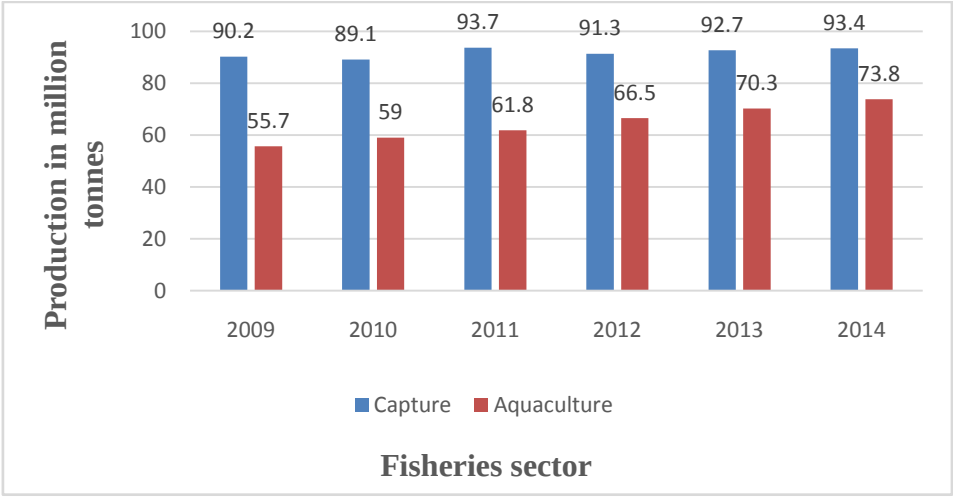


FIGURE 1. WORLD CAPTURE FISHERIES AND AQUACULTURE PRODUCTION FROM YEAR 2009 – 2014
SOURCE: FOOD AND AGRICULTURAL ORGANIZATION (FAO), 2016

III. POST-HARVEST FISH LOSSES

This refers to discarding of fish or selling of fish at relatively low price due to quality deterioration. This leads to potential loss of income for the fishers, processors and marketers. This also makes availability of fish to be reduced to consumers or fish with low quality value is supplied to them (Diei-Ouadi & Mgawe, 2011). It has been discovered that high level of post-harvest losses occurs during handling, processing, storage, transportation and marketing of fish (Singh et. al., 2012; Rahman et. al., 2013). Losses are as a result of poor processing practices causing damage to the fish; insect infestation; inadequate packaging; lack of storage facilities; lack of good roads leading to damage of fish products (Diei-Ouadi & Mgawe, 2011; Mungai, 2014). Post-harvest fish loss has been seen as a constraint to planning at the state and country level due to lack of qualitative and quantitative data.

3.1 Types of post-harvest fish losses

There are three types of post-harvest fish losses physical losses, nutritional losses and economic losses (Ames, 1990; Eyo, 2001).

Physical losses of fish are majorly as a result of poor handling and discard of by catch at sea due to being small or will not attract good value (Olusegun & Matthew, 2016; Diei-Ouadi & Mgawe, 2011). It can be defined as fish loss either entirely or losses which involve certain parts of the fish through insects' infestation, theft of by animal predation (Eyo, 1997). Dried fish which have been infested by beetles or maggots or fish that fall into fire during smoking are all classified under physical loss (Eyo, 1997; Kumolu-Johnson & Ndimele, 2011).

Nutritional losses is defined as nutritional value loss of fish due to spoilage or exposure to high temperature during smoking causing deterioration of fish to the extent that it is not fit for human consumption. Nutritional values decreases in fish as its

spoils because degradation of protein occurs due to bacteria causing spoilage (Eyo, 1997; Kabahenda et. al., 20009; Getu et. al., 2015). Actions of bacteria on the fish produces harmful odours and becomes unattractive for consumption (Kumolu-Johnson & Ndimele, 2011).

Economic losses occur when fish which is meant for human consumption is downgraded due to spoilage which leads to decrease in value. This loss leads to physical and financial losses of product as a result of quality fish deterioration. This fish will be sold out at a lower price against the amount it ought to be sold. This is as a result of lack of appropriate preservation through use of ice for fresh fish, poor transportation and inadequate market information, processed fish are stored for a longer period of time which encourages spoilage to set in and fish degradation occur resulting to selling in low prices (Eyo, 1997; Diei-Ouadi & Mgawe, 2011; Hassan, Rahman, Hossain, Nowsad, & Hossain, 2013).

Market losses are caused due to shortage existing between demand and supply which leads to fish price changes. If there is a fall in fish price due to oversupply, marketers experience market loss (Mungai, 2014; Olusegun & Matthew, 2016).

3.2 Causes of post-harvest fish losses

3.2.1 Fish spoilage

Fish is highly perishable and spoils immediately after death as soon as rigor mortis sets in (Getu et. al., 2015; Mohammed, 2015; Adeyeye, 2016). Rigor mortis is the stiffening of fish muscle tissue which starts between 1 – 7 hours after death. The sum of duration is calculated to be between 30 – 120 hours while fish that are suffocated and not preserved with ice indicate shorter period of fish muscle toughening between 32 – 93 hours. Studies have shown that rigor mortis stays for a longer period when fish has utilized less muscular movement prior to death and refrigerated immediately (Huss, 1995; Cook, 2008; Santoso & Yasin, 2010). Prior to and throughout the rigor mortis stage, fish should be handled properly during catching, removal from the net, throwing of fish should be done with little bruise on board and anaesthesia should be given to the fish (Shim & Cho, 2014). Reviewed literatures explains that immediately fish dies, device used for defence stops while enzymatic, oxidative and bacteriological spoilage set in to cause deterioration of fish quality (Diei-Ouadi & Mgawe, 2011). Assessment of post-harvest losses studies revealed that fish losses of about 10 to 12 million tonnes per year is caused by spoilage while estimate shows that 20 million tonnes of fish are discarded at sea yearly (FAO, 2010, Kumolu-Johnson & Ndimele, 2011).

Findings have revealed that high temperature leads to fish spoilage at different stages of fish production particularly in the tropical countries. Activities of micro-organisms, protein and fat oxidation in the fish body is increased (Ababouch, 2005; Mungai, 2014). Also, Diei-Ouadi and Mgawe (2011) support the findings stating that high temperature of about 20^0C create avenue for fish spoilage while low temperature of 5^0C slows down bacteria activity and spoilage rate. Signs of fish spoilage include slime formation, texture changes and discoloration with time and off-odours detection. Olusegun and Matthew (2016) revealed that high fish spoilage at the landing site was a s result of poor handling practices by the fishers. It was discovered that no washing, gutting and clean storage of fish was done on board or at the landing site.

3.2.2 Time

Studies by Bataringaya (2007) indicated that delay of fish arrival at the landing site in respect to time also cause fish rejection due to microbial growth. Rate of spoilage increase when landed fish are not iced immediately before selling to the middlemen. This is in agreement with Diei-Ouadi and Mgawe (2011) findings which stated that fresh fish spoilage rate is influenced by time and hours spent in the fishing nets. Also, Bolorunduro, Adesehinwa and. Ayanda, (2005) and Kumolu-Johnson and Ndimele, (2011) stated that time in between fish catch and preservation preparation also leads to fish losses. According to Abbas, Saleh, Mohamed & Lasekan,(2009) several biological and chemical changes occur in the dead fish immediately after capture which is likely to lead to rejection for consumption as a result of spoilage and being harmful to human health. Mungai (2014) suggested that fishermen should reduce the fishing cycle length in order to reduce post-harvest losses and also use ice on-board and adequate training should be provided on fish handling practices to reduce post-harvest losses.

3.2.3 Infrastructural facilities

According to Ghaly, Dave, Budge and Brooks (2010) over 30% of landed fish landed are lost due to bacterial activity as a result of lack of storage facilities and ice for preservation. This leads to softness of fish flesh or watery or tough and dry once bacterial spoilage set in. The fish becomes putrid and inedible for human consumption. Literatures (Kwangwa and Odongkara, 2005; Tesfay & Teferi, 2017) specified that lack of facilities, lack of awareness, poor transportation and

inaccessibility of ice are also part of what leads to fish losses. Table 1 shows the different stages and causes of post-harvest fish losses.

TABLE 1
STAGES AND CAUSES OF POST-HARVEST LOSSES IN FISH PRODUCTION

Stage	Causes	Loss type
Fishing	Fishing involving use of harmful methods such as poison, dynamite which result to harvesting of damaged fish	
	When fishing gear is set for a long period resulting to fish spoilage before gear is hauled.	Physical
	Fish discarded as bycatch or fall from the net	Physical
Fish on board	Exposure of fish to high ambient temperatures at sea during delay of landing of fish.	Physical, Nutritional
	Degut, wash and chill the fish on board is not done	Nutritional
	Mistakenly stepping on fish leads to physical damage	Physical, Nutritional
Unloading of fish	Lack of hygienic practices causing contamination	Nutritional
	Falling of fish from the basket on to the shore	Physical
	Fish exposed to high ambient temperature and kept on the ground for a long bargaining time	Nutritional
Fresh fish marketing	Lack of insulated container and inadequate application of ice	Physical, Nutritional
	During extra-large catches, inadequate preservation capacity e.g. processing equipment, ice	Physical, Nutritional
	Traders deliberately delayed purchasing of fish	Nutritional
During processing and packaging	Processing of spoilt or poor quality fish	Physical, Nutritional
	Infestation of blowfly through processing of fish under unhygienic conditions	Physical, Nutritional
	Over smoking or burning of fish due to uncontrollable heat intensity during processing	Physical, Nutritional
	Unsupervised drying of fish on ground, rocks or herbs	Physical, Nutritional
	Inadequate packaging method leading to fish breakage	Physical, Nutritional
Storage	Spoilage caused by growth of mould making fish damp	Nutritional
	Insect infestation on fish during storage	Physical, Nutritional
	Fish discoloration due to chemical changes	Nutritional
	Inadequate storage facilities	Physical, Nutritional
Distribution	Fish breakage during transportation	Physical
I.	Breakdown of transport vehicles resulting to delays and inaccessibility of production areas.	Physical, Nutritional
Marketing	Inadequate cold-storage facilities and lack of ice	Physical, Nutritional
	Selling of fish delay	Nutritional
	Fish supply to the market at the wrong time	Market
	Low purchasing power of consumers	Market

Adopted from Diei-Ouadi and Mgawe (2011)

3.3 Ways of reducing post-harvest fish losses

Reduction of post-harvest losses in fisheries sector becomes paramount after understanding the causes and stages at which it occurs in order to proffer solution. Handling practices is one of the major point of reducing fish post-harvest losses. According to Huss (1994) and Kyangwa and Odongkara (2005) the major practice for safeguarding fish quality and safety is through fish handling. This is in agreement with Das, Kumar, Debnath, Choudhury and Mugaonkar (2013) stating that fish quality depends on the handling method used during landing, processing, storage, packaging and transportation. Careful methods will retard spoilage, reduce losses and improve the quality of the marketed produce. Quality of fish and safety is the major concern of consumers which can be achieved through adequate hygienic practices among fishermen and fish processors (Balasubramaniam, Charles & Krishna, 2009; Singh et. al., 2012). Also, Olusegun and Matthew (2016) revealed that reduction of fishing time to minimum of 12 hours will help the fish caught to remain in good condition. In addition, good handling practices of fish on board by washing, gutting and storing in clean containers will help to reduce losses.

IV. CONCLUSION

Fishermen needs to be trained on proper fish handling and hygiene to ensure food safety production. Provision of infrastructural facilities such as cold storage equipment, processing facilities and good road network to maintain good quality of fish and ease transportation. Ice should be provided for fishermen during capture and landing in order to preserve the fresh fish for a longer period of time. Also access to loan/subsidies should be provided for fisher folks which will help to boost fish production level. Intervention regarding fisheries related policy on post-harvest loss should be set by the government to cover the future development of the fishery.

