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Interactions of Market Horticultural Productivity on Climate and Weather Variations in the Northern Senatorial District of Cross River State, Nigeria

Eneji V. C. O.[1], Ogar D. A.[2], Omoogun C. A.[3], Dunnamah A. Y.[4], Ojikpong C.[5] & Ekpo C.[6]

[1] Department of Geography (Rural Development), School of Environmental Sciences, Federal University of Technology, Yola, Nigeria

[2] Department of Forestry and Wildlife Resources Management, University of Calabar, Nigeria

[3] Department of Curriculum and Teaching, University of Calabar, Nigeria

[4] Department of Economics, Ahmadu Bello University, Zaria, Nigeria

[5] Department of Geography, College of Education, Hong, Adamawa State, Nigeria

[6] Science and Environmental Education, University of Abuja, Nigeria

Correspondence: Eneji V. C. O., Department of Geography (Rural Development), School of Environmental Sciences, Federal University of Technology, Yola, Nigeria. Tel: 234-808-504-0272. E-mail: vcogareneji@yahoo.com

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Abstract

Various definitions have severally been adduced to poverty as the major cause of most environmental problems ranging from resources depletion, species extinction, deforestation, erosion etc. Majorly man's activities on the biosphere has tended to impact negatively on the environment, this activities are primarily carried out to sustain man's existence on the planet earth. These activities include faulty farming practices, indiscriminate use of agrochemicals including fertilizers for agricultural purposes, urbanization and industrial development. This research took a cursory look at the activities of rural farmers cultivating vegetables and other market gardening activities in order to sustain themselves during dry season as safety net for shortfalls in family income and food. These activities are done along watershed and this involves clearing, tilling, use of inorganic manure, herbicides amongst others. Researches including this have shown that these farming practices are detrimental to the ecosystem and have contributed greatly to affecting the ecological balance of the supportive ecosystem. This research therefore set out to assess how these dry seasons agriculture carried out within the study areas has affected the environment vis a vis climate and weather variation. The research adopted field experiment where data were collected twice per year for a period of five years from the farm site and the local meteorological station at Ogoja. The authors used bar graphs to present data from the field. Using rainfall, temperature and soil moisture content data for five years, the authors gathered that this farming activities has impacted negatively on the environment affecting both weather and climate variation. It was also observed by the researchers that though the data for five years was very minimal for any positive generalization, but findings shows that there were some noticeable changes attributed to these activities. It was recommended that hand dug well or boreholes should be used as sources of water supply to avoid destruction of the watershed. It was also suggested that farm yard manure should be used instead of the inorganic manure that has become detrimental of late to both the environment and human health.

Keywords: watershed destruction, climate variation, food security, environmental degradation

1. Introduction

The economic and social main stay of most communities in the world today is agriculture. Agriculture, be it mechanized or peasant farming accounts for more than half of the world population's source of food and income. Agricultural production accounts for less than five percent of the gross world product not minding the size of its workforce and its contribution to society's survival (an aggregate of all gross domestic products) (Gordon et al., 2007). Poverty has been attributed as the root cause of most environmental problems in our world today. Because poor people are always faced with the problems of meeting their daily needs; food, shelter, health and other financial requirement they are forced to fall back to the land for their daily survival not minding the impacts of their action on the environment. The only place they can turn to for their survival is the land or the environment where they look at the resources within as their ancestral inheritance. They see these common pool resources as their last reserve where they can always fall back to for complimenting their sources of livelihoods. The pressure of the activities of man on the environment has increasingly affected the ecological system and its resources. This has also affected biodiversity and other species within the ecosystem. Man's activities on the environment has altered the ecological balance and functioning of the entire ecosystem. This interaction is due to the energy flow or the thermodynamic interactions taking place within the ecosystem. The sum total of these effects is specie extinction including specie loss, endangerment, disappearance and ecological problems like shortage in rainfall, climate change, flooding, increased heat, and fertility loss and food insecurity.

Chrispeels and Sadava (1994) saw the action of man in the environment as acting without thinking about the environment and the future generations. Pretty et al. (2000) observed that man has so impacted on the environment that he does not even think well about the influence of his activities on the environment. While Darlong (2004) observed that poverty is the reason behind man's unquenching action to compulsorily satisfy his basic needs. According to Maslow (1963), these needs are arranged in an ascending order, and once a particular need at the lower order is met, the next need on this hierarchy of human needs surfaces automatically. Maslow, (1963) listed human basic needs to include, physiological needs (food, shelter, clothing etc), safety need (security, consciousness and identification among a crowd of people), love (acceptance, love and belonging), self esteem (human dignity and respect, self integrity, self pride and honor) and self actualization (the sense of fulfillment and arrival in the social strata in any society, class formation and attainment).

Sol (2003) avers that most causes of environmental problems are caused by habitat decimation and destruction, urbanization, pollution and natural disaster. Eneji et al. (2009a) included faulty agricultural practices as another cause of environmental problems. In their submission, they looked at the major problems of the environment to include deforestation for whatever reason, agriculture, urbanization, infrastructural development, poor agricultural practices (peasant agriculture, rotational bush fallowing, shifting cultivation, mechanized agriculture with the concomitant indiscriminate and unregulated use of agrochemicals, herbicides, insecticides, pesticides, inorganic fertilizer), habitat decimation, pollution from both domestic, industrial and commercial production and consumption processes. Other forms of waste, from industrial, domestic and commercial activities all contributes in no small way to environmental degradation (Expedito et al., 1996; Alakali et al., 2006; Borger, 2008).

The activities of these rural farmers/horticulturalists in the study area in an attempt to increase their sources of income and improve their livelihoods have contributed in greater dimension to destroy the ecosystem leading to the variation of the climatic/weather conditions of the area. In these study areas, agriculture is still at the traditional level, with the use of local farm implements like hoe and cutlasses for clearing and cultivating land, bush burning is another method of clearing land for farming activities. In some communities, stumping is also done where there appear to have too many shrubs on the farmland. Besides the major staple food crops cultivated for family consumptions like yams, cassava, maize , banana, guinea corn, millet, long beans, potato, rice etc, most women and young people have resorted to cultivate rice, and some vegetables during the dry season to improve their income basically and to contribute occasionally to their feeding. In villages like Ugboro, Ukpah, Otukpuru, Akurinyi, Ukpada, Abuochiche, Beten, Nyanya, Gabu, Igoli and a pocket of other communities, they now cultivate rice twice on the same portion of land per year. The idea in itself is to guarantee food sufficiency and food security and also to increase their income, but the methods of cultivation with low agricultural inputs rather depending on the natural fertility of the soil for better yield has become worrisome of late. Young women and men now cultivate vegetables like pumpkin (*Telfairia occidentalis* hook) green vegetables (*Amaranthus spinosus*), okra (*Hibiscus esculantus*), ginger (*Zingiber offinaciale*), waterleaf (*Talinum triangulere*), curry, pepper, and other such annual short live crops along watershed. According to Akinkugbe, (2007) this type of market horticultural practices and short period agricultural activities destroys the ecosystem and the ecobalance leading to leaching of fertile soil, destruction of watershed and destruction of soil fertility fixing microorganism. The choice of cultivating along watershed is to have access to water close to their farm where they could do manual irrigation of the crops directly from the streams or rivers. A watershed is any area drain by any body of water or a stream. This therefore means that as they engage in this type of dry season agriculture/ horticulture, their choice of land is along watercourses, which are the main watershed of the area (Eneji, 2006). During land preparation, they deforest the land with machetes and cutlasses; burn the grasses, then till the land using local farm implements (hoe and shovels). In the process of clearing and cultivating, they destroy the watershed. The result of these unwholesome farming activities is the destruction of environment and the climatic variations in global and local climate regimes of the communities (Borger, 2008). This activities of local market gardeners in the area has contributed in time past to destroying the watershed and exposing the top soil to direct effect of solar radiation and heat. The burning of these grasses has also generated a lot of heat on the soil, thereby killing some microorganisms responsible for soil fertility replenishment and other nutrient recycling in the farm site under study (Silveira et al., 1996; Akatugba, 2004; Pretty, 2000; Field, 1997; World Bank, 1995).

In a research by Ojugo (2009) on the effect of tillage method on soil microclimate and yield of fluted pumpkin (*Telfairia occidentalis hook. f*), looking at the effect of tillage on ground cover, he observed that ground cover influenced surface runoff, the dynamics of soil moisture, and played an important role in soil and water conservation. Baker et al. (2000) observed that ground cover mainly refers to living land surface cover, non-decomposed and semi-decomposed plant litters mulched on the soil surface, once these ground cover are destroyed, the living part of the earth has been destroyed (Yaqub, 2007). In this study, they used *Paspalum notatum* and its litter to study the effects of ground cover on distribution of soil moisture along a slope.

The practice of slash and burn agricultural system has both negative and positive consequences. The positive consequences, which local farmers always capitalize on, is that the fire burns off all insects and crop pest which would have ordinarily affected or attacked their crops (Pretty, 1994). Other usefulness of burning farm sites includes easy way of discarding the thick grasses and packing them from the farmland. Scientifically, the major positive impact of bush burning is that in an acidic soil, the ash from the burnt grasses is very rich in alkalinity, so it helps to reduce the soil acidity hence allowing the neutralization of the acidic soil content and improving crop productivity. However, the point always forgotten even at that is that how did they test the soil to ascertain whether the soil is basic or acidic in nature to know whether it needs neutralization or improvement on the soil acidity (Pretty, 1994). Negative effect of bush burning for agricultural purpose by our local and peasant farmers is the killing of soil micro organism responsible for soil fertility improvement and decomposition of leaf litters and other living things amongst which are nitrosomonas, (nitrogen fixing bacterial in the stem and leave nodules of rhizomes and cover crops, azocotbacter, fungus, bacteria). Another is the reduction of soil water or moisture content by evaporation as a result of the heat from the burning. Most often when this type of crop production is practiced, the local farmers use all kinds of inorganic fertilizers to boast their crop productivity especially market horticulture. Synthetic nitrogen, along with mined rock phosphate, pesticides and herbicides has greatly increased crop yields in the study area (Suman & Chakesang, 2008). In these areas of study, inorganic chemical fertilizers like NPK 15:15:15, NPK 20:10:10, NPK 12:12:17:2, ammonium nitrates popularly known as urea and herbicides are ignorantly applied to the soil and water without knowing the chemical composition and implication for both the soil, water, animal and humans that may consume such crops or vegetables (Schneider, 1989; Eborge, 2002; FAO, 2008). The use of obnoxious chemical to kill grass and bushes around, some of these herbicides are those that have been disbanded from usage in other part of the world, but are now being used in Nigeria (Brady & Weil, 2002; Schneider, 1989; Gordon et al., 2007).

Micro climatic conditions and variations in ecological systems within the region have generated very complex weather and climatic conditions. To analyze the perceptions of communities practicing these horticultural systems in relation to their experience with eco-climate change, five unique sites practicing this traditional market gardening were selected. The mean annual rainfall varies from 2000-4000 mm with most rainfall concentrated from May to September.

1.1 Some Market Horticultural Practices

Vegetation clearing: (Slash and burn): the clearing of the topsoil's vegetation and the setting of fire to burn the grasses to enable land cultivation is slash and burn. This is also called land clearing and burning. This process exposes the top soil to direct solar radiation and thermal heat, while the burning also kills soil microorganism, which are responsible for the fixation of soil nutrient, recycling and decomposition of dead materials (Azotobacter and nitrosomonas and denitrifying bacteria).

Land preparation or cultivation (also call soil tillage): The ploughing of the top soil in preparation for planting or for the addition of nutrient, herbicides and pesticides. Land cultivation varies in intensity from zero to complete traditional tilling. Though this method may improve crop productivity through the warming of the soil, introduction of fertilizer and through weed and pest control, but the unintended effect leads to rendering the soil more exposed to ecological and climatic conditions such as erosion, it catalyses the decay or decomposition of soil organic matter releasing CO_2, and reduces the abundance and diversity of soil organisms while also increasing the average temperature of the area (Brown, 2008).

Nutrient/fertility management: This is the process of inputting crop nutrient or soil fertility to improved crop productivity. Fertility inputs or nutrient management has to do with the introduction of either organic or inorganic manure/fertilizers, farmyard or compost and phosphate minerals from rocks to boast crop yield. Manure is applied by spreading either dry or in liquid form on cropland or farm sites where crops and vegetables are cultivated. The commonest type of manure used by these farmers are the inorganic compound manure like NPK and Urea, though in some cases organic manure like farmyard manure, animal dung's and composting are also used. In this type of agriculture, other forms of soil fertility management like rotational bush fallowing and shifting cultivation are rarely practiced.

Soil water management: This is the practice of improvising water for crop growth. Attempting to manage soil moisture content and water by manually irrigating the land from the river or stream where such gardening is done along watershed involves altering the water condition of the watershed. This practice is done during the dry season where market gardening is carried out. The practice includes mulching, irrigation and regular watering of crops especially vegetables (Suman & Chakesang, 2008)

The study was carried out to assess how these agricultural practices of dry season farming can affect the climatic and weather condition of the study area and the larger community as a whole. The study also seeks to establish if the farming practice can affect local climate modification and environmental change.

2. Methodology

This study was located within the northern senatorial district of Cross River State; the Northern Senatorial District of Cross River State is made up of five local government areas: Ogoja, Yala, Bekwarra, Obudu and Obanlikwu Local government areas of Cross River State, Nigeria. However, the study was rather concentrated in Bekwarra and Ogoja local government areas. The specific study sites are located at Kwarikwata swamp in Igoli, Ogoja local government area, Illa stream in Gakem village, Unwaodaa stream and Uduo River, in Otukpuru village, Junction swamp in Abuochiche, Ityem River in Ukpah and Ugboro in Bekwarra local government area. These villages and farm sites for market horticultural productivity (market gardening) were purposefully selected because they have been engaged in market gardening activities over the years. They cultivate dry season/short period vegetables like fluted pumpkin (Telfairia occidentalis Hook. F), green vegetables (*Amarantus spinosus*), waterleaf (*Talinum triangulere*), maize (*Zea mays*), okra (*Habiscus esculantus*), scent leaves, (Cymbopogon Winterianus, Geranium Pelargonium), garden egg (*Solanu melongena* var., esculentum, *Solanum integrifolium*), pepper (*Capsicum annuum*) curry leaves (*Murraya koenigii*), onions (Allium, stellatum, Alum selpa), ginger (Zingiber officinale Roscoe) among others. The study area according to the 2006 national population census has an aggregate combine population of more than 350, 000 persons; with a gender ratio of 1: 3 (male: female ratio), their major occupation is predominantly peasant farming for feeding their immediate families with very little for market. Peasant farming is the predominant occupation of the people, a reasonable percentage of the population are civil/public servants and businesspersons. The area is bounded by Obudu and Boki local government areas in the East, while in the North, it is bounded by Vandeikya of Benue state, in the West, it s bounded by Okuku and Yache in Yala local government area, while in the South the area is bounded by Ikom all in Cross River State, Nigeria.

The study was carried out under a five years period. The authors used both Action and experimental research design. Using participatory research method involving semi-structured interviews (SSI) on focal groups of farmers, data was generated on agricultural land use pattern in these study areas. First, the authors designed a key informant interview and administered to the people involved in the cultivation of market horticulture, this was to enable us ascertain if there has been any noticeable change or variation in the weather and climatic condition of the area. These changes we tried to attribute to the horticultural production activities, which they have carried out over the years. The authors also administered interview and questionnaire to some people living around the areas where these farming practices are being carried out. This is to identify if there are changes in water, reduction in rainfall, river dry up, forest depletion, watershed destruction, and increase in temperature among other variables conceived to be associated with these farming practices. A total of 100 persons were interviewed and discussed with informally. During this five years period, the researchers collected soil samples from the farming sites during the farming season and exposed the soil samples to two types of treatments. We weighed the soil samples immediately there were collected from the farm sites, then expose the sample to the sun for some period and then weigh again to check for weight loss because of evaporation. We also heat some of the soil samples using a Bunsen burner in the laboratory to determine the rate of evaporation and to ascertain if any soil moisture is lost during burning and exposure to heat from direct sun light since the vegetation cover is regularly removed during the dry season. We took the temperature of the farm sites for both dry and rainy season for a period of five years using stationed thermometers and the amount of rainfall within the study period using a graduated rain gauge. During the same period of study, we also collected data from the meteorological station at Ogoja for temperature and rainfall. The yearly average for temperature and rainfall and also the average for every six months dividing the seasons into when they cultivate these market crops and when they don't, though we were not oblivion of the fact that temperature increases during dry season. We interviewed residents who have lived in the area for onwards of ten years and above to compare what they see now in the area as compared to what they had seen before these farming activities became prominent in the area. Results from the field sample study are as shown in the tables below.

3. Results

From Figure 1 below, there is a marked increase in temperature as a result of the farming activities practiced in the area and this has contributed to the rise in temperature over the study.