REFERENCES

[1] Abbas, K. A, Saleh, A M., Mohamed, A, and Lasekan, O. (2009). The relationship between water activity and fish spoilage during cold storage: *A review; Journal of Food, Agriculture &Environment* 7 (3&4): 86 -90.

[2] Adewolu, M.A. and A.J. Adeoti, (2010). Effect of mixed feeding schedules with varying dietary crude protein levels on the growth and feed utilization of *Clariasgariepinus* (Burchell, 1822) fingerlings. *J. Fish. Aquat. Sci.*, 5: 304-310.

[3] Adeyeye, S.A. (2016). Traditional fish processing in Nigeria: a critical review. *Nutrition & Food Science*, *46*(3).

[4] Balasubramaniam, S., J. Charles and Krishna Srinath(2009). Adoption of hygienic practices at Fish Landing Centres and Markets. *Fish. Technology.* 46: 177-184

[5] Bolorunduro, P.I., Adesehinwa, A.O.K. and. Ayanda, J.O. (2005). Adoption of Improved Fish Preservation Technologies in Northwestern Nigeria. Tropicultural, 23: 117-123.

[6] Cook, D. G. (2008). The effects of harvesting procedures on physiological and biochemical properties of Chinook salmon (Oncorhynchustshawytscha) white muscle prior to and during frozen storage.

[7] Diei-Ouadi, Y.and Mgawe, Y. I. (2011). Post-harvest fish loss assessment in small-scale fisheries: A guide for the extesion officer. *FAO Fisheries and Aquaculture Technical Paper*. 93p.

[8] Domingo, J. L., Bocio, A., Falcó, G., and Llobet, J. M. (2007). Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology*, *230*(2), 219-226.

[9] FAO.(2010). Fisheries and aquaculture topics: Food security and fisheries. Topics Fact Sheets. Text by Peter Manning, FAO Fisheries and Aquaculture Department, Rome.

[10] Gbolagunte, G. D., Salvador, A. F., and Enoghase, J. S. (2012). Evaluation of the microbial load on smoked fishfrom some markets in Ogun state, Nigeria. *African Journal of Microbiology Research*, *6*(7), 1376-1379.

[11] Getu, A., Misganaw, K., andBazezew, M. (2015). Post-harvesting and Major Related Problems of Fish Production. *Fisheries and Aquaculture Journal*, 6: 154.

[12] Ghaly, A. E., Dave, D., Budge, S., and Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: review. *American Journal of Applied Sciences*, *7*(7), 859.

[13] Hassan, M. N., Rahman, M., Hossain, M. M., Nowsad, A. A. K. M., and Hossain, M. B. (2013). Post-Harvest Loss and Shelf Life of Traditionally Smoked Shrimp Products Produced in Bangladesh. *World*, *5*(1), 14-19.

[14] Huss, H. H. (1995). *Quality and quality changes in fresh fish* (No. 348). Food & Agriculture Org.

[15] Ibengwe, L., and Kristófersson, D. M. (2012). Reducing Post-Harvest Losses of the Artisanal Dagaa (Rastrineobolaargentea) Fishery in Lake Victoria Tanzania: A Cost Benefit Analysis. Fisheries Training Programme 52pp.

[16] Kabahenda, M. K.,Omony, P., and Hüsken, S. M. C. (2009). Post-harvest handling of low-value fish products and threats to nutritional quality: a review of practices in the Lake Victoria region. *Fisheries and HIV/AIDS in Africa: Investing in Sustainable Solutions, The World Fish Center*.

[17] Kumolu-Johnson, C.A. and A.A. Jimoh, (1997). Quality assessment of fresh and frozen *Oreochromisniloticus* (Trewavas) in some markets in Niger State, Nigeria. J. Prospects Sci., 1: 145-148.

[18] Mohammed, A. E. I. E. (2015). *Post-harvest hazards at Khartoum and Almourda Fish markets* (Doctoral dissertation, UOFK).

[19] Nowsad, A. K. M. A. (2010). Post-harvest loss reduction in fisheries in Bangladesh: A way forward to food security. *Final report PR*, *5*(08), 171.

[20] Olusegun, O. J., and Mathew, O. S. (2016). Assessment of Fish Post Harvest Losses in Tagwai Lake, Niger State, Nigeria. *International Journal of Innovative Research and Development ISSN 2278–0211*, *5*(4).

[21] Rahman, M., Khatun, S., Hossain, M. B., Hassan, M. N., andNowsad, A. A. K. M. (2013). Present scenario of landing and distribution of fish in Bangladesh. *Pakistan Journal of Biological Sciences*, *16*(22), 1488.

[22] Santoso, J., andYasin, A. W. N. (2010). Charateristic Changes of Shark and Stingray Surimi as Affected by Compositioning and Chill Storage of the Mince Fish. *JurnalTeknologi Dan IndustriPangan*, *19*(1), 57.

[23] Shim, K. B., and Cho, Y. J. (2014). Improving the Effects of Cold Saturated Salt Solution Immersion on the Texture of Black Rockfish (Sebastesschlegeli) Muscle. *Turkish Journal of Fisheries and Aquatic Sciences*, *14*, 667-675.

[24] Singh, Y. J., Santhakumar, R., Pandey, D. K., Bharati, H., andDebRoy, P. (2012). Adoption of Hygienic Fish Handling Practices by Fishermen. *Indian Research Journal of Extension Education*, *12*(1), 36-38.

[25] Tesfay, S., &Teferi, M. (2017). Assessment of fish post-harvest losses in Tekeze dam and Lake Hashenge fishery associations: northern Ethiopia. *Agriculture & Food Security*, *6*(1), 4.

[26] Ward, A. (1996).*Quantitative data on post-harvest fish losses in Tanzania. The fisheries of Lake Victoria and Mafia Island.* Technical Report, 73pp.

[27] Ward, A. R. (1998). Field based fish loss assessment methodologies. In a *Report and proceedings of the sixth FAO Expert workshop.*

Effects of Adding Different Levels of Phytaseto Diet Containing Low Phosphorus on Performance, Shell Quality and Bone Parameters in Laying Japanese Quail

Yılmaz BAHTİYARCA[1*], Yusuf KONCA[2]

[1]Selcuk University, Faculty of Agriculture, Department of Animal Science, 42030 Selçuklu/ Konya
[2]Erciyes University, Faculty of Agriculture, Department of Animal Science, Melikgazi-Kayseri

Abstract— *This experiment was carried out to determine the effect of addition of different levels of phytase containing low phosphorus diets on performance, eggshell quality and bone characteristics in laying Japanese quail (Coturnix japonica). In the experiment, a total of 162 7-week-oldlaying Japanese quail were fed two different diets containing 0.33 % available phosphorus (AP, control) and 0.15 % AP supplemented with phytase 0, 500, 1000, 1500, 2000 phytase unites/g feed. Each diet was feed to groups having three replicates, each cages nine quail, in completely randomized design. Feed and water supplied for ad-libitum. Light was provided 17 hours daily. Egg production, egg mass, average daily feed consumption, viability, fat-free dried tibia weight were improved, as phytase level increased in the diet. However, final live weight, carcass weight, body weight gain, feed efficiency, tibia ash (%) and deformed egg ratio (%), some eggshell quality parameters measured in eggs collected at the end of the first period, were not affected by treatments. With regard to measured parameters, the responses of laying quail, to the increasing phytase levels, were not linear. The performance and bone parameters of the group fed with 1500 units were very close to that of the control group, even higher than the control group in some traits. Decreasing in shell weight of eggs collected at last three days of the experiment were not prevented any of the phytase levels. At the same period, shell thickness and breaking strength of the eggs of groups fed with 1500 and 2000 units phytase, were significantly lower than that of the control group. Manure ash percentage of phytase added groups were significantly lower than the control group.*

Keywords— phosphorus, quail, performance, egg quality, bone traits.

I. INTRODUCTION

In order to improve production, nutritionists endeavor to increase digestibility, availability of nutrients in the diet and to obtain more product consumed per unit feed, and therefore they consider total nutrient intake and retention as well as waste output. Although poultry is highly efficient in converting feed to egg or body gain, the presence of components such as antinutritional factors and indigestible nutrients in the feed may reduce production efficiency. These components in the feeds reduce nutrient digestibility and availability by either chemically or physically binding nutrients or by direct toxic action the animal (Nelson, 1971; Adeola and Cowieson, 2011). Availability of phosphorus (P) for monogastric animals in feed ingredients of plant origin is much lower than the P content of those ingredients, because of the greater portion of their total P is present as phytate. Phytate or phytic acid is both an antinutritional factor and an indigestible nutrient (Adeola and Cowieson, 2011). The P contained in the molecule is very low availability to monogastric animals, especially poultry. Because of its chelating ability with essential mineral elements such as zinc, manganese, iron, calcium, potassium, and proteins, phytate reduces the availability of these nutrients through the formation of indigestible complexes (Nelson, 1967).

Recently advances in enzyme technology have led to the development enzyme products to be used as feed additives. Many fungi, bacteria, and yeasts naturally produce the enzyme phytase and some of them already on the market. Nelson et al. (1971) found that when the preparation of phytase made from *Aspergillusficuum* was added to acorn-soybean diet containing low P, phytate phosphorus was almost completely hydrolyzed by the chickens. Recently, heightened environmental concerns over phosphates in animal waste have renewed the interest phytase (Sharpley et al., 2004; Toprak and Yilmaz, 2016)and many research with broiler(Yan et al., 2003) and quail (Sacakli et al., 2006; Saima et al., 2014)were conducted demonstrating enhancements of P availability by phytase addition to diets. Newkirk and Classen (1992) suggested that when phytase and xylanase enzymes were added to diets containing different levels of total P (0.4, 0.5, 0.6, 0.7%) in the laying hens, phytase reduced the feed efficiency and interacted with xylanase to influence egg production. Konca and Bahtiyarca (1996), Bahtiyarca and Parlat (1997),Sacaklı et al. (2006) showed that when the diets have low P were supplemented with phytase, body weight gain, feed conversion ratio was increased and also bone ash and strength were comparable with the control diet has adequate P. On the other hand, with addition of phytase to poultry diets sometimes

revealed controversy results (Adeola and Cowieson, 2011). Therefore the objective of the present study was to determine the effects of different levels of phytase addition to a diet containing low P on the performance, shell quality and bone characteristics in laying Japanese quail.

II. MATERIALS AND METHODS

In the experiment, a total of 162 7-week-old female Japanese quail were used. They were selected from 300 female quail on the basis of body weight. They fed a starter diet containing 23% crude protein, 12.5 MJ ME/kg, 0.80% Ca, 0.45% available P, 1.3% lysine, 0.50% methionine and 0.75% methionine+ cysteine and fed test diets until 7 weeks of age. The experimental diets were prepared based on corn-soybean meal containing adequate level (control, 0.33%) and low level (basal, 0.15%) of available P and the basal diet and supplemented with 0; 500; 1000; 1500 and 2000 units/kg of the phytase (total six groups). Diets compositions are presented in Table 1.

TABLE 1
COMPOSITION OF THE DIETS USED IN THE EXPERIMENT[1]

Ingredients	Adequate available phosphorus (Control diet)	Low available phosphorus (basal diet)
	%	%
Corn	49.5	49.0
Soybean meal (44 % CP)	25.5	25.5
Sunflower seed meal (30%CP)	6.2	8.0
Fish meal (64 % CP)	4.2	3.5
Vegetable fat	7.0	6.0
Limestone	5.3	6.7
Di-calcium phosphate (18 % P)	1.0	0.0
Salt	0.40	0.40
Mineral premix[2]	0.15	0.15
Vitamin premix[3]	0.40	0.40
L-lysine	0.21	0.22
DL-methionine	0.14	0.13
Total	100.00	100.00
Calculated composition		
Crude protein, %	19.92	19.98
Calcium, %	2.76	2.77
Available phosphorus, %	0.33	0.15
Lysine, %	1.27	1.27
Methionine, %	0.51	0.50
Metabolizable energy (MJ/kg)	11.70	11.70

[1]The diets containing phytase were obtained by supplementing thebasal diet with 0, 500, 1000, 1500, 2000 units/g phytase.
[2]Mineral premix furnished per kg of diet: 60 mg Mn; 50 mg Zn; 30 mg Fe; 5 mg Cu; 1 mg I.
[3]Vitamin premix supplied per kg feed: vitamin A, 5500 IU; vitamin D3, 1100 I CU; vitamin E, 11 IU; vitamin B2, 4.4 mg; Ca pantothenate, 9.6 mg; nicotinic acid, 44 mg; choline, 220 mg; vitamin B12, 6.6 µg; vitamin B_6, 2.2 mg; folic acid, 0.6 mg, biotin, 0.11 mg.

Phytase enzyme (Finase™, produced A. niger, Alko Biotechnology, Ltd., Rajamâki, Finland). Phytase preparation has 500.000 phytase units/g (a phytase unit is defined as the amount that liberates 1 nano mole of phosphorus from sodium phytate in one minute under standard conditions). It is recommended that the phytase enzyme should be added at a level of 250.000-1.000.000 phytase units/kg feed (Anonymous, 1993 a, b). The research was carried out for five periods and each

period 28 days (total 140 days) in randomized plot designs with three replicates of nine female quail each. (6 groupx9 quailx3 replicate=total 162 quail). The quail were housed in groups of nine birds in laying wire battery cages that were manufactured in Konya. Feed in mash form and water supplied ad libitum and continual lighting was provided during growing and laying periods.

Body weights of female quail were determined in groups of nine quail at the start and end of the experiment. Egg numbers and given feed were recorded daily and average egg production and feed consumption for every 28 days periods were calculated from these data. Egg weight was determined for all eggs collected at the last three days of every 28 days periods. Egg mass was found to be multiplying daily egg number by average egg weight for every period and feed conversion ratio was found by dividing of average daily feed consumption by egg mass. Shell parameters were measured for eggs collected from quail at 70, 71, 72thdays (1st period) and at the last three days (2ıid period) of the experiment. The surface area of the egg was calculated according to the equation of Carter (1975). The breaking strength of the eggs were determined with Test Instrument of Biological Materials using the procedure described by Öğüt and Aydın (1992). After the eggs were broken, their contents were removed and shell washed with tap water. Five shells from each replicate were selected at random. Two pieces of shell were taken from the equator of the egg and shell membrane was removed and thickness was measured using a micrometer. After the pieces of shell were combined with main shell, they were dried in oven for 24 hours at 105, and air-cooled and weighed. The weight of the shell with membrane was expressed as a percentage of whole egg weight and to calculate shell weight per unit surface area, the weight of shell plus membrane was divided by surface area.

To determine carcass weight all quail in group were sacrificed, defeathered, eviscerated (except heart and lungs) at termination of the experiment and were subsequently weighed per group all quail. Right tibiae of birds were cut, kept in a refrigerator for 24 hours and then cleaned from soft tissues to determine of tibia weight and tibia ash. Tibiae were dried in oven for 24 hours at 105 °C. Fat-free dried tibia weight and percentage tibia ash were determined according to the method of Said and Sullivan (1985). Manure was collected during last five days of the experiment and combined and dried in oven for 24 hours at 105 °C, and air-cooled. Dried manure was a shed in a muffle furnace for 6 hours at 550 °C and percentage of manure ash was calculated.

Statistical analysis was carried out using the statistical software SPSS. Data for all measurement were subjected to ANOVA procedure appropriate for the completely randomized design. Linear, quadratic and cubic effects were observed, however, in the tables only linear effects are given due to similar probability (P) values. Duncan's multiple range tests was used to separate differences between the means. All percentage values were transformed according to the equation below before analysis of variance was performed (Winer, 1971):

$$\text{Transformed value} = 2 \times \arcsin\sqrt{(\%/100)}$$

However, the percentage values are shown in the tables and figures for easy interpretation. Probability significance levels were accepted as $P<0.05$.

III. RESULTS

Dietary treatments did not significant effect on final body weight, body weight gain, and carcass weight of quail. Although for the first three periods of the experiment, there were no significant responses to dietary treatments with regard to egg number or percentage of egg production, egg number of the group fed low AP was significantly lower in period 4 and, egg numbers of the groups fed low AP+0 and 1000 units/g phytase were significantly lower ($P<0.005$) than that of control and 1500 units/g phytase group in period 5. In addition, the percentage egg production of the same groups were significantly lower ($P<0.05$) than that of the control and 1500 units/g phytase groups in periods 4 and 5 (Table 2). Egg number and egg yield of quail were generally increased; when the diet has low AP was supplemented with different levels of phytase. But an increase in egg production was not linear with increasing dietary phytase, especially egg production of quail receiving low AP+1500 units phytase/g was similar to those of control group, even slightly higher than the control group (Table 2). Dietary phosphorus and different phytase levels had no significant effect on percentage uncollectable egg (very thin shell or no shell) and mortality. However, mortality rate of the quail fed diet containing low AP was relatively higher when compared to control group.

The quail fed the diets containing low AP+0 and 1500 phytase levels had egg weight in period 5 significantly higher than that of C group. This effect was not seen in other periods and on average egg weight. But there were significant differences in egg mass among the dietary treatments. The egg mass of the group fed low AP was significantly higher than the control group in period 4. When the diet has low AP was supplemented with different levels of phytase, egg mass was improved. Egg mass of

the groups receiving 500, 100, 1500 units phytase in period 1 and 1500, 200 units phytase in period 4, and average egg mass of the group receiving 1500 units phytase were significantly higher (P<0.05) than the group fed low AP+0 unit phytase. Egg mass of the experiment groups were very close to those of control group (Table 3).

Dietary treatments had no significant effect on feed consumption in different periods of the experiment, but average daily feed consumption during the experiment of the quail fed low AP+500, 1500 and 2000 units phytase were significantly higher (P<0.05) than that of C group. However, feed conversion (feed/egg mass) of the quail fed low AP supplemented with phytase generally tended to slightly high. However, the groups fed low AP+0; 500 and 1500 units phytase had feed conversion significantly higher (P<0.05) than control in period 4. The group fed 1500 units phytase was very close to those of control group in feed conversion (Table 3).

TABLE 2

EFFECT OF ADDING A DIFFERENT LEVEL OF PHYTASE LO DIET CONTAINING LOW AVAILABLE PHOSPHORUS (AP) ON BODY WEIGHT, BODY WEIGHT GAIN, CARCASS WEIGHT, EGG PRODUCTION, UNCOLLECTABLE EGG RATIO AND VIABILITY IN LAYING JAPANESE QUAIL.

Parameters	Control (0.33%AP)	Adding phytase levels to diet has low AP (0.15%), units/g					Probability
		0	500	1000	1500	2000	
Initial body weight, g	153.3±2.60	156.3±1.45	161.0±1.53	158.3±2.03	160.7±3.33	157.7±1.20	0.197
Final body weight, g	231.7±4.41	245.0±9.29	244.3±1.45	230.3±10.17	236.0±4.04	236.7±2.19	0.465
Body weight gain, g	73.4±5.93	88.7±8.45	83.3±0.33	72.0±10.26	75.3±5.24	79.0±1.00	0.530
Carcass weight, g	155.1±4.95	156.9±6.30	160.9±4.38	153.4±7.72	158.1±5.35	157.6±4.15	0.201
		Average egg production, number/quail					
1stperiod [I]	18.6±0.27	17.3±1.01	19.6±0.61	21.0±0.88	20.3±0.54	18.9±1.63	0.123
2nd period	26.0±0.84	24.8±0.50	25.1±0.92	25.0±1.13	26.4±0.36	24.9±0.90	0.665
3rd period	26.1±0.31	24.4±0.61	25.6±0.38	23.1±2.13	26.1±0.45	25.7±0.58	0.277
4th period	26.5±0.50^{a11}	19.6±1.50^{b}	23.7±1.84^{ab}	21.5±2.52^{ab}	26.0±0.39^{a}	24.8±0.97^{a}	0.045
5th period	25.7±0.39^{a}	20.6±1.92^{b}	22.5±0.96^{ab}	20.0±1.50^{b}	25.6±0.47^{a}	23.8±1.92^{ab}	0.042
Total	122.9±0.46	106.7±1.11	116.5±0.94	110.6±1.63	124.4±0.44	118.1±1.20	0.079
		Average egg production, %					
1st period	66.5±0.99	61.7±3.62	70.1±2.17	74.9±2.09	72.6±1.94	67.5±5.82	0.152
2nd period	94.0±3.27	89.2±2.06	89.6±3.25	89.0±4.21	95.3±1.14	90.0±2.91	0.406
3rd period	93.2±1.08	87.1±2.23	91.5±1.37	82.5±7.63	93.0±1.60	91.7±2.09	0.277
4th period	94.6±1.77^{a}	70.1±5.35^{c}	84.5±6.60^{ab}	76.8±8.97^{bc}	92.7±1.42^{a}	88.5±3.37^{ab}	0.012
5th period	91.9±1.41^{a}	73.8±6.86^{b}	80.1±3.46^{ab}	71.4±5.32^{b}	91.5±1.71^{a}	85.1±6.89^{ab}	0.031
Average	88.1±1.04	76.4±3.68	83.2±2.57	78.9±4.96	89.0±0.52	84.6±3.04	0.067
Uncollectable eggs,% [III]	0.34±0.07	1.47±1.17	0.64±0.15	0.67±0.32	0.78±0.12	0.58±0.19	0.663
Mortality, %	0.0±0.0	11.1±6.41	3.7±3.70	0.0±0.0	0.0±0.0	3.7±2.70	0.249

[I] Periods from 1 to 5 correspond 4 weeks between weeks 7-10, 11-14, 15-18, 19-22, 23-26 respectively

[II] a-c: Means with no common superscripts within each row differ significantly (P<0.05).

[III] The eggs without shell or having very thin shell

TABLE 3

EFFECT OF ADDING A DIFFERENT LEVEL OF PHYTASE TO DIET CONTAINING LOW AVAILABLE PHOSPHORUS (AP) ON EGG WEIGHT, EGG MASS, FEED CONSUMPTION AND FEED CONVERSION RATIO IN LAYING JAPANESE QUAIL.