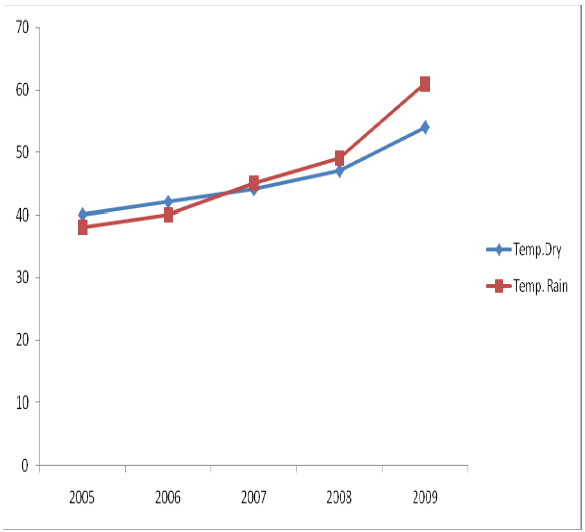

Figure 1. Temperature variations between dry and rainy season during the study period (ºC)

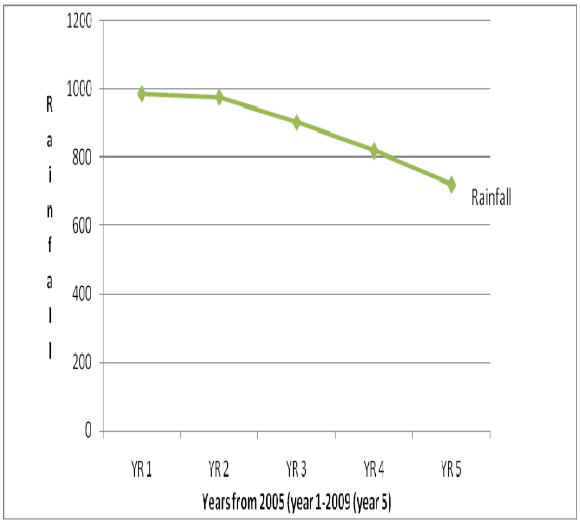

Figure 2. Annual rainfall variation within the study periods in the farm sites

There is thus a steady increase in the mean annual temperature figures over the years increasing from 2005 at 43.4 ºC to 54.8 ºC in 2009 during the dry season when they engage in market gardening, while there is also an increase in the mean annual temperature in the study areas during the rainy season from 40.7 ºC in 2005 to 50.2 ºC in 2009. When the variance is calculated within these study periods, there is a marked increase in temperature. From this analysis, it therefore means that the farming activities carried out during this period contribute to a change in temperature within the area under study.

Figure 2 also shows that there is reasonable reduction in rainfall due to deforestation, bush burning and direct exposure of the soil to heat from the sun. This is because of the land clearing and subsequent destruction of the watershed along the watercourses where they cultivate these farms during dry season. From the graph below, the rainfall values shows a reduction of -264.8 cm^3 within the study period. This is so because the soil moisture content evaporates at a rate higher than the normal. The second reason is that the watersheds are usually destroyed leading to the reduction in rainfall volume and frequency. This farming activity therefore has significant impact on the rainfall pattern and frequency.

From Figure 2 above, it is observed that there is a reduction in the soil moisture content, this shows a marked difference in temperature which was attributed to the exposure of the soil to direct heat from bush burning and solar radiation as a result of deforestation over a period of 5 years. The mean variance in temperature between the years 2005 and 2009 shows an increase temperature of 17.2 ºC, this shows an annual increment of 3.4 ºC.

The result is that there is an increment in temperature and a reduction in rainfall and soil moisture content in the area under study. From our personal interviews and observation, the main reason for people engaging in these farming activities is to improve their income and supplementing their food during times of want. The people also observed that there is well-established change in the environment, especially as the amount of heat observed these days is quite different and higher than what was the case some years ago. The respondents also complaint about the amount of rainfall which has also reduced as compared to what use to be the case in these areas. They attributed the cause of all these to bush burning and deforestation as being carried out in the area. Some informed people called the observed problem desertification.

Result from the KII also shows that people living within the study areas over the years have agreed to have noticed some changes, according to them, these changes may not only be due to the existence of the these farming practices only, but also because of the prevalent scarcity of water which force people to turn to the forest for this type of agriculture. Another reason adduced for this change in the forest condition was that cattle Fulani's move with their cattle into the forested areas of the zone, while this was never the case. Even at that some were blunt in their position that it is true that the gardening system is contributing in no small way to the feeding of the population, but the environmental cost associated with this farming system is too much for the community to bear.

4. Discussion of Findings

From the graphs and tables, the results shows that there is a marked difference in temperature, rainfall and soil moisture content and above all, observed changes in environmental and climatic conditions in the study areas. It was clear from our discussions that traditional practices in agriculture are overloaded with eco-climatic problems ranging from siltation, soil moisture content loss, reduction in rainfall, increase in temperature, increase in atmospheric carbon, soil fertility loss and biodiversity depletion and in some cases, extinctions. Because the practice of this farming pattern and system is to provide for immediate food shortage and to also carter for the urgent financial needs of those who become engaged in market gardening. This is further buttressed by the findings of Oamen (2004). This local innovation requires minimum supply of water and is practiced after the rainfall when water becomes scarce. They now depend on water from the streams and rivers, and farming along watershed, hence the destruction of watershed reducing rainfall in the farm area. Due to reduced rainfall and moisture in the soil, most land is becoming desertified. This phenomenon is rated as one of the major ten ecological problems in recent times. Desertification does adversely affect the socioeconomic development of any society. Desertification does not only cause serious harm to ecosystem, but also has adverse effects on many aspects of socioeconomic and cultural development of the entire region (World Bank, 1995). Some of the problems associated with desertification as a result of reduced moisture availability in the soil includes: Reduced soil quality and arable land size, reducing the quality of soil for crop productivity. Soil desertification from reduced moisture content and rainfall diminished biodiversity and bio-productivity. In this case, desertification has extremely serious ecological consequences. With this type of farming activities, it leads to soil moisture content loss which causes high soil and water loss, with some soil deposition in rivers and lakes; this worsens the whole ecological environment (World Bank, 1995). This also increases poverty and reduction of economic benefits. Because desertification destroys land and water balance, it also aggravates poverty.

The result from the field was further buttressed by the observation of residents within the study area during the Key informant interview. Even though most of those interviewed were illiterate, they agreed that they have noticed some changes in the forest conditions and also on the intensity of heat generated from the sun and the volume of rainfall which has decreased in recent times considerably. The deterioration of the ecological environment aggravates the contradiction between man and nature, making it difficult to establish a harmonious relationship among society, nature and ecological civilization (Yu et al., 2002; Brown, 2008). The activities carried out by these farmers' causes serious threats to the eco-climatic regime of the local people and this contribute in no small way in modifying the local climate of the environment. Some of the ecological implication of market horticulture as practiced by these people include but not limited to these listed here. Agriculture imposes external costs upon society through pesticides, nutrient runoff, excessive water usage, and assorted other problems.

Land transformation, the use of land to yield goods and services, is the most substantial way humans alter the Earth's ecosystems, and is considered the driving force in the loss of biodiversity. Estimates of the amount of land transformed by humans vary from 39-50%. Land degradation, the long-term decline in ecosystem function and productivity, is estimated to be occurring on 24% of land worldwide, with cropland over represented. The UN-FAO report cites land management as the driving factor behind degradation and reports that 1.5 billion people rely upon the degrading land. Degradation can be deforestation, desertification, soil erosion, mineral depletion, or chemical degradation (acidification and salinization).

Eutrophication, excessive nutrients in aquatic ecosystems resulting in algal blooms and anoxia, leads to fish kills, loss of biodiversity, and renders water unfit for drinking and other industrial uses. Excessive fertilization and manure application to cropland, as well as high livestock stocking densities cause nutrient (mainly nitrogen and phosphorus) runoff and leaching from agricultural land. These nutrients are major nonpoint pollutants contributing to eutrophication of aquatic ecosystems. This finding affirmed conclusions of Arnold (1992) and

Ogar (2005) who observe respectively that inorganic chemical fertilizers contribute an unintended outcome of over fertilizing water bodies which increases the growth of water hyacinth and also increase the carbon content of the water on the top preventing oxygen from going under for plants and animals to live on, here eutrophication arises.

Climate change can potentially affect agriculture through changes in temperature, rainfall (timing and quantity), CO_2, solar radiation and the interaction of these elements.

It has been established beyond all reasonable doubt that the gardening activities as carried out by these local farmers have contributed in no small way to negative modification of local climate and this can also result to climate change, reduction in water supply as a result of watershed destruction, water pollution, eutrophication and species extinction and endangerment, increased atmospheric carbon, temperature increase and reduction in rainfall and salinization of rivers and other water bodies.

5. Conclusion

These market horticultural farming activities had shown that they have deleterious effect on the environment and also on human health and wellbeing. The farming system also cause erosion and slope formation along cultivated area and along the water shed where they cultivate the rich forest soil for water availability assurance and fertility. Above all there is the destruction of the watershed that guarantees our steady supply of water, hence most water bodies in the area have completely become shrunken or dried up. Most of the forest areas have been depleted and some places have been completely laid bare as a result of these farming activities of market horticulture. This had led to most land becoming dry and empty, with just minor shrubs here and there in the farmland. This situation of climatic and weather change is noticed within Uduo river and Unwaodaa in Otukpuru, Junction spring in Abuochiche, Illa in Gakem, Ityem in Ukpah and Ugboro and Kwarikwata in Igoli, Ogoja. The case is becoming so critical that it has become a case for concern of late because this practice is detrimental to the environment. These ecosystem disturbances as a result of these market horticultural productivities have also been confirmed by the researches of other scholars like the Millennium Ecosystem Assessment. These complex trade-offs between increased agricultural production and declines in other ecosystem services as caused by agricultural changes to the hydrological cycle have been reviewed by the Millennium Ecosystem Assessment and the Comprehensive Assessment of Water Management in Agriculture Comprehensive Assessment of Water Management in Agriculture, Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture, Earthscan Publications (2007). This review research revealed that knowledge of these trade-offs is prolific and increasing, this also shows that we lack an integrated understanding of how agricultural modifications of the hydrological cycle regulate the prevalence and severity of weather and eco-climatic change in ecosystems (Gordon et al., 2007).

In view of the foregoing, it is clear that the farming activities carried out by these groups of farmers have potentially contributed in no small way to environmental deterioration and degradation in the area under study. It is necessary to mention here that though the time frame of five years was scientifically too short to record any reasonable climate change, but with careful observation and analysis followed by independent studies, we drew the conclusion that there is observed local climate modification and change in this area.

6. Recommendations

From the findings in the field, we came up with the following suggestions:

- There should be the introduction of the use of water from a reservoir or hand dug well to replace farming along water course.
- Such farms should be cultivated away from watershed and watercourses.
- The use of inorganic artificial fertilizer should be replaced with the use of organic manure like composting and farmyard manure from the leaf litters and the grasses removed from land clearance in the farm.
- Government should design a well articulated poverty reduction strategy that will reduce undue pressure on the environment.
- Proper laws should be made regarding the importation, use and control of most of the agrochemicals which have been bounded out of the country and Nigerian and most African countries are now found as a dumping ground for such chemicals which researches have found to contribute to more than 42% of cancer in Nigeria.
- Agricultural extension agents should also be sent to these rural communities to help in articulating and providing services on modern horticultural production methods which are ecologically friendly.

References

Akatugba, C. V. D. (2004). Evaluation of the utilization of common leafy vegetables in Southern Nigeria. *Journal of Vegetables Studies, 2*(1), 23-28.

Akinkugbe, J. H. (2007). The production processes and marketing of vegetables in selected urban centres in Edo and Delta States. *Journal of Business Management, 10*(1), 14-20.

Alakali, S. J., Ariahu, C. C., & Mkpa, A. (2006). Kinetics of osmotic dehydration of mango slabs. *J. Food Processing and Preservation, 30*(5), 597-607. http://dx.doi.org/10.1111/j.1745-4549.2006.00080.x

Arnold, J. E. M. (1992). Production of Forest Product in Agricultural and Common Land Systems: Economic and Policy Issues. In E. Sharma & Narendra (Eds.), *Managing The World's Forest*. Iowa: Kendal/Hunt Publishing Company.

Baker, J. B., McKinney, B., & Hurd, J. (2000) Initial Assessment of Social and Economic Factors Affecting Biodiversity Conservation Efforts in the Forests of the Lower Mekong Ecoregion Complex, IUCN and WWF. Hanoi.

Borger, J. (2008). *Feed the world? We are fighting a losing battle, UN admits*. The Guardian (London).

Brady, N. C., & Weil, R. R. (2002). Nitrogen and Sulfur Economy of Soils. *Elements of the Nature and Properties of Soils*. (pp. 386-421). Pearson Prentice Hall, Upper Saddle River, NJ.

Brown, L. (2008). *Global Water Shortages May Lead to Food Shortages-Aquifer Depletion*.

Chrispeels, M. J., & Sadava, D. E. (1994). Farming Systems: Development, Productivity, and Sustainability. *Plants, Genes, and Agriculture* (pp. 25-57.) in Jones and Bartlett, Boston, MA.

Darlong, D. S., (2004). Effects of mound and no-till on the yield of fluted pumpkin in Northern Nigeria. *AgroScience, 3*(2), 8-14.

Eborge, E. O. D. (2002). Comparative analysis of the growth and yield of yam under mound and ridge tillage. *Journal of Agricultural Development, 6*(2), 54-66.

Eneji, V. C. O. (2006). Urban and Rural Dwellers Consumption Pattern of Wildlife and its Impact on Biodiversity: The case for Bekwarra and Calabar Urban of Cross River State, Nigeria, Unpublished Degree thesis, University of Calabar, Calabar, Nigeria.

Eneji, V. C. O., Qi, G., Jian, X., Oden, S. N., & Okpiliya, F. E. (2009). A review of the dynamics of forest resources valuations, issues, arguments and concern. *Journal of Agriculture, Biotechnology and Ecology, 2*(2), 210-231.

Expedito, T. F., Silveira, M., Shafur, R., & Backle, M. A. (1996). Osmotic dehydration of pineapple: Kinetics and product quality. *Food Research Intl, 29*(3-4), 227-233.

Field, B. (1997). *Environmental Economics*, UK: Earthscan Publication.

Food and Agriculture Organization. (2008). Analysis of farming systems. Rome.

Gordon, G., Peterson, D., & Bennett, E. M. (2007). *Trends in Ecology & Evolution, 23*(4), 211-219.

Maslow, A. (1963). Hierarchy of human needs

Millennium Ecosystem Assessment. (2007). In L. Brown (Ed.), *Global Water Shortages May Lead to Food Shortages-Aquifer Depletion*.

Oamen, D. E. (2004). The influence of tillage on the yield of intercropped melon and yam in Southern Nigeria. *Agro Development and Environmental Protection Monograph, 8*, 5-8.

Odjugo, P. A. O. (2007). The effects of tillage systems and mulching on soil microclimate, growth and yield of white yam (Dioscorea rotundata) in Midwestern Nigeria. *Journal of Food, Agriculture and Environment, 5*(2), 164-169.

Ogar, E. A. (2005). Effects of fertilizer rates and cutting frequency on the marketable vegetable and pod yields in fluted pumpkin in south Eastern Nigeria. *AgroScience, 4*(1), 66-69.

Pretty, J. N. (1994). Alternative systems of inquiry for a sustainable agriculture. *IDS Bulletin, 25*(2), 37-49. http://dx.doi.org/10.1111/j.1759-5436.1994.mp25002004.x

Pretty, J. N. (2000). An assessment of the total external costs of UK agriculture. *Agricultural Systems,* 65(2), 113-136. http://dx.doi.org/10.1016/S0308-521X(00)00031-7

Schneider, K. (1989). *Science Academy Recommends Resumption of Natural Farming*. The New York Times. Retrieved from September 8, 1989, http://www.nytimes.com/1989/09/08/us/science-academy-recommends-resumption-of-natural-farming.html

Silveira, M., Expedito, T. A., Shafhur, R., & Ken, K. B. (1996). Osmotic dehydration of pineapple: Kinetics of product quality. *Food Research Int, 29*(414), 227-233. http://dx.doi.org/10.1016/0963-9969(96)00035-X

Sols, S. (2003). *Global Environmental Outlook 3*. Earth Scan Publications.

Tegtmeier, E. M., & Duffy, M. (2005). External Costs of Agricultural Production in the United States. *The Earthscan Reader in Sustainable Agriculture*. Retrieved from http://www.organicvalley.coop/fileadmin/pdf/ag_costs_IJAS2004.pdf.

World Bank. (1995). Overcoming Agricultural Water Pollution in the European Union.

Yaqub, A. A. (2007). The effects of leaves harvest on the yield of pumpkin fruits in the middle belt of Nigeria. *Journal of Environment and Agriculture, 5*(1), 78-86.

Yu, W. (2002). In L. R. Brown (Ed.). *Global Water Shortages May Lead to Food Shortages-Aquifer Depletion*.

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Assessment of Irrigation Pricing Policies: A Data Envelopment Analysis Approach

Giacomo Giannoccaro[1], Maurizio Prosperi[1], Francisco Alcón[2] & Julia Martin-Ortega[3]

[1] Dpt. SAFE, University of Foggia, Foggia, Italy

[2] Dpt. Economía de la Empresa-Área de Economía, Sociología y Política Agraria. Universidad Politécnica de Cartagena, Cartagena, Spain

[3] Social, Economic and Geographical Sciences, The James Hutton Institute, Aberdeen, Scotland UK

Correspondence: Giacomo Giannoccaro, Dpt. SAFE, University of Foggia, Via Napoli, 25-Foggia 71100, Italy. E-mail: g.giannoccaro@unifg.it

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Abstract

The European Water Framework Directive encourages pricing policy reforms in order to protect the environmental quality of water and promote its efficient use. This paper deals with two aspects of efficiency, namely economic and environmental efficiency, analyzed for different pricing methods. Volumetric pricing methods are compared with other indirect schemes (‘per area’, on ‘input’, on ‘output’ and ‘quota’) under three different water saving scenarios. The Data Envelopment Analysis (DEA) technique is used to assess the eco-efficiency of an eventual water pricing reform in the irrigated agricultural system of Capitanata, in Italy. Overall, findings point out that a pricing system based on ‘per area’ and ‘output’ will lead to the highest eco-efficiency, although this is not valid under any water pricing charge. The enforcement of water saving via pricing does not imply a higher eco-efficiency, mainly in the case of environmental efficiency. The use of the DEA approach appears useful in the assessment of water pricing policies where conflictive economic and environmental goals arise. It provides a methodology to support policy makers in the design of water policy pricing aimed at the enhancement of efficiency, both economic and environmental.

Keywords: eco-efficiency, data envelopment analysis, water pricing scheme, irrigation

1. Introduction

The enhancement of water use efficiency is one of the priorities of the European Water Framework Directive (WFD), which sets the target of achieving a sustainable use of water that guarantees the good ecological status of all water bodies. The most relevant impact from its implementation is expected on the agricultural sector, since it accounts for 60-80% of the overall demand (Dworak et al., 2007). To achieve its objectives, the WFD prescribes the use of economic tools and principles, such as the full cost recovery of water services, and the polluter pays principle (Martin-Ortega, 2012).