Parameters	Control	Adding phytase levels to diet has low AP (0.15%), units/g					Probability
	(0.33%AP)	0	500	1000	1500	2000	
		Average egg weight, g					
Istperiod[I]	12.3±0.16	12.0±0.09	12.2±0.18	12.0±0.12	12.1±0.10	11.8±0.25	0.274
2nd period	12.7±0.11	13.1±0.21	12.4±0.18	12.7±0.13	13.0±0.13	12.5±0.31	0.116
3rd period	12.7±0.02	13.1±0.24	12.6±0.17	12.8±0.10	13.l±0.19	12.5±0.16	0.113
4th period	12.6±0.05	13.4±0.38	13.0±0.23	13.0±0.23	13.5±0.15	13.0±0.17	0.131
5th period	12.6±0.03[c11]	13.8±0.28[a]	13.0±0.04[bc]	13.0+0.31[bc]	13.6±0.04[ab]	12.9+0.25[bc]	0.013
Average	12.6±0.02	12.7±0.18	12.6±0.08	12.7±0.13	13.1±0.11	12.5±0.21	0.523
		Average egg mass[III], g					
Ist period	8.2±0.21ab	7.4±0.39[b]	8.6±0.14[a]	9.0±0.28a	8.8±0.18[a]	7.9±0.53[ab]	0.037
2nd period	11.8±0.43	11.7±0.41	11.1±0.28	11.3±0.58	12.4±0.06	11.2±0.09	0.203
3rd period	11.810.12	11.4±0.46	11.5±0.17	10.6± 1.03	12.2±0.34	11.5±0.20	0.380
4th period	11.9±0.28[ab]	9.4±0.440[c]	10.9±0.77[abc]	10.0±1.19[bc]	12.5±0.28[a]	11.5±0.33[ab]	0.036
5th period	1l.6±0.21	10.2±0.96	10.4±0.48	9.3±0.55	12.4±0.23	11.0±1.00	0.059
Average	11.l±0.14[ab]	10.0±0.44[b]	10.5±0.31[ab]	10.0±0.59[b]	11.7±0.13[a]	10.6±0.24[ab]	0.042
		Average daily feed consumption, g/quail					
Ist period	28.2±0.08	28.0±0.07	28.1±0.25	28.5±0.28	28.5±0.11	28.0±0.20	0.348
2nd period	29.5±O.49	31.4±0.39	30.4±0.45	30.4±0.19	31.5±0.73	30.4±0.39	0.068
3rd period	29.8±0.57	32.0±0.25	31.6±l.26	30.8±0.45	32.9±0.49	32.0±0.53	0.079
4th period	28.2±0.96	29.1±1.52	32.6±0.80	31.7±2.01	32.7±0.17	31.2±0.48	0.080
5th period	26.1±0.24	27.7±0.66	28.6±l.19	27.5±0.04	29.1±0.99	28.2±l.02	0.211
Average	28.5±0.43[b]	29.8±0.45[ab]	30.4±0.50[a]	29.9±0.48[ab]	30.1±0.48[a]	30.1±0.45[a]	0.046
		Average feed conversion, feed/egg mass					
Ist period	3.5±0.09	3.8±0.19	3.3±0.06	3.2±0.11	3.2±0.06	3.6±0.28	0.083
2nd period	2.5±0.14	2.7±0.10	2.7±0.07	2.7±0.13	2.5±0.06	2.7±0.05	0.410
3rd period	2.5±0.03	2.8±0.09	2.7±0.10	3.0±0.29	2.7±0.11	2.8±0.019	0.450
4th period	2.4±0.03[c]	3.1±0.12[ab]	3.0±0.26[ab]	3.2±0.21[a]	2.6±0.07[bc]	2.7±0.07[abc]	0.012
5th period	2.3±0.03	2.8±0.24	2.8±0.22	3.0±0.19	2.4±0.12	2.6±0.17	0.090
Average	2.6±.0.0	3.0±0.11	2.9±0.12	3.0±0.14	2.17±0.06	2.9±0.09	0.061

[I] Periods from 1 to 5 correspond 4 weeks between weeks 7-10,11-14, 15-18,19-22,23-26 respectively

[II] a-c: Means with no common superscripts within each row differ significantly (P<0.05).

[III] Egg mass= Average daily egg yield per quail x average egg weight.

Although dietary treatments had no significant effect on the shell quality parameters determined at the first period of the experiment, they had a significant effect on the various shell quality parameters at the second period of the experiment. Eggshell surface area of the groups fed with low AP+500 and 2000 units phytase were close to those of group C, however, significantly lower than those of fed low AP+0 and 1500 units phytase. The groups fed low AP and low AP supplemented different phytase had shell weight (P<0.05) and shell weight per unit area (P<0.01) significantly lower than that of group C (Table 4). When the diet has low AP was supplemented with 500 units phytase, the shell thickness of other groups was significantly lower (P<0.05) than that of group C. In addition, while the groups receiving low AP+0, 1500 and 2000 phytase units had shell strength significantly lower (P<0.05) than that of C group. The groups receiving 500 and 1000 phytase units had shell strength comparable to those of C diet. The result of the experiment shows that the response of the laying quail as age increased was different with respect to various shell quality parameters when the diet has low AP was supplemented with phytase.

Fat-free dried tibia weight of the group fed low AP was significantly lower than that of group C (P<0.05).However, when phytase added to diet has low AP, fat-free dried tibia weight was improved and bone weights of the groups supplementing phytase were very close to that of group C. In addition, the groups fed low AP+500 and 1500 phytase units had bone weight significantly higher than those of group fed low AP. Percentage of bone ash of the groups fed low AP with and without were

comparable to those of group C. Manure ash content of the groups fed diets has low AP supplemented with or without phytase was significantly lower (P<0.05) compared to group C.

TABLE 4

EFFECT OF ADDING A DIFFERENT LEVEL OF PHYTASE TO DIET CONTAINING LOW AVAILABLE PHOSPHORUS (AP) ON SHELL QUALITY, FAT-FREE DRIED TIBIA WEIGHT AND ASH AND MANURE ASH IN LAYING QUAIL.

Parameters	Control		Adding phytase levels to diet has low AP (0.15%), units/g				Probability
	(0.33%AP)	0	500	1000	1500	2000	
First-period shell parameters							
Surface area, cm^2	24.90±1.15	24.73±0.44	23.65±0.15	24.01±0.17	24.44±0.22	23.78±0.29	0.496
Shell weight, g	1.03±0.01	1.09±0.02	1.00±0.01	1.04±0.03	1.05±0.01	1.01±0.02	0.062
Shell weight per unit surface area mg/cm^2	41.37± 1.77	44.08±0.49	42.28±0.30	43.32±0.87	42.96±0.63	42.47±0.25	0.608
Shell weight %	8.16±0.06	8.15±0.12	7.99±0.05	8.14±0.16	8.01±0.14	8.04±0.01	0.784
Shell thickness, mm	0.213±0.003	0.207±0.007	0.193±0.007	0.197±0.003	0.200±0.000	0.203±0.003	0.086
Shell strength, kg	0.84±0.05	0.86±0.06	0.96±0.04	0.90±0.08	0.74±0.05	0.82±0.007	0.273
Second-period shell parameters[11]							
Surface area, cm^2	$23.63±0.03^{abIII}$	$24.92±0.57^{a}$	$23.47±0.33^{b}$	$23.80±0.38^{ab}$	$24.85±0.31^{a}$	$23.20±0.56^{b*}$	0.045
Shell weight, g	$1.05±0.02^{a}$	0.93±0.02b	$0.93±0.03^{b}$	$0.94±0.01^{b}$	$0.94±0.03^{b}$	$0.92±0.01^{b*}$	0.012
Shell weight per unit surface area, mg/cm^2	$44.43±0.74^{a}$	$37.32±1.11^{b}$	$39.63±0.91^{b}$	$39.50±0.61^{b}$	$37.83±0.63^{b}$	$39.66±0.61^{b**}$	0.001
Shell weight %	$8.38±0.14^{a}$	$6.91±0.26^{b}$	$7.51±0.15^{b}$	$7.43±0.15^{b}$	$6.99±0.09^{b}$	$7.59±0.18^{b**}$	0.001
Shell thickness, mm	$0.217±0.007^{a}$	$0.197±0.003^{bc}$	$0.203±0.007^{ab}$	$0.193±0.007^{bc}$	$0.187±0.003^{c}$	$0.193±0.003^{bc*}$	0.024
Shell strength, kg	$0.55+0.05^{a}$	$0.36±0.07^{bc}$	$0.46±0.05^{ab}$	$0.42±0.04^{abc}$	$0.28±0.01^{c}$	$0.36±0.03^{bc}$	0.016
Fat-free dried tibia weight, g	$0.47±0.01^{ab}$	$0.39±0.02^{c}$	$0.46±0.03^{ab}$	$0.41±0.02^{bc}$	$0.49±0.02^{a}$	$0.42±0.02^{bc*}$	0.048
Tibia ash, %	61.1±1.50	59.2±2.88	57.9±0.46	58.5±0.63	58.2±0.87	58.2±0.44	0.534
Manure ash, %	$23.9±1.24^{a}$	$12.9±0.98^{b}$	$13.9±0.99^{b}$	$14.0±0.78^{b}$	$14.6±0.69^{b}$	$15.0±0.22^{b**}$	0.000

[I] First-period shell parameters were determined for eggs collected at the 70-7 l-72th days of experiment

[II] Second period shell parameters were determined for eggs collected at the last three days of experiment

*[III] a-c: Means with no common superscripts within each row differ significantly (*P<0.05; **P<0.01)*

IV. DISCUSSION

Results of the present experiment showed that egg production (as number and percentage), egg mass, average daily feed consumption, fat-free dried tibia weight and viability improved when added phytase level to diet has low AP. These parameters were comparable to those of control group fed diet has adequate AP. Final live weight, carcass weight, body weight, body weight gain, feed conversion ratio, uncollectable eggs ratio, some egg shell quality parameters measured in eggs collected at the end of the first period were not affected by treatments. Similar findings were observed by Sacakli et al., (2006) who reported that BW, BWG, FC, FCR, and carcass were not affected by the phytase addition (300 phytase unit) to low AP content (2 g/kg) quail diets in growing period. On the other hand, many trial resulted lower body weight, feed intake and feed efficiency by a low level AP diets and phytase addition to diets improved broiler performance (Denbow et al., 1995; Qian et al., 1996).

These findings were similar results of the meat type poultry and agree with previous reports of Simons and Versteegh (1991). They reported that laying hens fed diets with low AP supplemented with phytase had performance as high as those of fed diet with adequate AP. Results of the experiment that was conducted at Penn State University with enzymes aims to reduce fecal phosphorus levels of commercial pullets and hens, also showed that body weight, production and egg parameters equal to the control can be maintained with diet has low P added phytase (Patterson, 1996). In addition, Patterson (1996) narrated from Van der Klis et al. (1996) that egg production, egg weight and feed/egg ratio can be improved from a negative control diet (3.3 g/kg total P) with addition phytase at 200 unit/kg. Another report (van der Klis et al. 1991) showed that most portion of dietary phytate phosphorus was hydrolysed by addition phytase at 250 and 500 units/kg feed in WL-laying hens. However, Newkirk and Classen (1992) reported that in two lines of leghorn hens, egg production decreased with addition phytase to diets have different levels of total P. However in this study, with regard to egg production, egg mass, average daily feed consumption, the responses of laying quail to the increasing phytase levels were increased, but this increase was not linear.

These findings indicate that in order to improve availability of phosphorus addition phytase to diet has high phytate phosphorus is an effective application in laying quail as broiler chicks and laying hens.

As increase age, low dietary phosphorus resulted in a decrease in many of shell parameters and while adding increasing levels of phytase to diet containing low AP was improved some shell parameters, some of them were not affected. No data concerning the effect of phytase on shell quality was found in the literature.

Fat-free dried tibia bone weight of quail were significantly increased by addition phytase at 500 and 1500 units/g feed when compared with quail fed low AP and, quail fed diet with and without phytase had % tibia ash comparable to those quail fed adequate AP. These findings suggest that utilization of phosphorus was increased by addition phytase. An increase in body weight gain, feed efficiency, fat-free tibia weight, bone ash and strength due to adding phytase to diet has low AP were also seen by Konca and Bahtiyarca (1996) and Bahtiyarca and Parlat (1997) in young Japanese quail.