In this regard, a water-pricing reform would (directly or indirectly) “[. . .] provide adequate incentives for users to use water resources efficiently, and thereby contribute to the environmental objectives of this directive”, as stated by Article 9 of the WFD. This paper is aimed at providing scientific support to the implementation of the WFD in this respect, by assessing the efficiency of several water pricing policies for irrigation. The volumetric pricing is commonly believed to be more effective because payments are directly linked to actual delivered water (Dono, Giraldo, & Severini, 2010). However, its implementation is hindered by some technical difficulties, such as the application of metering devices to different kinds of water sources (streams, natural reservoirs, groundwater, wells, etc.); and their monitoring and surveillance (Molle & Berkoff, 2007). Moreover, volumetric systems are costly and, as it is pointed out by Tsur and Dinar (1997), in some areas the efficiency gains may not be sufficient to cover the costs for water pricing reform. This paper tries to address the research question of whether alternative pricing schemes may also be suitable (or eventually better) for the achievement of WFD’s environmental objectives in accordance with its economic efficiency principles.

The main added value of this research relates to the joint assessment of economic and environmental efficiency. Economic efficiency relates to the adequate allocation of the water resource among different irrigated crops, and its capacity to generate economic outputs. Environmental efficiency relates to the capacity of pricing schemes to reduce externalities caused by irrigated agriculture on the environment. These two concepts, when considered together, yield the measure of eco-efficiency. The assessment is performed by simultaneously considering technical input, negative environmental externality (i.e. undesirable) and economic outputs (i.e. desirable) (Färe, Grosskopf, Lovell, & Pasurka, 1989; Titeca, 1996; Kuosmanen & Kortelainen, 2005; Picazo-Tadeo, Gómez-Limóm & Reig-Martínez, 2011). This approach represents a contribution to the literature since at present, most studies concerning water productive efficiency follows an agronomic approach (yield per drop) or an engineering approach (conveyed water per available water) (see Malano & Burton, 2001) for a comprehensive list of the water efficiency indicators) and do not look at eco-efficiency as a measurement of global performance of irrigated agriculture, ignoring the fact that conflicting economic and environmental objectives may emerge.

Particular focus is placed here on insights into the improvement of eco-efficiency stemming from a water policy reform in agriculture adopting a macro-level perspective. More specifically, eco-efficiency is defined here as the ratio between economic value added and some indicators of environmental pressures such as nitrogen balance and risk of pesticides. The analysis is carried out at regional scale for the agricultural system as a whole. The study refers to the catchment of the irrigation basin named 'Consorzio della Bonifica della Capitanata' (CBC), which is located in the province of Foggia (Apulia region), Italy. Results are, nevertheless, expected to be of relevance at the wider level in Europe, where other water scarce regions face similar water management issues.

To address the research objectives, the overall effects of alternative hypothetical water pricing schemes are firstly simulated by a regional linear programming model (LP). Then, the assessment of the eco-efficiency is undertaken through a Data Envelopment Analysis (DEA). The use of simulated date to feed into DEA has already proven appropriate by the literature (e.g., Musolino & Rindone, 2009; Bono & Matranga, 2005). Raju and Kumar (2006) used DEA to select the most suitable irrigation planning alternative in the context of the Sri Ram Sagar Project in Andhra Pradesh (India). In this research, simulated data allows avoiding the interferences on the efficiency due to other external factors. As consequence, efficiency gains are more clearly related to the water policy change.

This combination of LP and DEA represents another added value of this research. Simply using the LP model outputs and accounting for the implied changes due to different pricing policies goes into largely argued problems of multi-input/multi-output production processes evaluation (Gómez-Limón & Sanchez-Fernandez, 2010). Indeed, inputs, outputs, and externalities can be measured in physical or value terms, but the most difficult task is the comparison of different performances. The greatest difficulty involves interpreting the combination of indicators selected to describe each policy effect, and therefore to be suitable as a practical administrative decision-support tool (Messner, 2006). Instead, DEA technique is expected to overcome the usual *trade-off* amongst the conflicting objectives as economic and environmental, as well as the possible commensurability and aggregation biases of different indicators.

The structure of this paper is as follows. Section 2 reports the main topics of water pricing policy options. Section 3 follows, where the empirical case and alternative pricing schemes are compared. Section 4, presents the methodology based on the two-stage DEA approach. Results are presented in Section 5 while conclusions are drawn in Section 6.

2. The Water Pricing Policies

It has been stated that the most suitable water pricing scheme to encourage users to limit their water is one based on volumetric billing use (Easter & Liu, 2005). The shortcomings of a volumetric approach have also been discussed in literature. For instance, Dono, Giraldo, and Severini (2010) analyze the economics effects and impacts of water usage of a volumetric approach with respect to an area based method. They point out that charge increases via volumetric schemes could stimulate groundwater withdrawal. Generally, criticisms of the volumetric methods for water irrigation management in semi-arid regions and other relevant water pricing methods have been argued (Viaggi, Raggi, Bartolini, & Gallerani, 2010; Molle, Venot, & Hassan, 2008). In addition, it has been largely recognized that the implemantation of direct methods in large irrigation districts implies the adoption of appropriate water metering devices, which in turn may require massive investment for their setting and monitoring (Molle & Berkoff, 2007; Burt, 2007).

There are many papers dealing with water pricing for irrigation and efficiency issue (Tsur & Dinar, 1995; Dinar & Subramanian, 1997; Johansson, Tsur, Roe, Doukkali, & Dinar, 2002). Basically, they show that the gain in efficiency is often not prevalent with respect to the implementation costs and obstacles related to volumetric pricing implementation (Tsur & Dinar, 1997).

While research focusing on economic issues, often at farm level, are largely available (e.g. Bazzani, Di Pasquale, Gallerani, & Viaggi, 2004; Ortega, de Juan, & Tarjuelo, 2004; Speelman et al., 2009), how volumetric pricing affects the environmental efficiency of irrigation systems have received less attention. Important contributions have been made by some authors in the field of policy analysis towards non-point source control in irrigated agriculture. Most of these papers refer to the cost and environmental effectiveness of measures such as eco-tax, permits trading, land use restrictions, nitrate pollution reduction at the farm (O'Shea & Wade, 2009) or at regional level (Martínez & Albiac, 2004; Volk, Lierscha, & Schmidt, 2009). However, most of the mentioned studies, which focus on farm impacts, are crop specific and none of these explicitly tackle the eco-efficiency of water use as a consequence of water pricing changes.

Moreover, there is the issue of whether alternative pricing schemes might perform better than volumetric billing when both economic and environmental efficiency are considered. To address this research gap, six water pricing schemes are compared here. Simple volumetric schemes and increasing block tariffs are the direct systems, whereas the indirect pricing schemes are area, input, output and quota pricing:

a) Volumetric schemes are based on the amount of water delivered. The price should be set equal to the marginal cost (i.e. financial, environmental and, resources costs) of providing the water from the economic optimal pricing rule. This pricing method, easily understood, leads farmers to use water efficiently until the economic marginal value equals the marginal cost. The main disadvantage of this method is that it requires meters and it has considerable implementation costs. In addition, environmental impacts of irrigation are not always linked with the amount of water used;

b) Increasing block tariffs involves varying the water price when water use for a set time period exceeds a set volume. The amount of the first block is considered as the basic amount of water needed to support a farm. Farmers pay a low rate for the first block but a higher price for subsequent ones. In a similar fashion to volumetric scheme, environmental consequences stemming from irrigation practices are not directly accounted for;

c) Per acre fees: pricing based on area is widespread around the world and, is still the most common pricing method in many developing countries. Basically, farmer's payment refers to the extension of irrigated area regardless of the actual demand of water. In some advanced regions, per area fees are differentiated taking into account the average irrigation requirement of each crop. Per area method can be easily implemented and is relatively less costly than direct methods. With reference to likely shortcoming, criticism rises to the fact that farmers do not have any economic incentive to adopt water-saving technologies;

d) Input pricing: this type of pricing scheme falls into indirect methods, given that payment for irrigation is based on other related inputs, such as seeds, plants, mulching materials, etc. Essentially, it is applied an extra charge to those inputs instead to pay directly for water. To some extent, this scheme is coherent with the polluter pays principle;

e) Output pricing: inversely to input methods, with this scheme payment is due to on the bases of economic value of irrigated production. Similarly to the value added tax, output scheme can be defined as percentage of market value of irrigated crops;

f) Quota: actually, this scheme is not purely considered as pricing method. In fact, with the quota scheme instead to vary price (scheme and/or charge), water allotment is progressively reduced. This method is recognized being more equitable and, in many developing countries is largely applied. Quota can be defined annually, per crop and, sometime under drought condition, on the bases of priority schedules. Inaccurate tariffs setting may lead to economic inefficiency, mostly if water charge results lower than the average marginal productivity.

3. Materials

3.1 Area Description

The case study relates to a flat area served by irrigation infrastructures, managed by the local irrigation district called 'Consorzio per la Bonifica della Capitanata' (CBC) and located in the south of Italy. The area covers 442 000 ha as a whole, but irrigation infrastructure covers only 135 000 ha. The infrastructure consists of a network of underground pipelines through which high-pressure water is conveyed to the farm plots. Therefore, the water supply is available on demand.

Water is stored in a complex system of dams, small rivers and artificial lakes, and annual availability depends strictly upon weather conditions, being an area characterised by a Mediterranean climate with cold wet winters and hot dry summers. Essentially, rainfall varies from 400 to 700 mm/year, but there are also recurrent periods of drought. The annual water stock amounts to 150 million m^3, and it is devoted to urban, industrial and agricultural uses. In the case of water shortage urban use is given priority. The irrigation board delivers annually about 106 million m^3 to irrigation purposes, but water restrictions are also common depending on rainfall patterns.

As a whole, agriculture is the main water user in the area, accounting for 80% of renewable resources, and while irrigated areas cover a smaller share of farmland (24%), in terms of value added, it shares 70% of total agricultural economic production. Thus, the agricultural sector accounts for 6.8% of GDP and around 11.7% of employment (CCIAA, 2009).

The irrigation network was completed in the 1960s. From then, traditional rain fed agriculture based on pastures and winter cereals, has been converting to more intensive agriculture (fresh cut vegetables, processed tomatoes, orchard fruits, olive groves, vineyards), which in turn has also been stimulating the withdrawal of groundwater sources. Indeed private wells have been largely drilled without strict control from the public authority. There is no official data on the actual groundwater extractions, and the share of water use from private wells annually varies according to CBC availability. Normally it is estimated at 45%, but raises dramatically during exceptionally long drought periods to up to 100% (D'Arcangelo, Nardella, & Rodio, 2005).

Water resources from CBC are allocated through a system of water rights, while groundwater is managed by farmers as a private asset. Although the CBC is a private consortium of landowners, it is empowered by the public authority (the Apulia Region) to manage the conveyed water from the reservoirs. Landowners have received water use rights by a system coupled with their own land, for an overall amount of water rights which is proportional to the land served by the infrastructure. The use rights are strictly linked with the land and water markets are not allowed. With regard to groundwater, farmers could drill private wells and set all of the necessary equipment for pumping, storage, and delivering water to the crop fields. Lastly, reform to control the excessive exploitation of this resource was introduced in 2008 through the Regional Law No 9 on May 2008 (PUGLIA, L.R.n.9/2008) which introduced the banning of new authorizations and the control by temporal licences for existing wells.

The CBC currently applies a volumetric pricing method, based on an increasing tiered rate system. The revenue collected by the CBC is currently covering the financial costs only, while resources and environmental costs are still not included. In the case of groundwater, farmers face only the private cost of water extraction.

According to the WFD requirements, the regional environmental agency started in 2008 a monitoring action aimed at defining the status of all water bodies in the region. The actions collected data depicting a critical status of quality worsening, mostly due to the salinization process caused by groundwater overexploitation that has induced the infiltration of sea water. In addition, pollutants such as nitrates, pesticides and organic carbon have been recognized amongst the most important environmental concerns about water quality status (PTA, 2009).

3.2 Specification of Alternative Water Pricing for the Area Study

Alternative pricing methods are implemented as follows:

a) P0. Baseline: Water pricing currently consists of a fixed annual fee per hectare (around 15 EUR/ha), and an increasing block system of tiered tariffs, therefore farmers pay accordingly to their actual consumption. A tariff of 0.09 €/m^3 for consumption up to 2050 m^3/ha, and 0.18 €/m^3 for additional 950 m^3/ha are applied. Finally, volumes exceeding 3000 m^3/ha are charged at 0.24 €/m^3. In the case of groundwater, as abovementioned, farmers carry only the private cost estimated at 0.09 €/m^3 (Noviello & Nardella, 2005; Giannoccaro, Prosperi, Valente, & Zanni, 2009); meanwhile, no resource and environmental costs are due;

b) P1. Full volumetric (Full Vol.): It is assumed that the current three-tiered rate system to CBC water is maintained. In addition, it is hypothesized as the introduction of a simply volumetric method for groundwater, reflecting the resource cost. In absence of any other estimate, a tariff of 0.03 €/m^3 is assumed. Therefore, farmers will pay the current private cost of 0.09 €/m^3 plus the new volumetric charge of 0.03 €/m^3;

c) P2. Input: The introduction of an indirect method in which the water charge is calculated on the input required by irrigated crops is assumed (e.g. plants or seeds, consumable irrigation equipments, ferti-irrigation materials). To reflect an indirect environmental tax on irrigation practices, farmers pay a sort of price surcharge on these inputs, regardless of the actual water consumption (from CBC and groundwater source). The surcharge is different for each crop, and set up on a basis of average crop water consumption;

d) P3. Output: The water consumption is charged proportionally to the gross return from irrigated crops, regardless of the water source. The charge rate applied to each crop is calculated as a ratio between the current value of its specific water consumption, and the corresponding gross return (vines 3%, horticultural crops 2.4-2.8%, olive orchards 1.9%);

e) P4. Area: A per-area pricing is assumed referring to the area suitable for the irrigation practice, regardless of water sources. A per-area hectare charge is set equivalent to the average CBC cost per hectare of irrigated area (82 €/ha);

f) P5. Quota: A constant water tariff (0.09 €/m^3) is applied, but subject to a rigid constraint on water availability for each farm. This method is popular among some farmers, as they claim that water prices should remain low and constant, regardless of water availability. Although it is not resulting from a real water market, they accept the concept that the availability may change according to the rainfall regime.

In addition, these water methods are analyzed under different water saving scenarios A, B, C, involving 10, 20 and 30% of water savings respectively. Tariffs are progressively increased according to pricing methods in order to get the established target of water saving. In the case of the quota scheme, tariffs are kept constant meanwhile water allotments are progressively reduced until each target scenario is obtained. Thus, moving from one pricing policy to another of them would give the policy maker a range of pricing feasibility that can be compared across the 6 pricing schemes that are used. As a whole, 18 water policy options are taken into account for running DEA analysis.

3.3 Data Collection

Data are obtained from Giannoccaro, Prosperi and Zanni (2010) where the effects of the water policy scenarios as mentioned above are performed through a linear programming model at the regional level, in which farmers are expected to maximize their profits. In other words, under all irrigation policy hypothesis farmers will pursue the optimal economic allocation of the resource. Therefore, water pricing changes could induce changes in farmers' behaviours towards use of water resource as well as all other farming inputs.

Farms were classified into three main groups according to farm size and cropping patterns (Table 1).

Table 1. Farm typologies in the area

	Small	Medium	Large	Total Basin
Number of farms	18,199	13,063	4,720	35,982
Average size (ha)	2.5	10	43	380,000
Irrigated land (ha)	1.2	2.9	11.6	80,000
Share on Basin farmland (%)	10	30	60	100
Share on Basin irrigated land (%)	3	47	50	100
Cropping patterns (within farm)				
Durum wheat	40%	68%	75%	
Orchards	50%	20%	9%	
Vegetables	3%	7%	7%	
Others	7%	5%	9%	

Source: Adapted from ISTAT (2000).

The resource constraint for water is specified in order to align with the water delivery schedule from the CBC (monthly), which delivers some 106 million m^3 between April and November. In the case of the non-CBC water source, there are constraints with regard to delivery, and availability is estimated at 89 million m^3 at the most.

The regional model is performed under short period assumptions with farm size and water rights being set as constant. As a consequence, the decision variables of the model are basically referred to the optimal cropping mix, which determines the utilization of production inputs (land, labour and capital) including water and chemicals, as well as economic outputs measured in terms of value added.

From the optimal solution of each water policy option, the variables of input, output and externalities are obtained. Detail of variables units are hold in Table 2.

Conventional inputs are land, labour, capital and water. The latter variable accounts for both surface water delivered by the CBC, and groundwater withdrawals. Value added refers to the difference between gross output (i.e. economic value of productions at the market prices) and intermediate consumptions (i.e. goods and services) used. Intermediate costs refer to seeds, fertilizers, pesticides, energy, and other services used in the farming processes. Finally, CAP regime and its relative financial supports are not included in the value added. Thus, it is a measure of the contribution of the agriculture (irrigated and rain fed as a whole) to the economic wealth.

Table 2. Inputs, output and externalities

	Conventional Resources					Environmental Externalities	
	Input				Desirable Output	Undesirable outputs	
	Land	Labour	Capital	Water	Value added	Pesticides Risk	Nitrogen Surplus
Units	10^3 hectares	10^3 hours	10^6 EUR	10^6 m^3	10^6 EUR	10^3 Kg	10^6 t

Source: own elaboration

Pesticide risk p_{jc} for crop c is modelled as follow:

$$p_{jc} = \sum_{t} a_{jt} 1000000 / DL50_t \tag{1}$$

Where a_{jt} amount of active matter t by unit of pesticide j; $DL50_t$ is the lethal dose 50 of the active matter t usually reported on commercial product. The indicator represents the weight (in kilograms) of the population of rats, 50% of which would be potentially killed by 1 Kg/L of the pesticide. In this context it represents a *proxy* of the environmental hazard (for chief details see OECD, 2001, p. 149).