The performance and bone parameters of the group fed with 1500 phytase units were very close values to those of control group, even higher than the control group in some characters. However, any of phytase level did not prevent a decrease in shell weight measured at the second period and a decrease in shell thickness and strength with 1500 and 2000 unit phytase were not prevented. The difference in source and activity phytase of enzymes, species of bird and inclusion rate were marked difficult interpretation of results obtained from the different experiment. The results of the present experiment indicated that with regard to performance and bone parameters, the responses of laying quail to the diet has low AP supplemented with increasing phytase levels were improved but this increase was not linear, and the responses of quail fed with 1500 phytase units were very close to or better than those fed adequate AP, but some shell parameters were impaired. Manure P content was decreased by lower AP consumption with feed. This result is expected because it has been known that when poultry fed diet containing low AP, phosphorus level of manure was significantly decreased.

V. CONCLUSION

Low dietary AP concentration may reduce production parameters in quail. The phytase addition to quail diets may help to improve production parameters. Tibia weight may decrease with low AP diets. Manure P content may be decreased via lower AP content in diets.

REFERENCES

[1] Adeola, O., Cowieson, A.J., 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89, 3189–218. doi:10.2527/jas.2010-3715Swick R.A. and F.J. Ivey, 1992: Phytase: The value of improving phosphorus retention. Feed management, Reprinted from January 1992.

[2] Anonymous, 1993 a: FineaseP in animal feed, Application sheet. February, Alko Biotechnology, Rajamaki, Finland.

[3] Anonymous, 1993 b: Finease FP 500, Product sheet. February, Alko Biotechnology, Rajamaki, Finland.

[4] Bahtiyarca, Y and S.S. Parlat, 1997: Effects of phytase on the performance and availability of phosphorus in corn-soybean meal diets by Japanese quail. ArchivfürGeflügelkunde, 58-65.

[5] Carter, T.C., 1975: The hen's egg estimation of shell superficial area and egg volume, using a measurement of frcsh egg weight and shell lenght and breadth aloıe or in combination. Brit. Poult. Sel. 16: 541-543.

[6] Denbow, D. M., V. Ravindran, E. T. Kornegay, Z. Yi and R. M. Hulet. 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci. 74:1831.

[7] Konca, Y. and Y. Bahtiyarca, 1996: Farklıseviyelerdefos for içerenrasy on laramayakültürlerive fitazilavesiningenç Japonbıldırcınlarındaper for mansvefos for unkullanımına et kisi. Ç.Ü. Zir. Fak. Ulusal Kümes Hayvanları Sempozyumu'96; 27-29 Kasım, Adana: 190-201.

[8] National Research Council (NRC), 1984: Nutrient requirements of poultry. 8th Revised edition, National Academy Press, Washington, D.C.

[9] Nelson, T.S., 1967: The utilizatioıı of phytate phosphorus by poultry-A review. Poultry Sci. 46: 862-871.

[10] Nelson, T.S., 1976: The hydrolysis of phytate phosphorus by chicks and laying hens. Poultry Sci. 55: 2262-2264.

[11] Nelson, T.S., T.R. Shieh, R.J. Wodzinski and J.H. Ware, 1971: Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nut. 101: 1289-1294.

[12] Newkirk, R.W. and H.L. Classen, 1992: The effect of dietary xylanase, phytase and phosphorus on the performance of laying hens. Abst. Poultry Sci. 72 (Suppl. 1): 17.

[13] Öğüt, H. ve C. Aydın, 1992: Konya ekolojikşartların day etiştirilenbazıelma çeşitlerinin poisson or anıveelastikiyet modüllerininbelirlen mesi. S.Ü. Zir. Fak. Dergisi, 2(3): 39-53.

[14] Perney, K.M., A.H. Cantor, M.C. Strow and K. Henkelman, 1993: The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. Poultry Sci. 72: 2106-2114.

[15] Pratley, C.A. and D.W. Stanley, 1982: Protein phytate interactions in soybeans. J. Food Biochem. 6: 243-247.

[16] Qian, H., H. P. Veit, E. T. Kornegay, V. Ravindran and D. M. Denbow. 1996. Effect of supplement alphytase and phosphorus on histological and othertibial bone characteristic and performances of broilers fed semi-purified diets. Poult. Sci. 75:618-626.

[17] Sacakli, P., A. Sehu, A. Ergun, B. Genc, and Z. Selcuk 2006. Theeffect of phytase and organic acid on growth performance, carcassyield and tibia ash in quails fed diets with low levels of non-phytate phosphorus. Asian Austral asian Journal of Animal Sciences 19 (2): 198-206.

[18] Sacakli, P., Sehu, A., Ergin, A., Genc, B., Selcuk, Z., 2006. The effect of phytase and organic acid on growth performance, carcass yield and tibia ash in quails fed diets with low levels of non-phytate phosphorus. Asian-Australasian J. Anim. Sci. 19, 198–202.

[19] Said, N.W. and TAV. Sullivan, 1985: A comparison of continuous and phased levels of dietary phosphorus for commercial laying hens. Poult. Sci. 64: 1763-1771.

[20] Saima, M. Z., U. Khan, M. A. Jabbar, M. Ijaz, and M.A.Q., 2014. Effect of Microbial Phytase Supplementation on Growth 24, 19–23.

[21] Sharpley, A.N.,Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C. andReddy, K.R., 1994. Managing agricultural phosphorus for protection of surface waters: Issues and options. Journal of Environmental Quality, 23(3), pp.437-451.

[22] Simons, P.C.M. and H.A.J. Versteegh, 1991: Phosphorus availability in relation to phytase supplementation in feeds for poultry. Mineral requirement and supply in farm animals. Editors: F. Sundstal, N.P. Kjos, O. Herstad. Proceedings from a Symposium at Department of Animal Sci. Agricultural University of Norway, 2 1991: 30-33.

[23] Swick, R.A. and F.J. Ivey, 1990: Effect of dietary phytase addition on broiler performance in phosphorus deficient diets. Abst. Poultry Sci. 69 (Suppl. 1): 133.

[24] Toprak, N.N. Yilmaz, A., 2016. The effects of phytase and beta-glucanase supplementation on performance, egg quality, some blood parameters, tibia and excreta characteristics of Japanese quails fed barley-based diets. Türk Tarım ve Doğa Bilim. Derg. 3, 126–135.

[25] Van der Klis, J.D., H.A.J. Versteegh and C. Geerse, 1991: The effect of dietary phytase and calcium level on the ileal absorption of phosphorus in laying hens. Proc. 19lh World's Poultry Congrees, 20-24 Scpt. Amsterdam, Vol. 3: 458-459.

[26] Yan, F., J. H. Kersey, J. H. Frittsand P. W. Waldroup. 2003. Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. Poult. Sci. 82:294-300.

Social Protection and Climate Resilience: A Review Of Sub-Saharan African Case Studies

Oladayo Nathaniel Awojobi[1], Mbenja Clovert Anamani[2]

[1]Department of Social Security, Bonn-Rhein-Sieg University of Applied Sciences, Sankt Augustin, Germany
[2]Anhalt University of Applied Sciences, Dessau, Germany, President and Founder, Rising Hope Foundation for Change (RHFC)

Abstract— *Social protection is mainly used for assisting the most vulnerable in the area of poverty reduction. However, international development scholars are arguing that social protection aside impacting the poor can also help in climate resilience. This study examines selected case studies in the social protection and climate resilient debate in Sub-Saharan Africa. Using qualitative and quantitative approaches in data collection, the study finds that social protection through cash transfers have been able to build climate resilience among participants of the scheme. Though findings from the study were minimal, a wide range of research needs to be carried out to determine the impact of social protection on climate shocks on a broader scale.*

Keywords— Climate Change, Cash Transfers, Social Protection, Sub-Saharan Africa.

I. INTRODUCTION

Climate change will affect many people globally due to the increase in temperature and greater variability of rainfall (Béné et al, 2013; Canonge, 2015; Kuriakose et al., 2012). It is argued that the impacts of climate change will pose tremendous challenges to individual livelihoods (Béné et al., 2013; Canonge, 2015).Climate change is real in Africa (Besada & Sewankambo, 2009). The Intergovernmental Panel on Climate Change (IPCC) has envisaged that harsh climate events across Africa are expected to increase resulting in disasters (Hulme et al, 2001).

Sub-Saharan Africa has been categorised as one of the regions that is most vulnerable to climate change due to its high exposure and poor adaptive capacity (Béné et al., 2013; Niang et al., 2014). Climate experts think that Sub-Saharan Africa is more susceptible to climate events due to its high poverty rate, droughts coupled with rain-fed agricultural production (Wild, December 2015b). This hypothesis is "based on two things: geographically and climatically Africa is exposed… Africa in general is already quite hot. Heat it up more and it's just downhill for animal production, plant production and human health" (Wild, 2015a). The African continent has witnessed severe climate events such as flooding, droughts and desertification (Awojobi, 2017; Awojobi & Tetteh, 2017). While no one is immune to the impact of climate change, the poor are the most vulnerable because they lack the capacity to adapt in the case of extreme weather events. Similarly, it is argued that the poor will particularly be disadvantaged as they have a low capacity for response and adaptation, as a result, they are at an expanded risk of losing lives and property in climate shocks and of having to depend on unpleasant coping strategies with long-term negative consequences for human development, such as pulling children out of schools, selling assets and involving in perilous jobs (Kuriakose et al., 2012).

Since the poor have been considered to suffer most in the event of climate change (Awojobi, 2017) There is need to consider how humanitarian and development approaches can help improve poor households' capacity to adapt to an extreme climate event (Jones et al., 2010). Social protection represents one approach when link with climate change adaptation measures (Ziegler, 2016). Social protection is a strong tool to protect people at a greater risk of climate-related adversity (Canonge, 2015).Furthermore, social protection instruments such as cash transfers, insurance, pension and employment guarantee schemes can be used to assist communities that are exposed to climate-related disasters (Béné et al., 2013; Ziegler, 2016).However, evidence illustrating how these measures can assist those affected to adapt and cope with climate challenges is still narrow (Béné et al., 2013).

This paper does not seek to expand on the concept of adaptive social protection developed by the Institute of Development Studies (IDS) and the United Kingdom (UK) Department for International Development (DFID), rather, it aims to provide a

concerted review of Sub-Saharan African case studies of current evidence about the role of social protection in reducing the impacts of climate change on poor communities and households

Section 2 of the paper expatiates on the methods adopted for the study. Section 3 accesses the evidence of the nexus between social protection and climate change. Section 4 is the discussion while section5 concludes.

II. MATERIALS AND METHODS

The following review looks at the nexus between social protection and climate change case studies from Sub-Saharan Africa. The selection of the sample case studies was based on a search for research articles in Google search engine examining the role of social protection in mitigating the impacts of climate change among households and communities.

The study uses the blend of quantitative and qualitative approaches to tackle the research problem. Both approaches have different advantages and in some cases the problem to solve dictate what types of approach is needed (Morrissey, 2009).

Quantitative approaches have been used basically as a means for searching the interface between social protection and climate change adaptation. The main approach has been used to undertake a statistical analysis of various proxies for social protection and the impacts of climate change and ascertains the level to which changes in climate events lead to change in adaptive social protection. One unique advantage of this method is that it underscores the interface between various aspects of social protection quality and climate change adaptation.

While quantitative approaches are considered to be unique in impact evaluation in social programmes. Most of the social protection programmes in Africa are meant for poverty reduction and not for climate change adaptation. As such, statistical analyses may well miss the connections between social protection and climate change adaptation, thereby achieving results which downplay the problem.