Nitrate surplus is the physical difference between nitrogen inputs and outputs from an agricultural system, per hectare of agricultural land (see OECD, 2001, p. 20).

Data on pesticides risk and nitrate surplus are from Giannoccaro et al. (2009). Values are calculated for each crop (per hectare of farmland) on the basis of technical and agronomic farming practices normally adopted by farmers in the area.

All the above variables are referred to the agricultural system as the aggregation of overall farms. The pay-off matrix obtained through the simulation process is reported in the appendix.

4. Methodology

Two main techniques are suitable for the analysis of the efficiency: the stochastic frontier analysis (SFA) and the data envelopment analysis (DEA). The typical statistical approach to evaluate the efficiency is characterised as a central-tendency approach, which evaluates producers relative to an average producer. To do that, in SFA the shape of function is prior defined. In contrast, DEA is an extreme-point method and compares each producer with only the 'best' producers[1]. This methodology is useful whenever there is no information about the relative importance among outputs or inputs, as it does not require assumptions *a priori* (Callens & Tyteca, 1999). Additionally, DEA deals with variables regardless of their unit measure, provided that these units are the same for every entity (Coelli, Prasada Rao, & Batteste, 1997). In this regard, we adopted the DEA since our research encompasses ecological aspects therefore, the unnecessary assignment of weights for environmental resources and impacts, allows us to proceed with a more objective analysis.

The first non-parametric analysis to compare multiple desirable and undesirable outputs is reported in Färe, Grosskopf, Lovell and Pasurka (1989). However, the general emphasis on the environmental issue has occurred later (Tyteca 1996, presents an exhaustive literature review), where externalities have usually been treated as 'undesirable outputs' of the production process (e.g., Fernandez-Cornejo 1994; Tyteca, 1996; Piot-Lepetit, Vermersch, & Weaver, 1997). Another possibility is to envision the undesirable outputs as inputs (see Seiford and Zhu (2002) for either approach). As in De Koeijer, Wossink, Struik and Renkema (2002) here environmental effects are modelled as conventional inputs.

In the present research, the DEA method is adopted with the aim of measuring the eco-efficiency of different irrigation pricing policies, as first applied by Korhonen and Luptacik (2004). Korhonen and Luptacik (2004) measured the eco-efficiency of 24 power plants in Europe in two steps. Essentially, conventional efficiency and the so-called environmental efficiency are estimated separately, then, by combining both efficiencies, the eco-efficiency score is obtained.

As mentioned in the introduction, eco-efficiency here is defined as the ratio between economic value added and environmental pressures. Indeed, this paper focuses on a broader measure of productivity (i.e. economic value added), which is achieved by using all the resources (capital, labour, land and water) available. At the same time, externalities (i.e. nitrate surplus and pesticides risk) are also taken into account[2]. The final result consists of an efficiency score that takes into account all resources used, as well as the externalities released to the environment. By construction, this score of eco-efficiency is upper bounded to one, the score that represents best performance. Moreover, the lower the score computed the lower eco-efficiency. Scores computed measures eco-efficiency in a Farrell-Debreu sense (Farrell, 1957), as they are assessing equiproportional or radial reductions of environmental pressures necessary to attain eco-efficiency.

Let us consider the *n* water pricing policies and the effects simulated, namely the amount of *m* inputs and *k* outputs estimated through the mathematical programming model. In particular, for $m=1,2,...i,$ the subscript for production inputs is assigned (here land, labour, capital and water), while for $m= i+1,i+2,...p,$ the externalities are specified (here nitrate surplus and pesticide risk indicators are seen as inputs); at the same time, for $k=1,2,..,r$, the subscript for conventional outputs is identified (here value added).

Firstly, the relative economic efficiency is performed by the Input-Oriented CRS model which assumes constant returns of scale[3]. In this step the efficiency of the policy *'0' (h_0)* is calculated through a linear programming model whose optimal solution provides the (positive) weights to be applied to outputs (μ_r) and inputs (ν_i), in order to find a ratio of output on inputs that ranges from 0 to 1:

$$\text{Max } h_0 = \left(\Sigma^{k}_{r=1}\ \mu_r\, y_{r0}\right) / \left(\Sigma^{m}_{i=1}\ \nu_i\, x_{i0}\right) \tag{2}$$

s.t.

$$\left(\Sigma^{k}_{r=1}\ \mu_r\, y_{rj}\right) / \left(\Sigma^{m}_{i=1}\ \nu_j\, x_{ij}\right) <= 1\ ,\ j = 1,2,\ldots,n$$

$$\mu_r,\ \nu_j => \varepsilon\ ,\ r=1,2,\ldots,k\ ;\ i=1,2,\ldots,m$$

$\varepsilon>0$ (Non-Archimedean)

Scores of radial efficiency, which in turn represents the proportional potential reduction of all conventional inputs (land, labour, capital and water) while value added are held constant, have been estimated by solving expression (2) for each water policy.

Secondly, the measurement of the environmental efficiency (g_0), through the calculation of the weights to be applied to the desirable outputs (μ_r) and the undesirable outputs (μ_s) is run according to the follow equation:

$$\text{Max } g_0 = \left(\Sigma^{k}_{r=1}\ \mu_r\, y_{r0}\right) / \left(\Sigma^{p}_{s=m+1}\ \mu_s\, y_{s0}\right) \tag{3}$$

s.t.

$$\left(\Sigma^{k}_{r=1}\ \mu_r\, y_{rj}\right) / \left(\Sigma^{p}_{s=m+1}\ \mu_s\, y_{sj}\right) <= 1\ ,\ j = 1,2,\ldots,n$$

$$\mu_r => \varepsilon\ ,\ r=1,2,\ldots,p$$

$\varepsilon>0$ (Non-Archimedean)

Specific environmental efficiency scores by policies have been calculated according to the expression (3).

At this point, it is possible to combine the results of both models as the output variables for the new DEA model (with the inputs equal to 1), in order to find an indicator for eco-efficiency.

The input-oriented version of this model estimates eco-efficiency by reducing both inputs and undesirable outputs while keeping desirable outputs constant. The eco-efficiency score thus indicates the extent to which both inputs and undesirable outputs can be reduced simultaneously.

5. Results and Discussion

This section reports and discusses firstly economic and environmental efficiency separately then the eco-efficiency analysis is shown. Table 3 shows the results of the DEA analysis.

Table 3. Efficiency scores for several policy options

Policy options	Economic efficiency	Environmental efficiency
A_P0.Baseline	0.95658	0.95729
A_P1.Full_Vol.	0.95658	0.95729
A_P2.Input	0.97913	0.95701
A_P3.Output	0.98335	1.00000
A_P4.Area	1.00000	1.00000
A_P5.Quota	0.96723	0.96360
B_P0.Baseline	0.93426	0.89527
B_P1. Full_Vol.	0.93466	0.89438
B_P2.Input	0.96018	0.88980
B_P3.Output	0.95065	0.88523
B_P4.Area	1.00000	0.92847
B_P5.Quota	0.88918	0.85174
C_P0.Baseline	0.94600	0.86403
C_P1. Full_Vol.	0.95582	0.87217
C_P2.Input	0.98499	0.81452
C_P3.Output	0.90537	0.75500
C_P4.Area	0.94834	0.79116
C_P5.Quota	0.87474	0.77874

Source: Own elaboration.

5.1 Economic Efficiency

On the basis of economic efficiency scores (first column in Table 3), only two options out of the 18 simulated reach rate 1, being the best efficiency score. They correspond to water pricing based on per area, at the water saving scenarios A and B. The lowest score is reached by the quota pricing for a 30% water saving (scenario C) with a value of 0.87474. The average efficiency of the sample is 0.95150 while median is 0.95620, and generally slight differences are found with the standard deviation being 0.035. According to these results it is not proven that the implementation of volumetric pricing will necessarily lead to a higher economic efficiency in comparison to other methods.

These outcomes show, in the first place, that water policy options taken into account here are rather efficient. These findings imply that there exists little difference in terms of technical efficiency among the different water pricing options. Thus, water policy reforms in the study area could scarcely increase efficiency of irrigated agricultural and, therefore of the agricultural system as a whole.

Nevertheless, some consideration should be discussed. Firstly, it should be noticed that while only 8 out of 18 options have a score lower than the mean or median, all of these fall into B and C water charge scenarios. Likewise, the per-area scheme which is the best option under A and B saving scenarios turns to an inefficient score in the case of scenario C. Indeed, it loses almost 6% of efficiency. B and C scenarios respectively consider a water reduction at basin level of 20 and 30% with respect to the current use, therefore powering water saving via pricing charges, does not seem lead to an improved (economic) efficiency of the agricultural sector. Secondly, current systems of water charge (Baseline) reach efficiency levels always under the mean. Regardless of the water saving scenario, the increasing block system currently implemented in the area is not efficient enough.

5.2 Environmental Efficiency

Environmental efficiency provides an indication of how the externalities can be reduced in the production process of agricultural systems, while continuing to produce the same value added.

The average efficiency of the sample (Table 3, column at right) is 0.89198 with the median at 0.89209. Generally, major differences are found when comparing this ranking with the one obtained by accounting only for economic aspects. In fact, the standard deviation is 0.074 and 9 out of 18 water options reach scores lower than the mean or median. Finally, only 2 out of 18 options are the most efficient, Area-based and Output-based methods. Both methods reach the highest level under water charge scenario A.

On the other hand, the worst efficiency level is shown in the case of the Output-based pricing alternative in the water saving scenario C, where almost 25% of inefficiency with respect to the most efficient alternative is found. Quota methods are always inefficient, regardless of the water saving scenario. The input pricing method reaches a lower efficiency due to an excess of environmental externalities.

Taking into account the most efficient options, it should be noticed that all water pricing policies take place in the water saving scenario A. At the same time, water saving scenarios B and C hold the worst options.

As in the case of economic efficiency, findings here suggest that an increase in water pricing charges does not result in an environmental efficiency improvement. In this regard, changes in the environmental impacts as well as value added for the Baseline pricing method are reported in Figure 1, where the 'status-quo' indicates the current level for the three variables, and the levels A, B, and C, report the change in these variables according to the water saving scenarios. As figure shows, the externalities (i.e. Nitrate surpluses and Pesticides risk) show a smaller decrease with respect to the value added along the water saving scenarios. As a consequence, if the enforcement of tariffs is applied value added goes down more than nitrate and pesticides indicators.

The fact that the overall-use of other non-water inputs decreases together with water use is found in most studies (Gómez-Limón, Riesgo & Arriaza, 2004; Manos et al., 2006; Bartolini, Bazzani, Gallerani, Raggi, & Viaggi, 2007; Speelman et al., 2009). Nevertheless, our approach allows for assessing the most efficient water pricing that ensures reducing these externalities as little as possible meanwhile the value added is held constant.

Overall, findings point out that a pricing system based on per area and output will lead to the highest efficiency although this is not valid under any water pricing charge. At the same time, this implies that the current pricing method (Baseline) is not the most environmentally efficient.

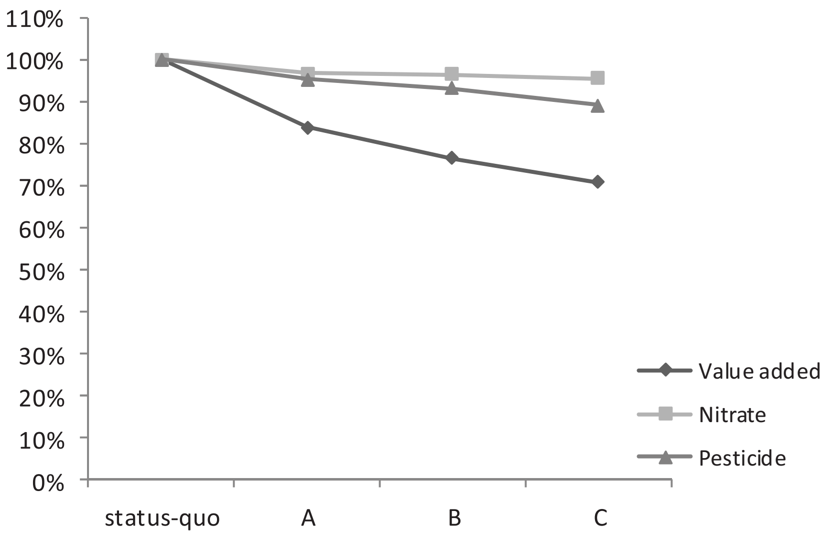

Figure 1. Change in value added, Nitrate and Pesticides indicatorsaccording to water saving scenarios for the Baseline pricing scheme

5.3 Eco-Efficiency

We turn now to the analysis of both economic and environmental efficiency simultaneously (i.e. second step of modified DEA technique). The eco-efficiency scores of different water pricing policies are presented in Table 4.

Table 4. Computed score of eco-efficiency

A_P0.Baseline	0.95729	B_P0.Baseline	0.93426	C_P0.Baseline	0.94600
A_P1. Full_Vol.	0.95729	B_P1. Full_Vol.	0.93466	C_P1. Full_Vol.	0.95582
A_P2.Input	0.97913	B_P2.Input	0.96018	C_P2.Input	0.98499
A_P3.Output	1.00000	B_P3.Output	0.95065	C_P3.Output	0.90537
A_P4.Area	1.00000	B_P4.Area	1.00000	C_P4.Area	0.94834
A_P5.Quota	0.96723	B_P5.Quota	0.88918	C_P5.Quota	0.87474

Source: own elaboration.

From the findings in Table 4, only three options out of the 18 simulated reach the highest eco-efficiency levels. They are the options A_P4.Area, B_P4.Area and A_P3.Output. It should be stressed that two out of three best options are pricing schemes based on per area, and two out of the three best options fall into the saving scenario A; the water saving scenario C does not account for any option. Again, the lowest score is reached by the quota method in the case of the water saving scenario C, with a value of 0.87474. The average eco-efficiency of the sample is 0.94924 while median is 0.95065, and generally slight differences are found with the standard deviation being 0.036. Finally, 7 out of 18 options have a score lower than the mean or menidan and, all of these, fall into B and C water saving scenarios.

According to the results of this analysis, it is not proven that the implementation of the volumetric pricing will necessarily lead to a higher eco-efficiency in comparison to other methods. The main result of this research is that the area-based pricing method is the most eco-efficient. At the same time, the output method where the water payment is linked with the economic return of irrigated crops emerges as an interesting alternative. Finally, as expected from the literature the quota scheme is always the least efficient.

Basically, while the results point out the relatively small magnitude of gains in eco-efficiency derived from the water policy reforms, major concerns arise from the charge levels, at which pricing policies are set. Indeed, both economic and environmental efficiency do not show any efficiency improvement if the water tariffs are enforced regardless of the pricing schemes implemented. This aspect is again found for the eco-efficiency indicators which combine the two previous indicators. If the charge is increased, only per area pricing shows an efficient performance, although extreme enforcement which takes place under the scenario C produces the same efficiency loss.

In regard to pricing schemes currently adopted within the irrigation basin (Baseline), findings here show that the present situation is not the most efficient, although magnitude of improvement in eco-efficiency by introducing a new pricing scheme (i.e. per area charge) is at least less than 5%. However, it is worth mentioning that the eco-efficiency score here refers to the overall agricultural sector thus smaller variation in the eco-efficiency score can results in larger reduction of inputs and environmental impacts, meanwhile the value added is kept constant.

In addition, taking into account the fact that volumetric schemes might imply an inherent additional cost and, since indirect methods, namely per area schemes are claimed to be easily implemented (Tsur & Dinar, 1997), they might be preferable, without significant losses in terms of efficiency.

In order to determine whether the final eco-efficiency score is mainly linked with the economic or environmental aspects, the Spearman’s rank correlation coefficient (R) is computed, which is useful to determine the measure of association between ranks obtained by two different approaches (Gibbons, 1971). Spearman R values of 1, 0 and -1, represent perfect association, no association and perfect disagreement respectively between the approaches. The result of non parametric test is 0.91 between the economic efficiency and eco-efficiency scores, meanwhile environmental efficiency and eco-efficiency reports a value of 0.61. This means that the radial eco-efficiency of water policies is highly correlated with the economic efficiency. On the other hand, it has been confirmed that environmental impacts are not always related to the water use, and water pricing, even more water pricing enforcement may not be suitable to increase the environmental efficiency. Thus, under the research circumstances, the agricultural performance in the area may be slightly driven by a water pricing reform. Other policy instruments such as incentives, tradable permits, tax, standard emission or compliance like in the case of CAP should receive more attention.

Finally, the comparison of the set options shows that their effect is not straightforward assuming a static framework for decision-making, which also justifies further research in this direction. This can also be caused by several issues related to the case study, such as climate condition that makes the elasticity of water substitution among other production inputs very low, reducing the room for farm adaptation.

6. Concluding Remarks

The reform of water pricing methods is one of the most re-called requirements for the enhancement of the efficiency of using water and of its quality status. In order to change the pricing schemes as well as the charges, policy makers require a clear overview of the different outcomes deriving from alternative water management policies, and tools aimed at decision support are needed in order to select the most suitable option.

In the present paper the relative economic and environmental efficiency of an agricultural system subject to alternative water pricing policies has been estimated using a methodology based on DEA. Water pricing policy has been evaluated taking into account economic and environmental goals in order to enhance the optimal pricing scheme as well as charging water sources considering the main agricultural system needs, in accordance with the ecological and economic principles of the WFD.

DEA evaluation overcomes the usual *trade-off* or compromise amongst the conflicting objectives as economic and environmental issues, taking into account the eco-efficiency as criteria for the options ranking.

The results obtained for the case study suggest that the potential gains of economic efficiency among the different water pricing options are relatively small. However, higher environmental efficiency gains could be achieved from water pricing scheme reforms. Despite the fact that the implementation of the volumetric pricing was expected to lead to a higher eco-efficiency, the area-based pricing method has proven to be the most eco-efficient in this case study. The output method also emerges as an interesting alternative. The enforcement of water saving via pricing charges does not imply a higher efficiency, mainly in the case of environmental efficiency.

For current pricing systems, if the 'full cost recovery' principle prescribed in the WFD is applied via a rise in price, the result will be a decrease in eco-efficiency. This aspect is crucial in the Capitanata area as well as in other Mediterranean regions where water for irrigation accounts for 70-80% of total water consumption.

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References

Bartolini, F., Bazzani, G. M., Gallerani, V., Raggi, M., & Viaggi, D. (2007). The impact of water and agriculture policy scenarios on irrigated farming systems in Italy: an analysis based on farm level multi-attribute linear programming models. *Agricultural Systems, 93*, 90-114. http://dx.doi.org/10.1016/j.agsy.2006.04.006

Bazzani, G. M., Di Pasquale, S., Gallerani, V., & Viaggi, D. (2004). Irrigated agriculture in Italy and water regulation under the European Union Water Framework Directive. *Water Resources Research, 40*(7), W07S04. http://dx.doi.org/10.1029/2003WR002201

Bono, F., & Matranga, D. (2005). *Measures of efficiency in the environmental context*. In S.I.S. 2005 - Statistica e Ambiente. Messina, Italy 21-23 September 2005.

Burt, C. M. (2007). Volumetric irrigation water pricing considerations. *Irrigation Drainage System, 21*, 133-144. http://dx.doi.org/10.1007/s10795-007-9025-3

Callens, I., & Tyteca, D. (1999). Towards indicators of sustainable development for firms: a productive efficiency perspective. *Ecological Economics, 28*(1), 41-53. http://dx.doi.org/10.1016/S0921-8009(98)00035-4

CCIAA (Camera di Commercio Industria Artigianato Agricoltura). (2009). *Rapporto economico 2007-2008*. Foggia, Italy: Osservatorio Economico Provinciale.

Charnes, A., Cooper, W., & Rhodes, E. (1978). Measuring the efficiency of decision-making units. *European Journal of Operational Research, 2*, 429-444. http://dx.doi.org/10.1016/0377-2217(79)90229-7

Coelli, T., Prasada Rao, D. S., & Batteste, G. E. (1997). *An introduction to efficiency and productivity analysis*. Boston: Kluwer Academic Publishers.

Cooper, W. W., Seiford, M. L., & Tone, K. (2000). *Data Envelopment Analysis*. Boston/Dordrecht/London: Kluwer Academic Publishers.

D'Arcangelo, G., Nardella, L., & Rodio, M.E. (2005). Analysis of water requirement and consumptions in a public district of recent irrigation: The case of sub districts 5b, 12 and 13. *Bonifica, 4*, 34-42.

De Koeijer, T. J., Wossink, G. A. A., Struik, P. C., & Renkema, J. A. (2002). Measuring agricultural sustainability in terms of efficiency: the case of Dutch sugar beet growers. *Journal of Environmental Management, 66*, 9-17. http://dx.doi.org/10.1006/jema.2002.0578

Dinar, A., & Subramanian, A. (1997). *Water pricing experience, World Bank Technical Paper 386*. Washington, DC: World Bank.

Dono, G., Giraldo, L., & Severini, S. (2010). Pricing of irrigation water under alternative charging methods: Possible shortcoming of a volumetric approach. *Agricultural Water Management, 97*, 1795-1805. http://dx.doi.org/10.1016/j.agwat.2010.06.013

Dworak, T., Berglund, M., Laaser, C., Strosser, P., Roussard, J., Grandmougin, B., ... Montesinos, P. (2007). *EU Water Saving Potential*, Report prepared for the EU Commission, Brussels. ENV.D.2/ETU/2007/0001r. Berlin, Germany.

Easter, K. W., & Liu, Y. (2005). *Cost recovery and water pricing for irrigation and drainage projects*. Agriculture and Rural Development, Discussion Paper No 20. Washington, DC: World Bank.

Färe, R., Grosskopf, S., Lovell, C. A. K., & Pasurka, C. (1989). Multilateral productivity comparisons when some outputs are undesirable: a nonparametric approach. *Review of Economics and Statistics, 71*, 90-98.

Farrell, M. J. (1957). The measurement of productive efficiency. *Journal of the Royal Statistical Society*, *Series A, General, 120*, 253-281. http://dx.doi.org/10.2307/1928055

Fernandez-Cornejo, J. (1994). Nonradial technical efficiency and chemical input use in agriculture. *Agricultural and Resource Economics Review, 22*, 11-21.

Giannoccaro, G., Prosperi, M., & Zanni, G. (2010). Assessing the impact of alternative water pricing schemes on income distribution. *Journal of Agriculural Economics, 61*(3), 527-544. http://dx.doi.org/10.1111/j.1477-9552.2010.00252.x

Giannoccaro, G., Prosperi, M., Valente, N., & Zanni, G. (2009). Valutazione degli impatti della politica di tariffazione delle acque irrigue sull'agricoltura della Capitanata (Puglia). In V. Gallerani, G. La Via & G. Zanni (Eds.), *Acqua e Agricoltura in Italia. Valutazioni di scenari e strumenti di supporto alle decisioni* (pp. 121-149). Milan, Italy: Franco Angeli.

Gibbons, J. D. (1971). *Nonparametric Statistical Inference*. New York: McGraw-Hill.

Gómez-Limón, J. A., Riesgo, L., & Arriaza, M. (2004). Multi-Criteria analysis of input use in agriculture. *Journal of Agricultural Economics, 55*, 541-564. http://dx.doi.org/10.1111/j.1477-9552.2004.tb00114.x

Gómez-Limón, J. A., & Sanchez-Fernandez, G. (2010). Empirical evaluation of agricultural sustainability using composite indicators. *Ecological Economics, 69*(5), 1062-1075. http://dx.doi.org/10.1016/j.ecolecon.2009.11.027

ISTAT (Istituto Nazionale di Statistica). (2001). *5th Censimento nazionale dell'agricoltura*. Rome, ISTAT.

Johansson , R. C., Tsur, Y., Roe, T. L., Doukkali, R., & Dinar, A. (2002). Pricing irrigation water: a review of theory and practice. *Water Policy, 4*(2), 173-199. http://dx.doi.org/10.1016/S1366-7017(02)00026-0

Korhonen, P. J., & Luptacik, M. (2004). Eco-efficiency analysis of power plants: An extension of data envelopment analysis. *European Journal of Operational Research, 154*, 437-446. http://dx.doi.org/10.1016/S0377-2217(03)00180-2

Kuosmanen, T., & Kortelainen, M. (2005). Measuring eco-efficiency of production with Data Envelopment Analysis. *Journal of Industrial Ecology, 9*, 59-72. http://dx.doi.org/10.1162/108819805775247846

Malano, H. M., & Burton, M. (2001). *Guidelines for benchmarking performance in the irrigation and drainage sector*. International Program for Technology Research in Irrigation and Drainage. Secretariat - Technology & Engineering. Rome: FAO.

Manos, B., Bournaris, T., Kamruzzaman, M., Begum, M., Anjuman, A., & Papathanasiou, J. (2006). Regional Impact of irrigation water pricing in Greece under alternative scenarios of European Policy: a multicriteria analysis. *Regional Studies, 40*(9), 1055-1068. http://dx.doi.org/10.1080/00343400600928335

Martin-Ortega, J., (2012). Economic prescriptions and policy applications in the implementation of the European Water Framework Directive. *Environmental Science and Policy, 24*, 83-91. http://dx.doi.org/10.1016/j.envsci.2012.06.002

Martínez, Y., & Albiac, J. (2004). Agricultural pollution control under Spanish and European environmental policies. *Water Resources Research, 40*, W10501. http://dx.doi.org/10.1029/2004WR003102

Messner, F. (2006). Applying participatory multicriteria methods to river basin management: improving the implementation of the Water Framework Directive. *Environment and Planning C: Government and Policy, 24*(2), 159-167. http://dx.doi.org/10.1068/c2402ed

Musolino G., & Rindone C. (2009). *An integrated LUTI and DEA procedure for strategic urban transport planning*. Proceedings of the European Transport Conference, Association of European Transport. Noordwijkerhout (NLD), 5-7 October 2009.

Molle, F., Venot, J. P., & Hassan, Y. (2008). Irrigation in the Jordan Valley: Are water pricing policies overly optimistic? *Agricultural Water Management, 95*, 427-438. http://dx.doi.org/10.1016/j.agwat.2007.11.005

Molle, F., & Berkoff, J. (2007). *Irrigation water pricing. The gap between theory and practice*. Wallingford, UK: IWMI/CABI. http://dx.doi.org/10.1079/9781845932923.0000

Noviello, N., & Nardella, L. (2005). I costi di produzione negli ordinamenti produttivi della Capitanata: Incidenza dell'acqua e dell'irrigazione. *Bonifica, 4*, 17-49.

OECD. (2001). *Environmental indicators for agriculture. Volume 3 - Methods and Results*. Paris: OECD.

Ortega, J. F., de Juan, J. A., & Tarjuelo, J. M. (2004). Evaluation of the water cost effect on water resource management: application to typical crops in a semiarid region. *Agricultural Water Management, 66*, 125-144. http://dx.doi.org/10.1016/j.agwat.2003.10.005

O'Shea, L. C. M., & Wade, A. J. (2009). Controlling nitrate pollution: An integrated approach. *Land Use Policy, 26*(3), 799-808. http://dx.doi.org/10.1016/j.landusepol.2008.10.017

Picazo-Tadeo, A., Gómez-Limóm, J. A., & Reig-Martínez, E. (2011). Assessing farming eco-efficiency: A Data Envelopment Analysis approach. *Juornal of Environmental Management, 92*, 1154-1164. http://dx.doi.org/10.1016/j.jenvman.2010.11.025

Piot-Lepetit, I., Vermersch, D., & Weaver, R. D. (1997). Agriculture's environmental externalities: DEA evidence for French agriculture. *Applied Economics, 29*, 331-338. http://dx.doi.org/10.1080/000368497327100

PTA (Piano di Tutela Acque). (2009). *Bollettino Ufficiale della Regione Puglia 2009*, 130 suppl., 4-8. Italy: Regione Puglia.

Raju, K. S., & Kumar, D. N. (2006). Ranking irrigation planning Alternatives Using data Envelopment Analysis.*Water Resources Management, 20*, 553-566. http://dx.doi.org/10.1007/s11269-006-3090-5

Seiford, L. M., & Zhu, J. (2002). Modeling undesirable factors in efficiency evaluation. *European Journal of Operational Research, 142*, 16-20. http://dx.doi.org/10.1016/S0377-2217(01)00293-4

Speelman, S., Buysse, J., Farolfi, S., Frija, A., D'Haese, M., & D'Haese, L. (2009). Estimating the impacts of water pricing on smallholder irrigators in North West Province, South Africa. *Agricultural Water Management, 96*, 1560-1566. http://dx.doi.org/10.1016/j.agwat.2009.06.014

Tsur, Y., & Dinar, A. (1995). *Efficiency and Equity Considerations in Pricing and Allocating Irrigation Water*. Policy Research Paper 1460. Washington, DC: World Bank.

Tsur, Y., & Dinar, A. (1997). The relative efficiency and implementation costs of alternatives methods for pricing irrigation water. *The World Bank Economic Review, 11*, 243-262. http://dx.doi.org/10.1093/wber/11.2.243

Tyteca, D. (1996). On the Measurement of the Environmental Performance of Firms- A Literature Review and a Productive Efficiency Perspective. *Journal of Environmental Management, 46*, 281-308. http://dx.doi.org/10.1006/jema.1996.0022

Viaggi, D., Raggi, M., Bartolini, F., & Gallerani, V. (2010). Designing contracts for irrigation water under asymmetric information: Are simple pricing mechanisms enough? *Agricultural Water Management, 97*, 1326-1332. http://dx.doi.org/10.1016/j.agwat.2010.03.014

Volk, M., Lierscha, S., & Schmidt, G. (2009). Towards the implementation of the EuropeanWater Framework Directive? Lessons learned from water quality simulations in an agricultural watershed. *Land Use Policy, 26*, 580-588. http://dx.doi.org/10.1016/j.landusepol.2008.08.005

Notes

Note 1. For a more in-depth discussion of DEA, the interested reader is referred to Cooper, Seiford, & Tone (2000) or the seminal work by Charnes, Cooper and Rhodes (1978).

Note 2. Nitrate surplus and pesticide risk were chosen as indicators of the main agriculture pressures on the region according to the assessment carried out by the regional environmental agency (see PTA, 2009). In this regards, environmental variables should be referred to the relevant environmental issues according to each case study.

Note 3. By relying on input oriented efficiency measures the approach takes an input perspective. Farmers maximize profit and respond to water policy changes by changing their cropping mix, which in turn affects the inputs use on farm. In addition, it is assumed that the farming exhibits constant returns to scale (CRS). In fact, under the short period assumptions they are constrained by the technology frontier and their individual constrains (i.e. farm size and water rights), so that the inefficiency scale does not face.

Appendix

Pay-off matrix

Policy	Land	Labour	Capital	Water	Value added	Pesticides Risk	Nitrogen surplus
	10^3 hectares	10^3 hours	10^6 EUR	10^6 m^3	10^6 EUR	10^3 Kg	10^6 t
A_P0.Baseline	372.698	20.757	176.148	170.414	71.402	705.016	33.624
A-P1. Full_Vol.	372.698	20.757	178.156	170.408	71.402	705.016	33.624
A_P2.Input	377.059	21.149	179.222	174.433	74.464	758.586	32.519
A_P3.Output	378.848	21.416	180.039	172.648	75.731	784.552	31.163
A_P4.Area	379.525	21.852	181.568	171.276	78.580	742.750	33.088
A_P5.Quota	372.730	20.841	175.844	173.911	72.488	711.049	33.802
B_P0.Baseline	373.253	20.280	172.841	151.474	65.153	687.872	33.545
B_P1. Full_Vol.	373.261	20.271	175.176	151.112	65.035	687.311	33.539
B_P2.Input	377.747	20.671	175.828	152.667	67.545	745.416	31.669
B_P3.Output	378.149	20.559	175.246	151.456	66.356	762.805	30.990
B_P4.Area	378.783	21.188	177.903	152.575	70.438	725.091	31.860
B_P5.Quota	371.990	19.878	169.409	152.655	62.366	692.107	33.350
C_P0.Baseline	372.403	19.872	168.535	137.826	60.193	658.483	33.208
C_P1. Full_Vol.	372.332	19.926	171.483	136.952	60.432	654.936	33.166
C_P2.Input	377.479	20.097	171.487	132.227	60.127	718.277	30.867
C_P3.Output	376.850	19.494	169.023	133.793	55.922	736.342	30.806
C_P4.Area	376.966	19.933	168.814	134.222	58.764	703.896	31.256
C_P5.Quota	371.382	19.272	164.871	137.479	55.518	673.871	32.676

Source: Own elaboration from Giannoccaro et al. (2010).

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Using Microwave Energy for the Removal of Ammonia From Municipal Wastewater: Continuous Flow Lab-Scale System

Fahid K. J. Rabah[1] & Mohamad S. Darwish[1]

[1] Faculty of Civil and Environmental Engineering, The Islamic University-Gaza, Palestine

Correspondence: Fahid K. J. Rabah, Faculty of Civil and Environmental Engineering, The Islamic University-Gaza, Palestine. Tel: 97-259-952-8047. E-mail: frabah@iugaza.edu.ps

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Abstract

This work was initiated to investigate the possibility of using a continuous flow Micro Wave (MW) radiation system with heat exchanging for ammonia nitrogen removal from municipal wastewater after the success of using the system as a batch reactor in our previous work. The effects of initial ammonia concentration, detention time, and initial temperature were investigated. The optimum detention time and initial temperature were determined using synthetic wastewater and found to be 8.5 minutes and 62 ºC, respectively. These optimum conditions are valid within the range of 60 to 120 mg NH_3-N/L. The residual concentrations under optimum conditions were 3.5 ± 0.4, 4.4 ± 0.4, 5.1 ± 0.2, and 6.9 ± 0.5 mg NH_3-N/L for initial concentrations of 60, 70, 80, 100, and 120 mg NH_3-N/L, respectively. These concentrations meet the 5 mg NH_3-N/L standard for recharging wastewater to groundwater in many countries such as Jordan .Similar results were obtained using real wastewater samples (3.1 ± 0.2, 4.0 ± 0.1 and 4.9 ± 0.3 mg NH_3-N/L for initial concentrations of 65.7, 73.8, and 94.6 mg NH_3-N/L, respectively). The developed system proved to be practical in the continuous flow mode that mimics the actual operations in wastewater treatment plants. It was concluded that MW energy could be one of the most effective methods for large scale removal of ammonia nitrogen from municipal wastewater.

Keywords: ammonia, microwave radiation, municipal wastewater, nitrogen

1. Introduction

Several methods have been applied to remove ammonia from municipal wastewater such as biological nitrogen removal (BNR), chemical precipitation, ion exchange, ozonation, and air stripping. Biological nitrogen removal is the most commonly used method worldwide. However, this method is sensitive to environmental conditions such as ambient temperature and pH. Moreover, conventional BNR methods require high energy for oxygen supply for the nitrification process (Gujer, 2010) and require high construction cost. Using chemical precipitation for ammonia removal may generate new pollutants in the treated wastewater, because of the required chemical additives (Demirer et al., 2005). For ion exchange technique, the cost of resins' regeneration is high, because nitrate and sulfate anions should be eliminated from the treated wastewater before discharging to the environment (Bochenek et al., 2011). Ozonation method consumes high amounts of energy, which seems economically unfeasible (Yang et al., 1999). Other methods like supercritical water oxidation and air-stripping methods require high operation energy, due to the high temperatures and pressures needed (Bermejo et al., 2008; Değermenci et al., 2012).