Qualitative analyses, in contrary, have primarily been used to elicit information for beneficiaries of social protection schemes regarding the impacts of any of the social protection schemes in response to extreme climate events. Furthermore, they have been used to underscore the level to which social protection schemes lead to climate change mitigation in the study areas. Despite the advantages of qualitative analyses, their shortcoming is that they do not present pragmatic measures of the robustness of the connections between the various aspects of social protection and the impacts of climate change.

III. FINDINGS

3.1 Social protection and climate resilience: review of selected evidence

The instruments of social protection include cash transfers, insurance, cash for work and social pension. This paper will focus on the role of cash transfers in building climate resilience among their beneficiaries. Cash transfers in Africa are mostly conceptualized for the poor and are designed to uplift them above the national poverty line and mitigate their vulnerability to risks and shocks (Awojobi, 2017). Scientific evidence has affirmed that cash transfers also favour those affected by extreme climate events (Awojobi, 2017). For example, in a study on cash transfers and climate-resilient development in Zambia, Lawlor et al, (2015), through the randomized roll-out of Zambia's Child programme and a panel of 2,515 households. The study finds that cash transfer decreases the possibility of embracing negative coping strategies connected with poverty traps and increases the possibility of embracing positive coping strategies. In addition to such findings, Lawlor et al. (2015), find among households that faced agricultural shocks, cash decreases their possibility of decreasing food consumption by 14 percentage points and increases the possibility of spending savings by 6 percentage points. Also, the study finds that cash transfer increases the possibility of using social services (such as seeking medical service, help from government or NGO) by 2 percentage points in the event of agriculture shocks and by 12 percentage for other shocks (Lawlor et al., 2015).

Supporting Lawlor et al.'s (2015) are those of Asfaw et al. (2016) whose work in Zambia found through the use of impact evaluation data from the Zambia Child Grant Programme (CGP) along with set of novel weather variation indicators that in the context of limited rainfalls that affected agricultural production, cash transfers were able to mitigate the impact of climate

shock. However, despite the positive correlation between climate shock and the cash transfers, they did not find a statistically significant correlation between the cash transfers and long-term average rainfall variable (Asfaw et al., 2016).

Similar findings came out of the work by Eriksen et al. (2005) who investigated the dynamics of vulnerability: locating coping strategies in Kenya and Tanzania. While they found a significant correlation between the capability of people to draw on supplementary sources of income and the capacity to tolerate droughts, they did find that various households had inadequate access to the preferred coping strategies as a result of inadequate skills, labour and capital (Eriksen et al., 2005).

In a more comprehensive study in Zimbabwe and Niger Republic by CARE International (2017),based on quantitative data, describes how cash transfers were able to build resilience on beneficiaries of the cash transfer programme. According to CARE International (2017) findings, cash transfer had a net positive impact on approximately all measures of absorptive capacity amongst recipient households across both the Zimbabwe and Niger country social interventions. However, the magnitude of the impact differs depending on what indicators are measured. CARE International (2017) affirms that the cash transfers contributed to mitigating the hike of food insecurity and the use of negative coping mechanisms and strengthening consumption (CARE International, 2017).

In addition, CARE International (2017) states the different impacts of the cash transfer interventions under three other core resilient capacities which are anticipatory, adaptive and transformative CARE's programming explores to bolster. According to CARE International, (2017), the impacts of the cash transfers on measures of adaptive capacity differed across the Zimbabwe and Niger country programme. In Zimbabwe, the findings claim that the receipt of cash transfer is positively correlated with increases in off-farm income generation. In Niger, a higher percentage of the participating households (60%) declared access to and use of improved seed varieties than non-participating households (43%), suggesting a cut in the risks that beneficiaries face (CARE International, 2017). Similarly, in transformative capacity, CARE International (2017), findings reveal that 26% more cash beneficiaries took part in various social networking than non-cash transfer beneficiaries. However, CARE International (2017), made it explicit that this not centrally a causal relationship but rather suggestive of the likely impact of a cash transfer programme. Still on CARE International (2017) findings, this time in the area of anticipatory capacity, the study indicates that in Niger, a positive impact on access to and use of climate information by the beneficiaries of the cash for work scheme (46%) compared to non-beneficiary households (41%). The impact was attributed to extra income and the participation in the programme helped beneficiaries' access to platforms they may not have otherwise known about (CARE International, 2017). However, female-headed households of the cash beneficiaries occur to be less likely to access and make use of climate information in planning and farm management practices as compared to the beneficiaries of male-headed households (CARE International, 2017). The study did not find any impact in Zimbabwe due to the non-availability of anticipatory-focus data.

IV. DISCUSSION

Social protection in Sub-Saharan Africa is often used for supporting the most vulnerable due to their precarious conditions. While those designing social protection programmes for their target groups do not take cognizance of the impact of climate events affecting the poor. The Institute of Development Studies (IDS) and the United Kingdom (UK) Department of International Development (DFID) have developed the concept "adaptive social protection" (Ziegler, 2016). This is because of the poor being the most vulnerable to climate change. It is argued that during extreme climate events, the poor suffer most. For instance, when there is excessive flooding, the poor lack resources to assist themselves to migrate (Awojobi, 2017).

Linking social protection to climate change is one concept that researchers in the development field have been canvassing. Instruments of social protection are bound to fail if they do not recognize both the short and long-term and stresses connected with climate change (Davies & Leavy, 2007). Current evidence has shown that social protection especially cash transfers have impacts on the beneficiaries of cash transfers when extreme climate events occurred, for details (see Asfaw et al., 2016; Awojobi, 2017; CARE International, 2017; Lawlor et al., 2015). While evidence in the correlation between social protection and climate change is still minimal, governments in Africa should devise methodology in incorporating social protection into climate change adaptation in regions that are vulnerable to climate change. This is one way that the poor can feel the presence of government when there are caught up in extreme climate events.

V. CONCLUSION

The study examines the impacts of social protection on climate resilience in Sub-Saharan Africa. Using a mixed method approach, the study was able to find a positive correlation between social protection (cash transfers) and climate resilience in some selected case studies. Though, cash transfers are designed purposely for the poor for them to live above the national poverty line. There is concern from development experts who think that linking social protection with climate change will help in assisting the poor who are affected by climate change in building resilience.

Evidence from this study was minimal because the scope of the study was concentrated in Sub-Saharan Africa making the search for case studies to be limited. A comprehensive study will be needed in order to articulate the positive correlation between social protection and climate change on a larger scale.

REFERENCES

[1] Asfaw, S., Carraro, A., Davis, B., Handa, S., & Seidenfeld, D. (2016). Cash Transfer Programmes for Managing Climate Risk: Evidence from a Randomized Experiment in Zambia. In *2016 AAAE Fifth International Conference, September 23-26, 2016, Addis Ababa, Ethiopia*. African Association of Agricultural Economists (AAAE).

[2] Awojobi, O. (2017). Cash transfers and climate change resilience in Africa [socialprotection.org]. Retrieved from http://socialprotection.org/learn/blog/cash-transfers-and-climate-change-resilience-africa

[3] Awojobi, O. N. (2017). CLIMATE CHANGE AND MIGRATION NEXUS: ASSESSING THE EVIDENCE. *International Journal of Agriculture and Environmental Research*, *3*(5), 3990–4001.

[4] Awojobi, O. N., & Tetteh, J. (2017). THE IMPACTS OF CLIMATE CHANGE IN AFRICA: A REVIEW OF THE SCIENTIFIC LITERATURE. *Journal of International Academic for Multidisciplinary*, *5*(11), 39–52.

[5] Béné, C., Cannon, T., Davies, M., Newsham, A., & Tanner, T. (2013). *Social Protection and Climate Change* (A paper prepared for the OECD-DAC Task Team on Social Protection). Organisation for Economic Co-operation and Development.

[6] Besada, H., & Sewankambo, N. (2009). CIGI Special Report: Climate Change in Africa: Adaptation, Mitigation and Governance Challenges. Retrieved from https://www.cigionline.org/sites/default/files/climate_change_in_africa_3.pdf

[7] Canonge, J. (2015). Social protection and climate change. Retrieved November 30, 2017, from http://www.social-protection.org/gimi/gess/RessourcePDF.action?ressource.ressourceId=53810

[8] CARE International. (2017). The impact of cash transfers on resilience: A multi-country study. CARE International, 89 Albert Embankment, London SE1 7TP.

[9] Davies, M., & Leavy, J. (2007). Connecting social protection and climate change adaptation. *Institute of Development Studies*.

[10] Eriksen, S. H., Brown, K., & Kelly, P. M. (2005). The dynamics of vulnerability: locating coping strategies in Kenya and Tanzania. *Geographical Journal*, *171*(4), 287–305. https://doi.org/10.1111/j.1475-4959.2005.00174.x

[11] Hulme, M., Doherty, R., Ngara, T., New, M., & Lister, D. (2001). African climate change: 1900-2100. *Climate Research*, *17*(2), 145–168. https://doi.org/10.3354/cr017145

[12] Jones, L., Jaspars, S., Pavanello, S., Ludi, E., Slater, R., Arnall, A., … Overseas Development Institute (London, E. (2010). *Responding to a changing climate: exploring how disaster risk reduction, social protection and livelihoods approaches promote features of adaptive capacity*. Washington, DC: Overseas Development Institute (London, England).

[13] Kuriakose, A. T., Heltberg, R., Wiseman, W., Costella, C., Cipryk, R., & Cornelius, S. (2012). Climate-Responsive Social Protection. Retrieved November 30, 2017, from http://www.fao.org/fileadmin/user_upload/fsn/docs/HLPEII/CRSP_-_SP_Discussion_Paper_1210.pdf

[14] Lawlor, K., Handa, S., Seidenfeld, D., & Zambia Cash Transfer Evaluation Team. (2015). Cash Transfers and Climate-resilient Development: Evidence from Zambia's Child Grant Programme. Innocenti Working Paper No.2015-03, UNICEF Office of Research, Florence.

[15] Morrissey, J. (2009). *Environmental change and forced migration*. Refugee Studies Centre, Oxford.

[16] Niang, I., Ruppel, O., Abdrabo, M., Essel, A., Lennard, C., Padgham, J., & Urquhart, P. (2014). Africa. In: Climate change 2014: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. Retrieved from https://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-Chap22_FINAL.pdf

[17] Wild, S. (December 2015b). FACTSHEET: Why Africa is vulnerable to climate change. Retrieved October 16, 2017, from https://africacheck.org/factsheets/factsheet-why-africa-is-vulnerable-to-climate-change/

[18] Wild, S. (Novermber 2015a). FACTSHEET: Why do many scientists think Africa will be hardest hit by climate change? Retrieved October 16, 2017, from https://africacheck.org/factsheets/factsheet-why-do-many-scientists-think-africa-will-be-hardest-hit-by-climate-change/

[19] Ziegler, S. (2016). Adaptive Social Protection–linking social protection and climate change adaptation. *GIZ: Discussion Papers on Social Protection*, (27).