In the last few years, microwave (MW) energy became a promising technology. It was studied for several purposes related to environmental applications like treatment of heavy metal contaminated soil (Jou, 2006), remediation of petroleum contaminated soils (Chien, 2012) and hydrocarbon-contaminated soil (Robinson et al., 2012). Focusing on wastewater treatment, MW energy had been applied for several purposes, such as the treatment of ballast water inoculated with invasive organisms (Boldor et al., 2008), remediation of phenol-polluted wastewater (Xiao et al., 2007), treatment of petroleum refinery wastewater (Sun et al., 2008) and safe disposal of highly-contaminated pharmaceutical wastewater (Yang et al., 2009).

After their bench-scale study, Lin et al. (2009) developed a continuous pilot-scale MW system to remove ammonia nitrogen from a real coke-plant wastewater. Under different operating conditions, the ammonia removal could reach about 80%, with initial ammonia concentrations of 2,400-11,000 mg/L.

Recently, via batch-reactor experiments, the authors of this research studied the characteristics of ammonia removal from municipal wastewater using MW energy and high removal efficiency was achieved. It was found that radiation time and pH had significant influence on the removal of ammonia nitrogen from municipal wastewater, while the initial ammonia concentration had lower effect (Rabah & Darwish, 2013).

Following our successful demonstration of ammonia nitrogen removal in batch experiment, a continuous flow bench-scale system was designed and investigated in this research, where treatment of synthetic and real wastewater was carried out. Three factors were investigated: detention time, initial ammonia concentration and initial temperature. A heat exchanger was applied to recover heat energy from the effluent stream in order to utilize it in heating the influent stream and to raise its initial temperature.

The objective of this work was to investigate the possibility of using a continuous flow MW radiation system equipped with heat exchanger after the success of using the system as a batch reactor in our previous work (Rabah & Darwish, 2013). Moreover, an important objective was to study the performance of this continuous system and to determine the optimum conditions for ammonia nitrogen removal from municipal wastewater.

2. Materials and Methods

2.1 Experimental Setup

Figure 1 shows a schematic diagram of the experimental apparatus. A domestic microwave oven (700 W, 2450 MHz, Dura brand XB2316, UK) was used as the source of the MW radiation. Three holes were drilled in the MW body, one on the top cover to insert the ammonia gas evacuation pipe and two at the side to insert the influent and effluent wastewater pipes. A 300 ml glass vessel was placed inside the MW oven and was connected to the three mentioned pipes as shown in the figure. The wastewater was pumped from a 4 L tank into the glass reactor using a peristaltic pump (SP311, VELP Scientifica). A heat exchanger was used to allow for thermal energy recovery by inserting the influent wastewater pipe in the U-shaped tube that is filled with the continuous hot wastewater flow coming out of the MW. The heat exchanger could be bypassed by installing a bypass pipe controlled by two valves that divert the hot effluent wastewater from the MW directly to the outlet of the system. The ammonia evacuation pipe is connected to a condenser to recover the water vapor and return it back to the effluent stream. Two sampling ports were used to draw samples for the required measurements (one on the influent side, the second at the effluent side as shown in Figure 1).

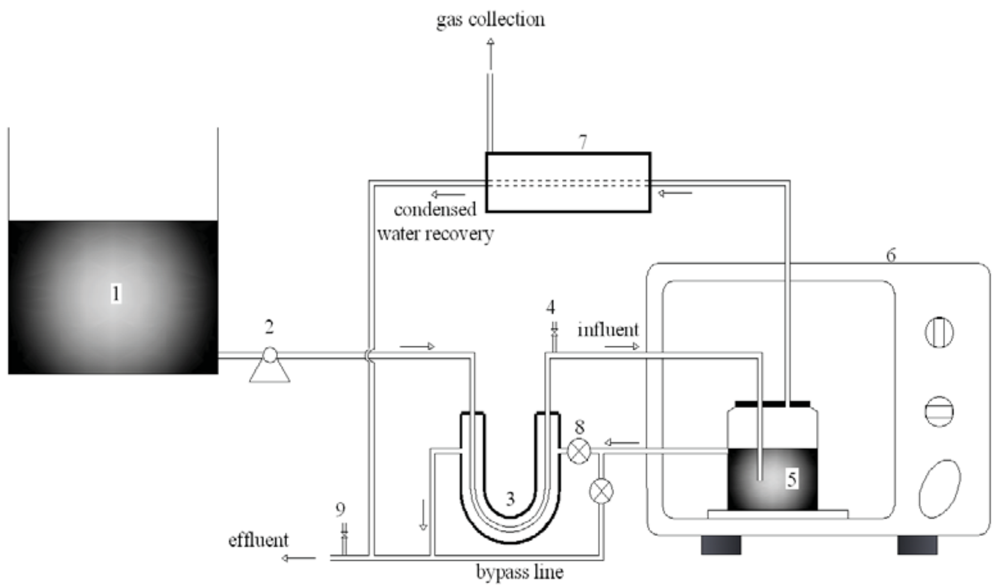


Figure 1. Schematic diagram of the experimental apparatus

(1) Wastewater tank (2) Peristaltic pump (3) Heat exchanger (4) Influent sampling port (5) Glass vessel (6) MW oven (7) Condenser (8) Valve (9) Effluent sampling port.

2.2 Materials and Analytical Methods

Synthetic wastewater (WW) solution was prepared with ammonium chloride (99.5%, analytical reagent, HiMedia Laboratories, Mumbai, India) and distilled water. The initial pH of the solution was adjusted at 11 using sodium hydroxide solution (NaOH, 2.0 mol/L, 97.5%, Chemie, Mumbai, India). Real wastewater was taken from the effluent of Gaza wastewater treatment plant.

Temperature of WW was measured by a thermometer and the ammonia nitrogen concentration was measured using Nessler standard method (APHA, AWWA, & WEF, 1989). In all the experiments, for statistical purposes, each condition was tested 3 times and an average value was reported together with its corresponding standard deviation.

2.3 Experimental Program

In this study, three sets of experiments were performed as follows:

First set: using synthetic WW with no heat exchanger.

Second set: using synthetic WW with heat exchanger.

Third set: using real WW with heat exchanger.

The main difference between experiments performed with heat exchanger and those without heat exchanger was the value of the initial temperature (T_i) of the WW when it enters the MW. With no heat exchanger, T_i was always 20 ºC (temperature of the source synthetic WW). With heat exchanger, T_i was in the range of 59 to 71 ºC as shown later in this paper. Energy recovery and cooling the treated effluent are the two main goals of using the heat exchanger.

The effects of initial ammonia concentration, detention time, and initial temperature were investigated. Four detention times (5, 10, 15, and 20 min) and four initial ammonia concentrations (60, 80, 100, and 120 mg NH_3/L) were tested in each of the first and second sets of experiments. For each detention time, 4 runs were performed, where each run corresponds to one of the 4 tested ammonia concentrations (60, 80, 100 or 120 mg/l). Thus, the total number of runs performed was 16 in each set of experiments (32 runs in the two sets).

In the third set of experiments three concentrations (55, 70, and 85 mg NH_3/L) were tested with the optimum detention time of 10 minutes (3 runs). The value of the three ammonia concentrations are based on the characteristics of the real wastewater samples collected from Gaza wastewater treatment plant at different times. The optimum detention time was determined from the results of the first and second sets of experiments as shown later.

In each experimental run, a WW sample was taken every 5 minutes for analysis purposes and the residual ammonia concentration (C) was determined. The system was operated until a steady state residual ammonia concentration (C) was attained.

The detention time of wastewater in the MW reactor was controlled by changing the wastewater pumping flow rate according to following equation:

$$\theta = \frac{V}{Q}$$

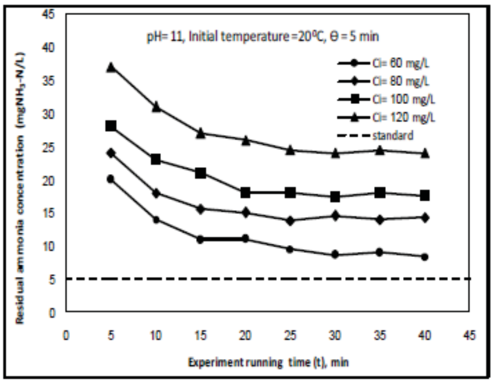
Where θ is the detention time in min., V is the volume of the glass vessel in ml, and Q is the wastewater flow rate in ml/min.

The initial pH for all sets of experiments was set at 11 which was found in our previous work to be the optimum value for removing ammonia nitrogen from municipal wastewater using MW radiation.

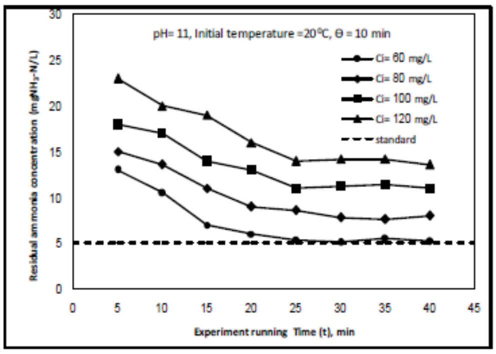
3. Results and Discussion

3.1 Performance of the System Using Synthetic WW

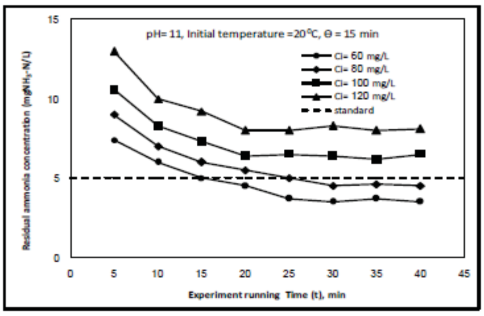
Figure 2 (a, b, c, & d) illustrates the performance of the system without using the heat exchanger (first set of experiments-16 runs) while Figure 3 (a, b, c, & d) illustrates its performance when using the heat exchanger (second set of experiments-16 runs). The two figures show the relation between the residual ammonia concentration (C) and the experiment running time (t). Each experiment lasted for 40 minutes. It was observed that the ammonia residual concentration (C) decreased with time at the beginning of each experiment and reached a steady state value after 20-30 minutes in all the experiments. It was also observed from the two figures that higher ammonia removal was achieved by increasing the detention time (θ).



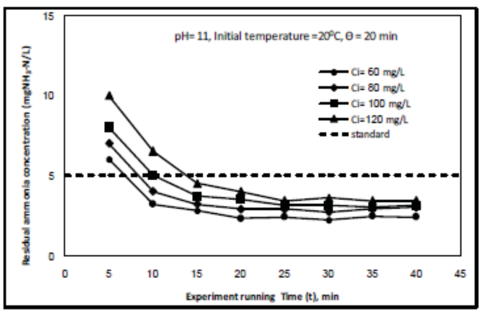
(a) θ =5 min, T_i =20 ºC



(b) θ = 10 min, Ti = 20 ºC



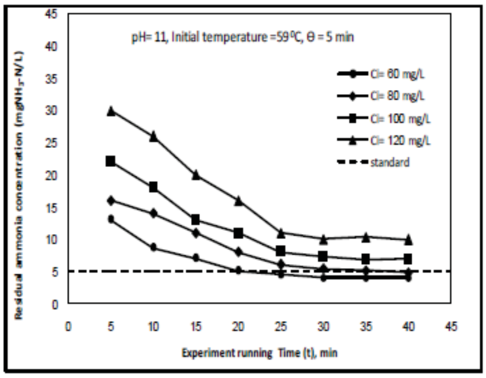
(c) θ = 15 min, T_i = 20 ºC



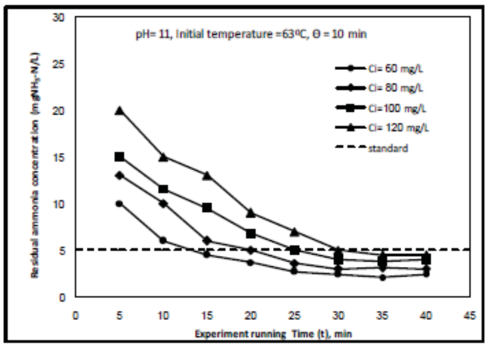
(d) θ = 20 min, T_i = 20 ºC

Figure 2. Residual ammonia concentration versus time without heat exchanger.

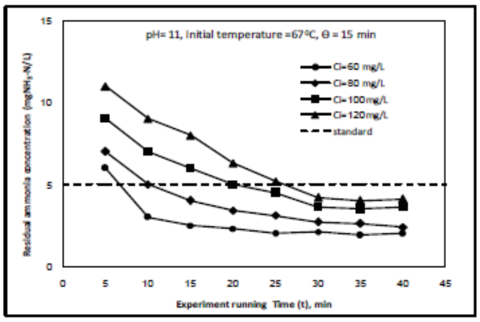
(C_i = initial ammonia concentration)



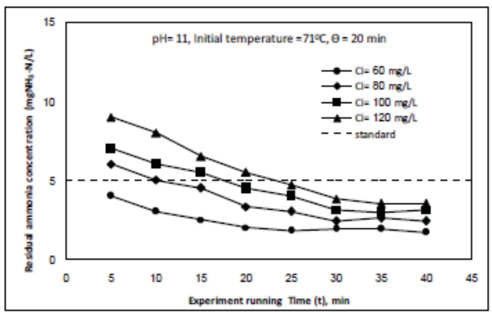
(a) θ =5 min, T_i =59 ºC



(b) θ = 10 min, Ti = 63 ºC



(c) θ = 15 min, T_i = 67 ºC



(d) θ = 20 min, T_i = 71 ºC

Figure 3. Residual ammonia concentration versus time with heat exchanger.

(C_i = initial ammonia concentration)

For example, when θ was 5 min (Figure 2-a) the residual ammonia steady state concentration (C) was 8.4 ± 0.3 mg/L while it was 2.4± 0.2 mg/L (for an initial concentration of 60 mg/l) when θ was 20 min (Figure 2-d). The same was observed when comparing Figure 3-a and Figure 3-d (for an initial concentration of 60 mg/l) as C was 4.1±0.4 mg/l and 2±0.1 mg/l for detention times (θ) of 5 min and 20 min, respectively. Moreover, C attained in the experiments with heat exchanger (i.e. results in Figure 3) was lower than that attained in the experiments without heat exchanger (i.e. results in Figure 2). For example, when θ was 5 min (Figure 2-a) and the initial temperature (Ti) was 20 ºC, (C) was 8.4 ± 0.3 mg/L (for an initial concentration of 60 mg/l) while it was 4.1 ± 0.4 mg/L when θ was 5 min and T_i was 59 ºC as shown in Figure 3-a indicating better removal efficiency when using heat exchange (86.7% versus 93.2% removals). More elaboration on the effect of detention time and heat exchange effect are given in the following subsections in addition to the effect of the initial ammonia concentration.

3.1.1 Effect of Detention Time

To investigate the effect of detention time (θ), in each run, the initial ammonia concentration (C_i) was fixed at a certain value (60, 80, 100 or 120 mg/l) while the detention time was increased in the range of 5 to 20 minutes. Figure 4 illustrates the effect of detention time (i.e. MW radiation time) on the steady state residual ammonia concentration (C) for C_i values of 60, 80, 100 and 120 mg/l when the initial temperature (T_i) was kept at 20 ºC via bypassing the heat exchanger. The general observed trend was that C decreased nonlinearly with the increase of detention time inside the MW reactor for all the ammonia initial concentrations.

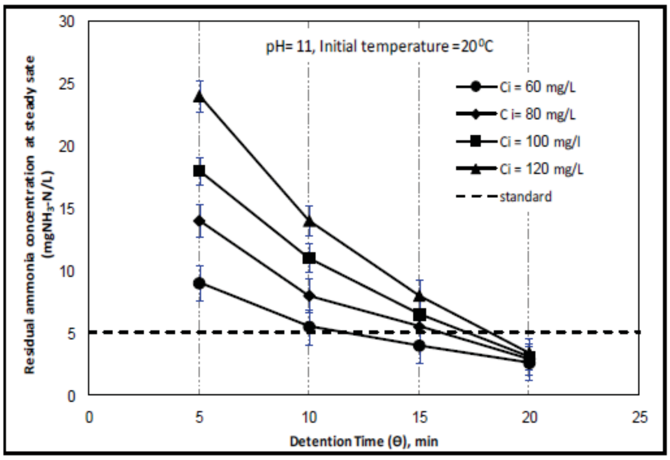


Figure 4. Effect of detention time on ammonia residual steady state concentration without heat exchange (T_i constant at 20 ºC)

For example, for a C_i value of 80 mg NH_3-N /L, the steady state residual ammonia concentrations were 14, 8, 6 and 3.5 for detention times of 5, 10, 15, and 20 min, respectively. The same trend was observed for the remaining concentrations as interpreted from Figure 4. The improved performance with the increase of detention time is attributed to the increased heating energy induced by the MW radiation that increased in turn the water molecules random motion leading to the increase of ammonia mass transfer out of the liquid phase. To make sense of the removal efficiency, the ammonia residual (C) is compared to the ammonia standard for wastewater reuse for recharge to the groundwater which is 5 mg NH_3-N/L (JISM, 2006). The standard concentration was achieved at 11, 16, 18 and 19 min for C_i values of 60, 80, 100 and 120 mg NH_3-N /l, respectively (Figure 4).

3.1.2 Effect of Initial Temperature

The initial temperature (T_i) was constant at 20 ºC in the first set of experiments when the heat exchanger was bypassed. However, in the second set of experiments, when the heat exchanger was employed, T_i increased with the increase of detention time (θ). T_i was 59, 63, 68, and 71 when the detention time was 5, 10, 15, and 20 min, respectively. Figure 5 illustrates the effect of initial temperature on the steady state residual ammonia concentration (C) for T_i range of 59 to 71 ºC. The removal efficiency when T_i was 20 ºC can be observed from Figure 4. Since T_i and θ are dependent variables when using the heat exchanger, it was necessary to isolate the effect of θ while studying the effect of T_i. This was achieved by comparing the value of C at T_i of 20 ºC at a specified detention time (5, 10, 15, or 20 minutes) with C at the corresponding T_i (59, 63, 68, or 71 ºC). For Example, by reading Figures 4 and 5, when θ was 5 minutes, T_i was either 20 or 59 ºC and when ϴ was 20 minutes, Ti was either 20 or 71 ºC. It was observed that the removal of ammonia increased by the increase of initial temperature. For example, for C_i = 100 mg/L and θ =10 minutes, C was 11.2 ± 0.5 mg/L (from Figure 4) and 3.8 ± 0.3 mg/L (From Figure 5) when T_i was 20 and 63 ºC, respectively. An important result was also concluded regarding the achievement of a specified standard residual value such as 5 mg NH_3-N/l for the purpose of recharge (Jordanian standards, 2006). The results indicate that a specified standard can be achieved by lower detention time when using higher T_i values by means of heat exchanger. For example, for C_i = 100 mg NH_3-N/L, the standard concentration required 15 minutes detention time to be achieved, while it was achieved at a detention time of 8.5 minutes only when the heat exchanger was used. This finding is very important in terms of the economical feasibility of such a system. Lower detention times mean lower reactor volume and lower power consumption per treated cubic meter of WW.