Institution of Prosecution against the Act of Transporting In a Vehicle a Bulk Consignment of Poultry Meat Unfit for Human Consumption – General Interest

Lasantha Krishan Hirimuthugoda[1*], Asela Sanjaya Weerakkody[2], Ranawaka Arachchilage Chaminda Ranawaka[3], Perampulla Mudiyanselage Lakshan Madushanka Munasinghe[4], Gamage Priyantha Perera[5], Udaya Isaac Rathnayake[6], Amarasinghe Mudiyanselage Kamala Hemalatha[7], Sudirikku Hennadige Padmal De Silva[8], Prem Madarasinghe[9]

[1]Post Graduate Institute of Medicine, University of Colombo, Sri Lanka
[2,3,4,5]Medical officer of Health office, Bulathsinhala, Kalutara, Sri Lanka
[6]Regional Director of Health Services office, Kalutara, Sri Lanka
[7,8]National Institute of Health Sciences, Kalutara, Sri Lanka
[9]Food Safety Consultancy Services, Kegalle, Sri Lanka

Abstract*—Storage temperature plays a major role in causing changes in quality and safety of poultry meat. Product should retain its desired sensory, chemical, physical, functional and microbiological characteristics. Authorized Officers are expected to guide and inspect on food safety and security measures of the country. After conducting comprehensive food outlet inspections covering the whole Medical Officer Health (MOH) area and legal prosecutions over violation of law related to food safety, inspections of food transporting vehicles were conducted. Twenty vehicles were inspected and 7 out of them were released after giving strict advice, 3 were detained to produce before courts. By entering a plea of guilty, first two defendants were admitting their guilt and fined 67 USD each. Last case was charged against the chicken company transporting chicken at temperature of $+5^0$ Centigrade (standard -18^0 Centigrade), where company lawyer admitted a plea of guilt, but pleaded to release all chicken worth of 2700 USD. Usually in Sri Lankan context, temperature of container was measuring by thermometers as a spot reading of whole passage of cold chain of frozen foods. Upon the expert opinion and scientific explaining by the MOH of the impact of releasing this bulk of chicken on the health of the general public, the Magistrate made decision against the company with strict advice to maintain all vehicles in good sanitary condition, fined 67 USD and ordered to destroy the entire bulk quantity of chicken worth 2000 USD under the supervision of the MOH. This would set future reference/ benchmark in field of food safety in Sri Lanka to supervise and examine of temperature record by Authorized officers using smart phones on data lodgers, which records should be maintained by all frozen food companies thorough out their cold chain, and it can be used as weighted and firm evidence for their legal submissions in future as a complete reflection of cold chain.*

Keywords— Shelf life, food safety, enactment of food act, poultry meat, unhygienic transport, public health legislation.

I. INTRODUCTION

Shelf-life is considered as "the time period within which food is safe to consume and/or has an acceptable quality to consumers"[6]. Upon storage and distribution for a certain period, foods are exposed to a wide range of environmental conditions such as temperature, humidity, oxygen and light[11]. Storage temperature plays a major role in causing changes in quality and safety of chicken during storage. Since most degradation reactions are "arrhenius" type reactions, higher temperatures are known to speed the rate of degradation.

A typical frozen product will spend part of its shelf life in a bulk cold store, a refrigerated vehicle or container, a distribution store, a retail display cabinet or institutional frozen food storage cabinet, a period out of refrigeration during the journey from the retail outlet to home and time in a home freezer[12]. Due to these fluctuations in temperature, the food product may become microbiologically unsafe before or very close to the end of their sensory quality shelf life if temperature-abused[10].

During shelf life, the product should retain its desired sensory, chemical, physical, functional and microbiological characteristics, as well as accurately comply with any nutritional information printed on the label. Periodic determination of shelf life helps to provide assurance that the product remains consistent over time with respect to quality [7]. Many frozen meat products probably have a shelf life over two years if product quality, processing and packaging (PPP) factors are well

managed [12]. P. Zeuthen, et al., demonstrated the estimated shelf life by different temperatures for various frozen foods [10]. At 20 centigrade, chicken can be consumed after 1000 days, but if it was at 10 centigrade, it needed to be consumed before 200 days [10]. So, with declining of stored temperature maximum shelf life varies inverse proportionately.

1.1 Importance of maintenance of standard temperature

Environmental factors such as temperature, humidity and light can trigger several reaction mechanisms that may lead to food degradation. There is considerable evidence in literature that temperature plays a major role in causing changes in food quality during storage. Higher storage temperatures generally lead to increased quality deterioration.

Fluctuating temperatures cause more detrimental change in frozen foods than storage at constant temperature[11]. Food products may become microbiologically unsafe before or very close to the end of their sensory quality shelf life if temperature fluctuates [10]. But it is not practicable to store frozen foods at a steady temperature of -18 ˚C.

1.2 Microbial growth in frozen meat products

Minus 34˚C is the lowest temperature at which a microorganism has been reported to grow[5]. *Lactobacillus sake* and *Lactobacillus curvatus* have been shown to be common species in frozen meat products. *L. sake* seems to form the predominant part of the spoilage population.

Spoilage lactic acid bacteria produce mostly lactic and acetic acids during logarithmic growth. Spoilage of vacuum packed meat is characterized by the development of sour acid odours and taste [5]. Methane, ethiol and dimethyl sulfide may contribute to the sour acid odour.

1.3 Pathogenic microbes with significant public health impact in frozen meat products

Pathogenicity of certain microorganisms is a major safety concern in processing, handling, and storage of foods. Upon ingestion of small quantities, microorganisms such as *Salmonella* species and *Escherichia coli* strains cause infection. Others such as *Aspergillus flavus, Clostridium botulinum* and *Staphylococcus aureus* produce chemicals in foods that are toxic to humans [9]. The more common food-borne pathogens such as *Salmonella*, *Staphylococcus aureus* and *Clostridium perfringens* belong to the group of "mesophiles". Table 1 shows some reported minimum pH values for the growth of some/certain food-borne organisms. Sri Lankan standards for microbiological specifications of comminuted meat products are shown in table 2. The emergence of low infectious dose pathogens presents a significant challenge to predictive microbiology[10].

TABLE 1
REPORTED MINIMUM PH VALUES FOR THE GROWTH OF SOME FOOD BORNE ORGANISMS [5]

Food-borne organism	Minimum pH for growth
***Clostridium botulinum*, Group 1**	4.6
Escherichia coli	4.5
Lactobacillus brevis	3.16
***Salmonella* spp.**	4.05
Staphylococcus aureus	4.0

TABLE 2
MICROBIOLOGICAL SPECIFICATIONS OF COMMINUTED MEAT PRODUCTS[4].

Microorganism	Limit
Staphylococcus aureus	Not more than 100 per gram
***Escherichia coli*(indicator)**	Absent in 1 g
***Escherichia coli* O157 : H7**	Absent in 1 g
***Salmonella* spp.**	Absent in 25 g

II. CASE PRESENTATION

In the area of the Medical Officer of Health (MOH), Bulathsinhala, where I the first author was appointed as the Medical Officer of Health, is having a burden of malnutrition with high prevalence compared to other MOH areas in Kalutara District. There had been many programmes conducted on food availability, but little none on food security. Pre-planning for several strategies to establish food safety and security measures in the area was done for a duration of one month with the participation of Public Health Inspectors (PHII), minor staff, and support of the Food Administration Unit of the Ministry of Health. After conducting comprehensive food outlet inspections covering the MOH area and legal prosecutions over violation of law related to food safety[8], focus was shifted to inspections on primary food transporting vehicles, as most of food items were transported from outside to Bulathsinhala.

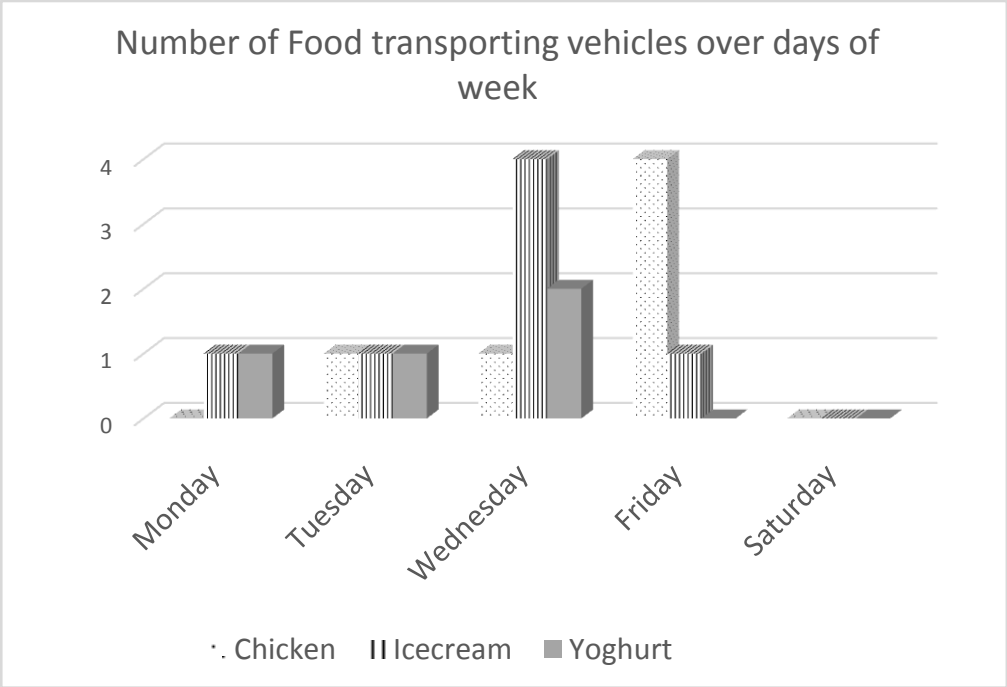


FIGURE 1. NUMBER OF DIFFERENT DIARY PRODUCT TRANSPORTING VEHICLES REACHING OVER DAYS OF WEEK

Situation analysis was carried out on dates of arriving of primary transporting vehicles to the town and its content of food items, with the support of minor staff and shop owners. The dates with the items were transformed to graph and it clearly showed that the most number and different type of primary food transporting vehicles were coming on Fridays of every week.

Inspections of food transporting vehicles were conducted since/ from 7.00 am to 11.00 am for the convenience of legal procedures before courts, as it was necessary to submit productions on the day of seizure itself. Twenty vehicles were inspected and 7 out of them were released after giving strict advice, 3 were detained to produce before courts. Almost immediately after arrest a defendant was brought into court for an arraignment. At the arraignment the defendant was read her rights and was informed of the charges against them.

[1] Flour Lorries belonging to reputed companies of Sri Lanka, transporting flour in unsanitary/ insanitary conditions.

[2] Three-wheeler selling bakery items, transporting bread exposing to dust.

[3] Lorry with chicken belonging to a reputed company of Sri Lanka, not maintaining the standard temperature. (Standard -18 c, was at +5 c)

III. LEGAL PROCEDURE

None of the team members / authorized officers had experience in similar type of legal procedures earlier. It was a novel experience to all and I the first author/ MOH was named to provide expert evidence for the cases as Medical Officer of Health. By entering a plea of guilty, first two defendants were admitting their guilt and fined 67 USD each. The last case was

charged against the chicken where the company lawyer admitted a plea of guilt, but pleaded to release all chicken worth of 2700 USD.

The court requested the expert opinion from the Medical Officer of Health on this matter and was replied (verbatim): "*I am qualified with MBBS, MSc in Community Medicine and currently following MD in Public health. This company was charged under 18 (a) the sentence of penal section of No 26 of 1980 food act,* ***where the nature of offence involves injury to the health of the public….etc,[9]***

So, it is not possible me to release this huge amount of chicken for public consumption. Madam, Kindly grant your permission to destroy this whole set under my supervision".

To this statement the lawyer representing the chicken company requested to send a sample of this chicken to the National Institute of Health Sciences which is the Government-approved food analysis laboratory, for analysis, and to keep the whole consignment of chicken in a sealed cold room.