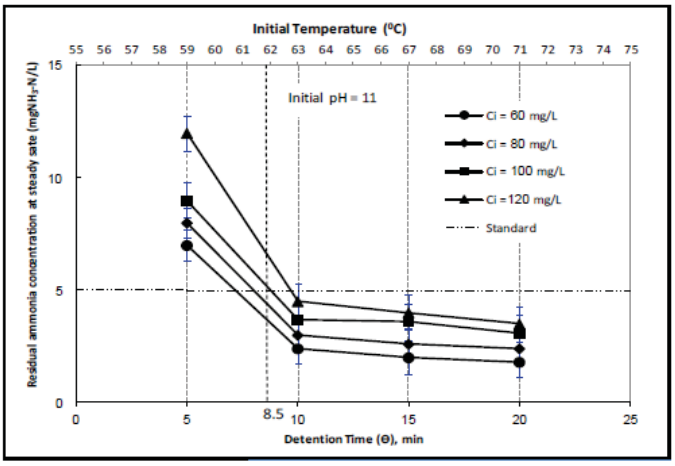


Figure 5. Effect of initial temperature on ammonia residual steady state concentration (with heat exchanger)

3.1.3 Effect of Initial Ammonia Concentration

Four initial ammonia concentrations (C_i) were tested in this work, namely: 60, 80, 100 and 120 mg NH_3-N/l. These concentrations are in the typical range of ammonia concentrations in real municipal wastewater (CMWU, 2010). To investigate the effect of C_i , the detention time (θ) was fixed at a certain value (5, 10, 15 or 20 min) while C_i was changed to one of the above mentioned 4 concentrations. Figure 6 illustrates the effect initial ammonia concentrations (C_i) on the steady state residual ammonia concentration (C) when the initial temperature (T_i) and pH were kept constant at 20 ºC and 11, respectively.

It is observed from Figure 6 that at low detention time (θ) the increase of initial ammonia concentration (C_i) resulted in higher ammonia steady state residual concentration (C) (i.e. lower removal efficiency). For example, when θ was 5 minutes, C increased from 26 to 38 mg NH_3-N /L when C_i increased from 60 to 120 mg/L. On the other hand, it was observed that at high θ the effect of C_i on C decreases and becomes negligible. For Example, when θ was 15 minutes, C increased from 6 to 11 mgNH_3-N /L only when C_i increased from 60 to 120 mg NH_3-N/L. Moreover, when θ was 20 minutes, C was almost constant when C_i increased from 60 to 120 mg NH_3-N/L (i.e. C increased from 3 to 3.5 mg/L only).

This phenomenon can be attributed to the variations of ammonia mass transfer rate due to the change of the heating energy induced into the wastewater by microwave radiation. At low detention time the heating energy in the wastewater is low and has a corresponding low constant ammonia mass transfer rate. So when C_i increases C also increases. On the other hand, at high detention time the heating energy in the wastewater is high and has a corresponding high constant ammonia mass transfer rate that is enough to overcome he increase in C_i and consequently results in constant value of C for all values of C_i.

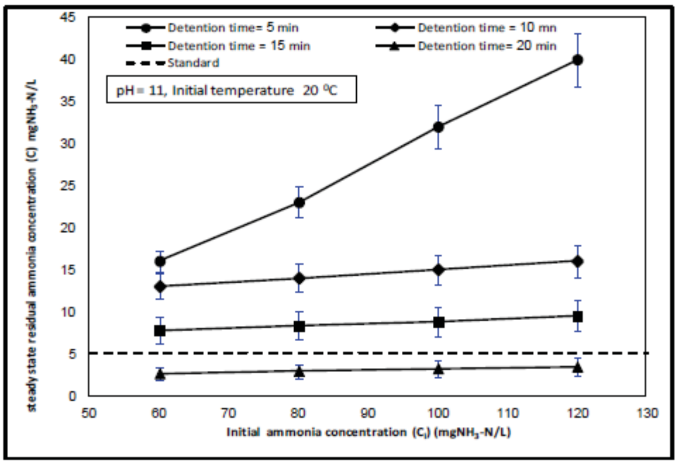


Figure 6. Effect of initial ammonia concentration on ammonia residual steady state concentration (without heat exchanger)

3.2 Performance of the System Using Real WW With Heat Exchanger

The third set of experiments was performed to study the performance of the system when treating real wastewater. The experiments were performed using three wastewater samples that were collected from Gaza wastewater treatment plant effluent. Samples characteristics and the sampling dates are given in Table 1.

Table 1. Characteristics of the real wastewater samples

Sample #	Collection date	pH	BOD_5 (mg O_2/L)	TSS (mg/L)	NH_3 (mg NH_3-N/L)	Fecal Coliform (Cell/100 ml)
1	03/01/2012	7.3	115	75	65.7	3190
2	03/13/2012	7.1	135	126	94.6	4370
3	04/03/2012	7.5	90	92	73.8	2460

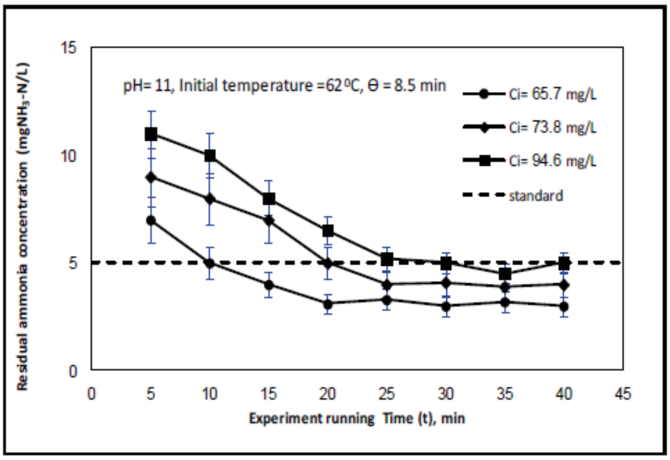


Figure 7. Performance of the continuous microwave system in removing ammonia from real domestic wastewater

The third set of experiments was run using the optimum conditions determined from the first and second sets of experiments that were performed on synthetic wastewater as indicated above. The detention time used was 8.5

minutes since it was found to be enough to achieve a residual ammonia concentration of 5 mg NH_3-N/L (as indicated in item 3.1.2). The initial temperature (T_i) was 62 ºC as a result of using the heat exchanger. The pH was set at the optimum value of 11. Three runs were performed where each run corresponds to one of the three initial ammonia concentrations presented in Table 1 (i.e. 65.7, 73.8, 94.6 mg NH_3-N/L). Figure 7 shows the performance of the system in removing ammonia from real domestic wastewater under the described conditions. It was observed that the ammonia residual concentration (C) decreased with time at the beginning of the process then it reached a steady state value after 20-30 minutes for the three runs. The residual steady state ammonia concentration (C) was found to be 3.1 ± 0.2, 4.0 ± 0.1 and 4.9 ± 0.3 mg NH_3-N/L, for the initial concentrations 65.7, 73.8, and 94.6 mg NH_3-N/L, respectively. This indicates that the ammonia standard concentration of 5 mg NH_3-N/L can be achieved under conditions described in this study. It was also observed that the ammonia removal efficiency and the residual ammonia concentrations are similar to that of the synthetic wastewater. This indicates that other constituents in the municipal wastewater such as organic matter and suspended solids have no significant effect on the ammonia removal by MW radiation.

4. Conclusion

This research was carried out to explore ammonia nitrogen removal from municipal wastewater using a continuous microwave system equipped with heat exchanger. The effects of ammonia initial concentration, radiation time, and wastewater initial temperature were investigated. It was concluded from the experiments performed on synthetic wastewater that the optimum conditions for this system are using a detention time of 8.5 minutes that leads to an initial temperature of 62 ºC when using the heat exchanger. These conditions are valid for the range of initial concentrations between 60 and 120 mg NH_3-N/L a concentration range typical for municipal wastewater. The residual ammonia concentrations under these conditions were 3.5 ± 0.4, 4.4 ± 0.4, 5.1 ± 0.2, and 6.9 ± 0.5 mg NH_3-N/L for initial concentrations of 60, 70, 80, 100, and 120 mg NH_3-N/L, respectively. Similar ammonia removals were achieved with real municipal wastewater samples that were treated under the same conditions. The residual steady state ammonia concentration (C) was found to be 3.1 ± 0.2, 4.0 ± 0.1 and 4.9 ± 0.3 mg NH_3-N/L, for the initial concentrations 65.7, 73.8, and 94.6 mg NH_3-N/L, respectively. It is concluded from these results that the optimum conditions for ammonia removal from real wastewater by MW radiation are: detention time of 8.5 minutes, pH of 11, and using heat exchanger (to achieve initial temperature of 62 ºC or better). These conditions apply for initial ammonia concentrations in the range of 65 to 95 mg NH_3-N/L which is the typical range of concentrations in municipal wastewater. This study suggests that MW radiation operated in a continuous mode is a promising technology for removal of ammonia nitrogen from municipal wastewater.

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References

APHA, AWWA and WEF. (1989). Standard Methods for the Examination of Water and Wastewater (17th ed.). Washington, DC: APHA.

Bermejo, M. D., Centero, F., & Cocero, M. J. (2008). Supercritical water oxidation of feeds with high ammonia concentrations: Pilot plant experimental results and modeling. *Chemical Engineering Journal, 137*(3), 542-549. http://dx.doi.org/10.1016/j.cej.2007.05.010

Bochenek, R., Sitarz, R., & Antos, D. (2011). HYPERLINK Design of continuousion exchange process for the wastewater treatment. *Chemical Engineering Science, 66*(23), 6209-6219. http://dx.doi.org/10.1016/j.ces.2011.08.046

Boldor, D., Balasubramanian, S., Purohit, S., & Rusch, K. A. (2008). Design and Implementation of a Continuous Microwave Heating System for Ballast Water Treatment. *Environmental Science and Technology, 42*(11), 4121-4127. http://dx.doi.org/10.1021/es7024752

Chien, Y. (2012). Field study of in situ remediation of petroleum hydrocarbon contaminated soil on site using microwave energy. *Journal of Hazardous Materials, 199-200*, 457-461. http://dx.doi.org/10.1016/j.jhazmat.2011.11.012

Coastal Municipalities Water Utility. (2010). *Wastewater lab tests results*, Gaza, Palestine.

Değermenci, N., Ata, O. N., & Yildiz, E. (2012). Ammonia removal by air stripping in a semi-batch jet loop reactor. *Journal of Industrial and Engineering Chemistry, 18*(1), 399-404.

http://dx.doi.org/10.1016/j.jiec.2011.11.098

Demirer, S. U., Demirer, G. N., & Chen, S. (2005). Ammonia removal from anaerobically digested dairy manure by struvite precipitation. *Process Biochemistry, 40*(12), 3667-3674. http://dx.doi.org/10.1016/j.procbio.2005.02.028

Gujer, W. (2010). Nitrification and me-A subjective review. *Water Research, 44*(1), 1-19. http://dx.doi.org/10.1016/j.watres.2009.08.038

JISM. (2006). Water-Reclaimed domestic wastewater. Technical Regulation, Jordanian Institution for Standards and Metrology, Jordan.

Jou, C. G. (2006). An efficient technology to treat heavy metal-lead-contaminated soil by microwave radiation. *Journal of Environmental Management, 78*(1), 1-4. http://dx.doi.org/10.1016/j.jenvman.2004.11.020

Lin, L., Chen, J., Xu, Z., Yuan, S., Cao, M., Liu, H., & Lu, X. (2009). Removal of ammonia nitrogen in wastewater by microwave radiation: A pilot-scale study. *Journal of Hazardous Materials, 168*(2-3), 862-867. http://dx.doi.org/10.1016/j.jhazmat.2009.02.113

Rabah, F. K., & Darwish, M. S. (2013). Characterization of Ammonia Removal from Municipal Wastewater Using Microwave Energy: Batch Experiment. *Environment and Natural Resources Research, 3*(1), 42-50. http://dx.doi.org/10.5539/enrr.v3n1p42

Robinson, J. P., Kingman, S. W., Lester, E. H., & Yi, C. (2012). Microwave remediation of hydrocarbon-contaminated soils-Scale-up using batch reactors. *Separation and Purification Technology, 96*(21), 12-19. http://dx.doi.org/10.1016/j.seppur.2012.05.020

Sun, Y., Zhang, Y., & Quan, X. (2008). Treatment of petroleum refinery wastewater by microwave-assisted catalytic wet air oxidation under low temperature and low pressure. *Seperation and Purification Technology, 62*(3), 565-570. http://dx.doi.org/10.1016/j.seppur.2008.02.027

Xiao, Y. B., Wang, P., Jiang, H., Xu, H., Shi, S., & Huang, J. (2007). Treatment of phenol wastewater by microwave-induced ClO_2-CuOx/Al_2O_3 catalytic oxidation process. *Journal of Environmental Sciences, 19*(12), 1510-1515.

Yang, M., Uesugi, K., & Myoga, H. (1999). Ammonia removal in bubble column by ozonation in the presence of bromide. *Water Research, 33*(8), 1911-1917. http://dx.doi.org/10.1016/S0043-1354(98)00364-9

Yang, Y., Wang, P., Shi, S., & Liu, Y. (2009). Microwave enhanced Fenton-like process for the treatment of high concentration pharmaceutical wastewater. *Journal of Hazardous Materials, 168*(1), 238-245. http://dx.doi.org/10.1016/j.jhazmat.2009.02.038

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Combined Analysis of Results on Single and Multiple Contacts: Potting Soil, Sand and Clay Transfers to Cloth for the Estimate of Soil Adherence to Human Skin

Alesia C. Ferguson[1], Zoran Bursac[1] & Wayne Johnson[2]

[1] College of Public Health, University of Arkansas for Medical Sciences, Arkansas, United States

[2] Engineering Studies, Armstrong Atlantic University, Georgia, United States

Correspondence: Alesia C. Ferguson, College of Public Health, University of Arkansas for Medical Sciences, Arkansas, United States. Tel: 1-501-526-6662. E-mail: aferguson@uams.edu

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Abstract

Humans can be exposed to contaminated soil and household dust via contact with environmental surfaces. A mechanical device was used to measure the transfer of soils from one surface to the other to provide estimates of adherence factors (mg/cm^2) used in estimates of human exposure to contaminants found in soils/household dust. This paper presents the analysis and comparison for thetransfer of clay, potting soil, and sand from aluminum to cloth samples for single and multiple contacts under varying environmental and contact time and pressure conditions. Adherence for clay was over 73 times that of sand and over 114 times that of potting soil. Clay particles with their softer texture and finer composition compared to potting soil and sand, are more likely to compress and adhere to surfaces producing higher transfers. Contact time and temperature had no effect, however: contact pressure, soil loading on the aluminum surface, and environmental humidity did have an effect on soil transfer.

Keywords: human exposure assessment, soil contamination, soil adherence, transfer factors

1. Introduction

The contamination of soil with chemical byproducts, due to manufacturing and agricultural operations is prevalent throughout the United States, leads to exposures for children and adults under varying circumstances and scenarios. The variation of chemicals andtheir concentration outdoors are affected by the type and magnitude of industrial, commercial, mining, and agricultural activities in the area; wind and climate patterns affecting the transport and deposition mechanisms; the originating soil type by regions; location and motion of bodies of water; and land use development (Hendryx, Fodorko, & Halverson, 2010; Isikli, Demir, Akar, Urer, Kalyoncu, & Canbek, 2012; Jarup, 2003). Housing and occupational settings close to processes releasing contaminants (i.e., heavy metals, dioxins) pose greater health risks for residents and workers due to drift, track in, and take home of these contaminants (Hendryx et al., 2010; Kuo, Wang, Yeh, Kuo, & Chou, 2010; Isikli et al., 2010; Nawrot et al., 2006).

House hold dust that accumulates over time and becomes persistent in the indoor environment is the main exposure source to a number of contaminants such as allergens, metals, pesticides, volatiles, and other mutagens or carcinogens for infants (Butte & Heinzow, 2002; Hwang, Park, Young, & Hammock, 2008; Roberts et al., 2009). The sources of contaminants in household dust can be from the outdoor sources listed above or indoor sources resulting from activities (e.g., smoking, pets in the home) or chemicals and products used in the home (e.g., new carpets, and sofas, pesticides, cleaning products) (Guo et al., 2012; Hwang et al., 2008; Thorne, Cohn, May, Arbes, & Zeldin, 2009).

The recommendations for safe levels of indoor contaminants in soils and household dust are limited and typically based on sparse human exposure and dose factors, soil/dust characteristics, chemical characteristics, and other environmental factors. In an analysis of the very potent tetrachlorodibenzo-p-dioxin (TCDD), a level of 1 ppb TEQ soil guidance was deemed a reasonable screening level for residential soil based on the most sensitive determinants of human dose and risk (Paustenback, Fehling, Scott, Harris, & Kerger, 2006). In a different analysis on the relationship between exposure to lead-contaminated soil, the impact on public health, and past assessments, a blood lead level (BLL) rise in young children of 3-7 mcg/dl was observed for every 1000 ppm increase in soil or dust concentration (Centers for Disease Prevention and Control, 1992). There continues to be a need to determine safe levels of various contaminants in household dust and their potential effect on human health, where uptake can occur through the skin, inhalation, or ingestion. For contaminants where the main route of exposure is dermal, improved estimates of exposure based on human activity (i.e., number of contacts with surfaces) and exposure parameters such as adherence of soils and/or dust to the skin is important in improving risk estimates.