The reply by the MOH when the magistrate sought the opinion of MOH at the above juncture was as follows *(verbatim):*

"*Madam, my objection, according to the storage instructions mentioned on the packaging the product should be kept at -18 degrees Centigrade. At the time of detection it was kept at + 5 degrees Centigrade. Thereby, exposed to deterioration of quality and safety of the product for a considerable period of time. Though there has been no apparent issue at this moment, with the passage of time, during the remaining shelf life the quality of the product will be adversely affected in terms of nutritional value, flavour, appearance, and safety".*

The Magistrate accepted the opinion of the Medical Officer of Health and made decision against the company with strict advice to maintain all vehicles in good sanitary condition, fined 67 USD and ordered to destroy all chicken worth 2000 USD under the supervision of the Medical of the Medical Officer of Health.

IV. CONCLUSIONS

Chemical, physical and microbiological changes are the leading reaction mechanism of food deterioration [11]. Lipid oxidation may produce changes in meat quality parameters such as color, flavour, odour, texture, and even nutritional value[1]. Microbial contamination can cause public health hazards and economic loss in terms of food poisoning and meat spoilage. Lack of proper temperature maintenance at any stage definitely leads to growth of microbes and lipid oxidation of chicken. A formal discussion with the Approved Additional Government Analyst in Sri Lanka revealed that any chemical analysis for chicken had never been carried out by them, and if done any microbial analysis sometimes would give "no growth report" in similar type of situations mentioned above.

Regulatory provisions related to above-mentioned food safety violations are defined under the Gazette Notification No. 1724/26 dated 26.01.12 titled Food (Hygiene) Regulations – 2011[2] published under the Food Act No. 26 of 1980.

The Provision 10 (6) of Food (Hygiene) Regulations – 2011 reads as:

"Where necessary, either conveyances or containers used for transporting foodstuffs shall be capable of maintaining foodstuffs at appropriate temperatures and allow those temperatures to be monitored". Contravention of the above provisions are punishable under the Section 18 (1) (a) of the Food Act No 26 of 1980 as amended by the Section 14 (1) (a) of the Food (Amendment) Act No 20 of 1991 and to fine not less than five thousand rupees (35 USD) and not exceeding ten thousand rupees (67 USD) or to an impressments for a term not less than six months. When considering the above fine with the recently introduced Motor Traffic law prescribing a fine of 170 USD for overtaking a vehicle on the left, a maximum fine of 67 USD for dealing with insanitary foods which are injurious to health is grossly inadequate.

It is noteworthy that there are clearly defined legal provisions related to unhygienic foodstuff/ food. The Section 2(2) of the Food Act No. 26 of 1980 states ***" No person shall import, sell or distribute any food manufactured, prepared preserved, packaged or stored for sale under insanitary conditions"*** and ***"Insanitary condition"*** is defined as " ***such conditions or circumstances as may contaminate food with dust, dirt or filth or render food injurious to health"***. In this regard special attention need to be focused to the terms ***"such conditions or circumstances"*** used by the legislators to define "Insanitary Condition" in a boarder manner giving opportunities to officers to express their views in wider perspectives when giving expert evidence in the Court of Law **.**

Maintaining of secure and proper shelf life for chicken is an essential aspect of public health in the island and Sri Lanka is still in the developing phase and the national nutritional status is in unsatisfactory levels especially among children. Despite the above the Sri Lankan legal system and legislatures related to food security have been comprehensively formulated to safeguard the health of the public.

In most European countries, cold chain of chicken is maintained to deliver safe food to people[13]. By contrast in Sri Lanka neither it is properly monitored nor supervised by legally authorized officials nor having any established system to retrieve records of temperature maintenance at any given time and at any stage of the cold chain.

Measuring and monitoring the cold chain lies with as a vacuum of making a great challenge on food safety measures. Therefore, implementing a system of placing a data lodger which records temperature fluctuations inside of any one set of bulk chicken is necessary as a future strategy to be enacted, which strategy is to be similar to the currently used monitoring mechanism for the cold chain of vaccines[3]. Using a data lodger is a matter of providers in each stage of cold chain of poultry meat and it would give an additional warranty for the next level purchaser before receiving to his outlet.

This context will be a next/forthcoming advancement in the field/realm of food safety in our country to supervise and inspect of temperature record by Public Health Inspectors through data lodger using their smartphones and it can be used as weighted and firm evidence for their legal submissions in future.

List of abbreviations:

1. MOH Medical Officer of Health
2. PHII Public Health Inspectors

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: Not applicable

Competing interests: The authors declare that they have no competing interests

Funding: Self-funded

AUTHORS' CONTRIBUTIONS

LK planned, designed, completed literature survey and participated in implementation of the study (Food Inspection Measures) as Authorized Officer for food safety of study context. LK was the major contributor in preparing the manuscript. A S, R A C, P M L M and G P participated actively for implementation of food safety measures as the Authorized Officers. U I was the coordinating and supervising district officer defined by food law of Sri Lanka. P performed by contributing to the manuscript preparation, especially the sections related to food law of Sri Lanka. K supported by submitting all references related microbiological and chemical standards of Sri Lanka. SHP supervised manuscript making and carried out the final drafting. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

All staff of the Office of MOH and the staff of the National Institute of Health Sciences, Kalutara, Sri Lanka.

AUTHORS' INFORMATION

LK was post graduate trainee, reading for a Doctorate in Public Health at the Post Graduate Institute of Medicine, University of Colombo, Sri Lanka and was qualified with MBBS in Faculty of Medicine, University of Colombo and Masters in Public health.

REFERENCES

[1] Aguirrezábal, M.M., M.C. Domínguez, J. Mateo and J.M. Zumalacárregu. 2000. The effect of paprika, garlic and salt on rancidity in dry sausages. *Meat Sci.* 54(1):77-81.

[2] Food (Hygiene) Regulations 2011. No: 1742/26. Government notitfication: The Gazette of the Democratic Socialist Republic of Sri Lanka; 26 January 2012.

[3] Epidemiology Unit, Ministry of Health, Sri Lanka. 2015. Immunization and Cold chain: available at: http://www.epid.gov.lk/web/index.php?option=com_content&view=article&id=139&Itemid=440&lang=en. Accessed 02 January 2017.

[4] Institute of Sri Lanka Standards. Amendment No : 3 To SLS 1218 : 2001, Specification for communited Meat Products [http://www.slsi.lk/web/images/PDF_upload/amds/amd%20164.pdf]

[5] Korkeala, Björkroth, J. Hannu, K. Johanna. 1997. Microbiological Spoilage and Contamination of Vacuum-Packaged Cooked Sausages. *J. Food Prot.* 6:610-737.

[6] Labuza, Ted, Dan Belina, F. Diez.: Food Safety Management in the cold chain through "expiration dating" In. Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, 55108 USA: 30.

[7] Man, C.M.D. (ed.). 2015. Shelf Life, 2 ed. John Wiley & Sons, West Sussex, UK.

[8] No 26 of Food Act. In.: Democratic Socialist Republic of Sri Lanka; 1980.

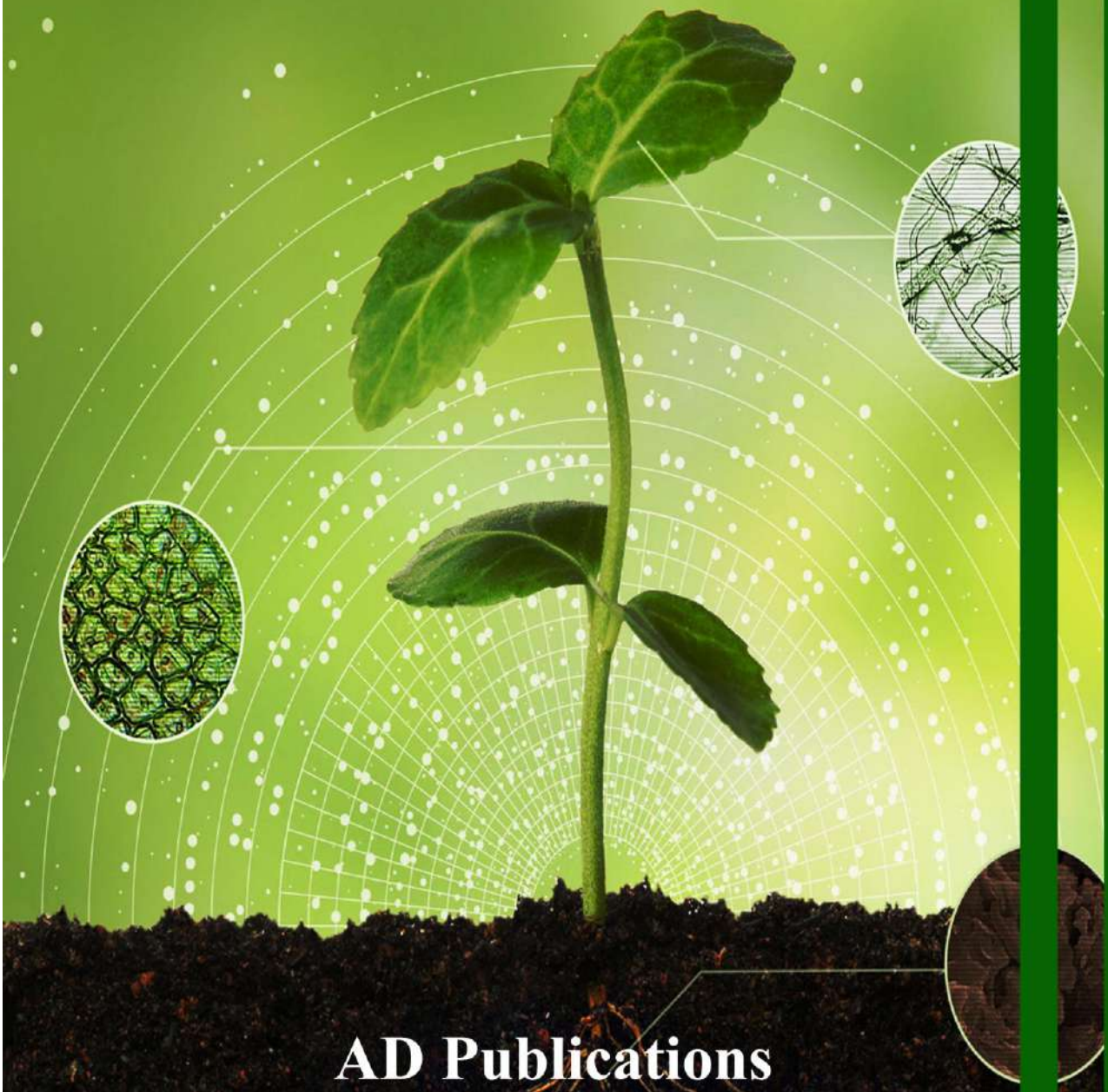
[9] Shelf Life Evaluation of Foods. In. Edited by C. M. D. Man AAJ; 1994.

[10] Shimoni, Eyal and T.P. Labuza. 2000. Modeling pathogen growth in meat products: future challenges. *Trends Food Sci. Technol.* 11(11):394-402.

[11] Singh RP: Scientific principles of shelf life evaluation. In: *Shelf Life Evaluation of Foods.* Edited by C.M.D. Man, Jones A, 2 edn. USA: Aspen Publishers, INC; 2000: 3-25.

[12] Symons H: Frozen foods. In: *Shelf Life Evaluation of Foods.* Edited by Man CMD, Jones A, 2 edn. USA: Aspen Publishers, INC.; 2000: 296-317.

[13] Zeuthen, P. , J. C. Cheftel, C. Eriksson, T. R. Gormley, P. Linko and K. Paulus (ed.). 1989. Processing and quality of foods: *High Temperature/Short Time (HTST) Processing: Guarantee for High Quality Food with Long Shelflife*, Elsevier Science Publishers LTD, Sweden.



AD Publications
Sector-3, MP Nagar, Rajasthan, India
www.adpublications.org, www.ijoear.com, info@ijoear.com