In an exposure assessment modeling approach, adherence values (mg/cm^2) can be combined with contaminant concentration pg/mg to determine the amount of contaminant on the area of skin (pg/cm^2) as an estimate of dermal exposure. Dose estimates can be calculated by determining the uptake rate through the skin once the exposure estimate on the skin is derived. The document, Risk Assessment Guidance for Superfund, presents a more comprehensive approach [refer to Formula (1)] for the calculation of chemical dose from dermal contact with soil (Environmental Protection Agency [EPA], 1989).

$$AD = (CS \times CF \times SA \times AF \times ABS \times EF \times ED)/(BW \times AT) \quad (1)$$

where:

AD = Absorbed dose (mg/kg/day)

CS = Chemical concentration in soil (mg/kg)

CF = Conversion factor (10-6 kg/mg)

SA = Skin surface area available for contact (cm^2/event)

AF = Soil-to-skin adherence factor (mg/cm^2)

ABS = Absorption factor (unitless)

EF = Exposure frequency (events/year)

ED = Exposure duration (years)

BW = Body weight (kg)

AT = Averaging time (period over which exposure is averaged-days)

In a modeling approach, such as the equation above, it is necessary to determine the frequency of soil contact events per year (i.e., exposure frequency (EF)). This approach is on a macro-scale, but there are occasions within a short timeframe that multiple contacts with soil occur (e.g., 10 contacts with soil over a 10 minute time period). Thus, more accurate models must estimate the total loading of soil on skin based on a series of multiple contacts and limitations of adherence and loading at the skin surface to obtain a realistic estimate of exposure and dose. Models exist that use this micro-activity approach for estimating the loading of a soil (and therefore contaminant) on the skin surface (Canales, 2004; Zartarian, 1996; Zartarian et al., 2000). Reduction in the amount of soil on the skin is also considered based on removal events at the skin surface (e.g., hand washing, and wiping events) along with consideration of maximum loads feasible on the skin surface.

The adherence of clays, resulting from single contacts, has been previously presented in relation to artisans exposed to clays contaminated with dioxins (Ferguson, Bursac, Johnson, & Davis, 2012). Adherence studies of sand and potting soils were also previously published to provide data needed to model children's dermal exposure in and around the home (Ferguson, Bursac, Coleman, Biddle, & Johnson, 2008; Ferguson, Bursac, Coleman, Biddle, & Johnson, 2009 a, b; Ferguson, Coleman, Bursac, & Johnson, 2009c). Adherence measures for multiple contacts for clay are presented for the first time in this paper. In addition, this paper compares the mass loading of three types of soils to cloth (representing skin) for single and multiple contacts and can aid the researcher in modeling efforts to estimate exposure to contaminants in soils or household dust where adherence values in mg/cm^2 is needed. Clay, sand, and potting soil represent varied soil matrices with potentially different adherence potentials to the human skin as afunction of texture, moisture, and size structure. In applying adherence values for soils from any study, the exposure modeler should be aware of the diverse exposure scenarios, soil types/mixtures, and the potentially varied distribution of contaminants in those soils. The distribution of contaminants is not only based on original sources of contaminants, but on their ability to bind, break down, or leach in the various soil types (Engle, Cogger, & Stevens, 1991). Here cloth is used to represent human skin (as has been done in other adherence studies of contaminant exposures), and can provide a comparison for various soil types. Past experiments from this group have used cadaver skin for adherence experiments. It is recognized that the characteristics of cloth and skin vary and can influence adherence.

2. Method

Experiments for mass transfer of soil types were conducted using a mechanical chamber designed to control and measure the contact pressure and duration of contact between two plates. Mass transfer was measured from an 8.97cm^2 aluminum plate laden with clay, sand, or potting soil to cloth samples attached to an actuated plate. Temperature and relative humidity probes were used to closely monitor and record the environmental conditions in the chamber. Previous results were published for adherence experiments conducted with potting soil and sand (Ferguson et al., 2008; Ferguson et al., 2009 a, b, c) and for clay based on single contacts (Ferguson et al., 2012). Those past experiments were combined with these multiple clay experiments to look at single, multiple, and total transfers for the overlapping pressures of 20, 30, 40, and 50 kPa (60 kPa was excluded for sand and potting soil), and for 10, 30, and 50 seconds (60 seconds was excluded for sand and potting soil). Only adherence for cloth from aluminum surfaces was analyzed for the soil models bringing the total number of experiments to 236. The adherence material of skin and the adherence surface of carpet were also excluded for sand and soil, based on no comparison with contacts for clay. Previously, for potting soil and sand adherence tests, the soil sizes of < 139.7 μm and from 139.7 up to 381 μm were analyzed separately; these two sand and soil sizes were analyzed separately and together to determine any difference in adherence results. Chamber temperature, chamber relative humidity, and soil mass before and after contact were recorded in all experiments.Further descriptions for potting soil and sand can be found in the previous publications for single and multiple contacts for adherence to cloth and human skin from carpet and aluminum surfaces (Ferguson et al., 2008; Ferguson et al., 2009 a, b, c).

2.1 Mechanical Chamber

The chamber consists of an outer and inner housing; air cooling, heating, and humidification devices; a changeable pneumatic cylinder and associated equipment and various temperature, pressure, and relative humidity transducers. National Instruments is the manufacturer of a USB 6009 data acquisition device (DAQ) which was used along with LabVIEW Virtual Instruments (VIs) to interface all instruments in the chamber with a personal computer. LabVIEWVIs provided a user interface to the instruments via the USB 6009 and recorded all sensor information (temperature and relative humidity) over the user-specified and chamber-controlled contact times and pressures. Chamber operation and use of the LabVIEW interface to control and record experimental parameters is well described in all previous experimental result papers and results and on the chamber design (Johnson, Ferguson, Hager, Sanou, & Shenoda, 2006; Johnson, Ferguson, Hager, Sanou, & Shenoda, 2007).

2.2 Contact Pressure and Time Range

Contact pressures (i.e., force applied over a surface area) and time of contact can potentially affect the mass transfer of chemicals. Some contact pressures were indicated by pediatric and occupational therapy literature for grip pressures exerted by children and adults (Dunn, 1993; Gurram, Gouw, & Rakheja, 1993; Link, Lukens, & Bush, 1995; Robertson & Dietz, 1988). The motions and pressures involved in making clay pottery might be expected to include a wider range of contact pressures over those encountered for situations of sand and potting soil contact (e.g., children playing in playground and around the home). Contact times can vary broadly for various contact scenarios encountered for these soil types, but increased contact times beyond a maximum point may not affect transfer rates, only the chemical uptake into the skin.

2.3 Clay, Sand, Soil and Cloth Preparation

As a comparison with soil transfer to human cadaver skin, cotton sheets were purchased from a fabric store. Soil transfer to human cadaver skin was previously conducted for sand and potting soil (Ferguson et al., 2008; Ferguson et al., 2009 a, b, c), but, however, were not performed for clay contacts. The cotton sheets were 200 thread count, 100% combed cotton sateen, with woven strips (Gramercy Park Sheets, New York, NY, USA). These were also cut into appropriate sizes and attached to the 8.97 cm^2 circular upper plate using o-rings. The clay was obtained through the Arkansas Art Center located in downtown Little Rock, AR. Within the studio section of the art center, artisans and students use this clay to mix with other materials to make pottery and related products. Dermal exposure can occur during the handling of clay containing dioxins. Dioxins can also be released for inhalation exposure during the kiln process where heat is applied to the finished products (Ferrario, Byrne, &Schaum, 2004; National Center for Environmental Assessment, 2008).

Play sand and lawn soil were purchased from Home Depot. The play sand is manufactured by LaFarge, North America (Pavestone Item # 55141), while the enriched lawn potting soil (i.e.: made of peat moss, a wetting agent, and fertilizer) is manufactured by Scotts, Inc. (product # 79259750). Sand and lawn soil were placed in open buckets to equilibrate with room conditions. Both were then dry-sieved to obtain two sizes < 139.7 µm and < 381.0 ≥ 139.7 µm, due to the apparent wide distribution of particle sizes. The authors have previously noted that the majority of the particle size distributions of the original sand and potting soil ranged between 66.4 to 139 µm (95.24%) for sand and was greater than 1524 µm (72.92%) for potting/lawn soil (Ferguson et al., 2007). Clay, sand or soil were not wetted. Humidity in the chamber was recorded and statistical correlations were made between this recorded humidity and adherence values.

2.4 Experimental Procedure

The experimental procedure was the same for each soil type. Clay, sand, or potting soil was distributed over 96.05 cm^2 aluminum samples placed over the bottom plate with full coverage in the center over the 8.97 cm^2 contact area. The chamber was cleaned and dusted with a static brush between experiments to make sure it was free of any clay particles. The aluminum samples with loaded clay, sand, or potted soil were weighed on an analytical Mettler Toledo balance (Columbus, OH, USA: model # AB-265-S) and then placed into the bottom plate of the chamber (this is 'Mass 1'). The upper plate with an attached cotton sheet was connected to the pneumatic cylinder within the chamber. After controlled contact was achieved between the upper and lower plates and recorded by the Virtual Instrument, the aluminum sample loaded with clay, sand, or potting soil was removed from the chamber and again weighed to obtain the difference (i.e., Mass 2). This difference ('Mass 1'-'Mass 2') corresponded to the amount of soil that adhered to the cotton sheet, and can be considered 'First Transfer'. For 'Second Transfer', the same aluminum sample loaded with the soil type (i.e., 'Mass 2') was placed carefully back in the chamber and a second controlled contact between the upper plate and lower plate was initiated and recorded by the Virtual Instrument under the same pressure and time of contact parameters as the first contact. The soil loaded aluminum sample was then removed from the chamber and again weighed to obtain 'Mass 3'. The difference (negative or positive) between 'Mass 2' and 'Mass 3' corresponded to the amount of soil that adhered to or was removed from the same cotton sheet for 'Second Transfer'. 'Total Transfer' is 'Mass 1'-'Mass 3'.

2.5 Statistical Analysis

All statistical analyses were performed using SASv9 (SAS Institute Inc., Cary, NC). The logarithmic transformation of the soil mass transfer amounts (i.e., mass adherence values) was performed to normalize the distribution. Descriptive statistics, including means and standard deviations for continuous variables and proportions for categorical variables were generated. Testing equality of means for clay mass adherence between individual experimental conditions was done using two sample t-tests and one-way analysis of variance (ANOVA) with Tukey-Kramer adjustment for multiple comparisons. Distribution of soil mass adherence was tested for normality using Kolmogorov-Smirnov test. The effect of experimental conditions on the transfer of soil as well as interactions was determined using two variations of a fixed effects model; in the first model there are three types of soils. Sand and potting soil are further separated by two particle sizes in the second model [refer to Formulas (2) and (3)].

$$\text{Log(transfer)}=b0+b1(\text{time})+b2(\text{pressure})+b3(\text{clay/sand/soil})+b4(\text{amountbefore}) +b5(\text{temperature})+b6(\text{humidity}) \quad (2)$$

And

$$\text{Log(transfer)}= b0+b1(\text{time})+b2(\text{pressure})+b3(\text{clay/sand small/sand large/potting soil small/potting soil large})+ b4(\text{amount(3) before})+b5(\text{temperature})+b6(\text{humidity}) \quad (3)$$

This model accounted for the amount of soil before the transfer as well as the relative humidity and temperature of the chamber. Estimated adjusted means for experimental conditions were exponentiated back to express them in the original units. All of the results were considered significant at the 0.05 alpha level.

3. Results

Results will be first presented for the **First Transfer** which is defined as the mass transfer of a soil type after the first contact between the plate with the cloth sample and the plate with the aluminum surface loaded with the soil type (i.e. 'Mass 2'-'Mass 1'). Results will then be presented for the **Second Transfer**, defined as the mass transfer of a soil type after a second contact between the same plate and cloth sample and the same plate with the aluminum surface loaded with the soil type (i.e., 'Mass 3'-'Mass 2'). Lastly, results will be presented for the **Total Transfer** which is the addition of the amount transferred in the first contact and the amount transferred in the second contact (i.e., 'Mass 3'-'Mass 1'). Table 1 summarizes a number of mass transfer variables across all soil types for 236 combined mass transfer experiments, where each experiment involves two contacts between a surface containing the soil media and the cloth samples used to represent human skin. The number of experiments for sand and potting soil were 95 and 93, respectively, where two soil sizes were tested for both soil types. More information on the size distribution contained in the sand and potting soils used can be found in a previous publication (Ferguson et al., 2009b). A total of 48 experiments were performed using clay without any size separation due to the consistent and fine texture of the clay. Temperature across the 236 experiments was 77.8 ± 0.9 ºF and average relative humidity was 28.9 ± 9.2%.

Table 1. Means analysis for mass transfer

Parameter	Mean transfer	Median transfer	Standard deviation	Minimum transfer	Maximum transfer
First Transfer	8.71	1.95	13.36	0.01	44.10
Log(First Transfer)	0.93	0.67	1.69	-4.61	3.77
Second Transfer	0.64	0.13	5.18	-18.32	69.75
Total Transfer	9.35	2.13	14.83	0.00	71.61
Log (Total Transfer)	1.00	0.76	2.61	-3.91	4.27
Mass Soil Before	2934.13	2861.08	761.19	1736.47	6360.50
Mass Soil After	2925.42	2848.43	753.12	1733.22	6321.90
Mass Soil After 2^{nd} Contact	2924.78	2857.47	752.39	1733.00	6316.20
Humidity (%)	28.94	25.50	9.24	15.10	48.25
Temperature (ºF)	77.77	77.70	.91	75.40	81.00

Note: (mg/ 8.97cm^2 area) for all soil types combined (n = 236): non-adjusted data.

Table 2. Means analysis for mass transfer by soil type

Soil Type	Measure	Mean transfer	Standard deviation	Minimum transfer	Maximum transfer
Sand	First Transfer	2.21	2.22	0.09	11.92
n=95	Log (First Transfer)	0.36	0.99	-2.41	2.48
	Second Transfer	0.64	7.23	-4.07	69.75
	Total Transfer	2.85	7.44	0.10	71.61
	Log (Total Transfer)	0.43	0.95	-2.30	4.27
	Mass Soil Before	2775.26	647.39	1959.96	3826.65
	Mass Soil After	2773.05	648.45	1952.94	3826.31
	Mass Soil After 2nd Contact	2772.41	647.92	1953.30	3826.01
Potting Soil	First Transfer	2.37	3.19	0.01	24.74
n=93	Log (First Transfer)	0.19	1.41	-4.61	3.21
	Second Transfer	-0.17	2.12	-18.32	4.84
	Total Transfer	2.20	2.24	0.00	12.22
	Log (Total Transfer)	0.27	1.20	-3.91	2.50
	Mass Soil Before	2599.26	406.05	1736.47	3732.09
	Mass Soil After	2596.89	405.65	1733.22	3731.46
	Mass Soil After 2nd Contact	2597.06	405.75	1733.00	3731.94
Clay	First Transfer	33.86	7.19	10.05	44.10
n=48	Log (First Transfer)	3.49	0.30	2.31	3.79
	Second Transfer	2.21	4.15	-2.71	27.60
	Total Transfer	36.07	7.95	11.80	57.70
	Log (Total Transfer)	3.55	0.29	2.47	4.06
	Mass Soil Before	3897.39	718.03	2752.70	6360.50
	Mass Soil After	3863.53	716.67	2716.10	6321.90
	Mass Soil After 2nd Contact	3861.32	716.51	2713.30	6316.20

Note: transfer expressed as mg/8.97 cm^2area, n=236, and non-adjusted data.

3.1 First Transfer

The mean transfer to cloth samples before log transformation for adherence of sand, potting soil, and clay combined from aluminum surface based for the First Transfer was 8.71 ± 13.3 mg/8.97 cm^2. The average sand, potting soil, or clay used initially on the aluminum surface was 2934.1 ± 761.2 mg. Kolmogorov-Smirnov test for normality again showed that the distribution of the outcome (sand/soil/clay transfer) was skewed ($p < 0.01$) warranting a log transformation. Transformation of the outcome (soil transfer) reasonably normalized the distribution. After log transformation, log mass transfer for First Transfer was on average 0.93 ± 1.7 mg/8.97 cm^2. Table 2, shows a breakdown for First Transfer before and after log transformation for the different soil types. In the univariate analysis for parameter effect on First Transfer, adherence of both soil sizes of sand and potting soil showed significance over clay in the model ($p < 0.0001$) as seen in Table 3. When soil sizes are separated for sand and potting soil, the mean log transfer for sand for soil size 139.7-381 µm was- 0.17 mg/8.97 cm^2 and for soil size of < 139.7 µm was 0.91 mg/8.97 cm^2, while for potting soil for soil size 139.7-381 µm was-0.31 mg/8.97 cm^2 and for soil size of < 139.7 µm was 0.70 mg/8.97 cm^2. Both types of soils show greater adherence for the smaller sized particles. Neither contact pressure nor contact time showed any effect on adherence for the soil types in the univariateanalysis.

Table 3. Univariate analysis

Effect	Mean for First Transfer over a 8.97 cm^2 area	Standard deviation	p-value	Mean for Total Transfer over a 8.97 cm^2 area	Standard deviation	p-value
Pressure (kPa)						
20	0.82	1.84	0.1706	1.00	1.76	0.4472
30	0.94	1.69	0.3335	0.85	1.77	0.2099
40	0.70	1.88	0.0769	0.93	1.61	0.3132
50	1.25	1.30		1.23	1.32	
Time in Seconds (s)						
10	0.84	1.64	0.7289	0.82	1.61	0.3793
30	1.00	1.80	0.8202	1.15	1.60	0.6926
50	0.94	1.66		1.04	1.64	
Soil Types Sand (139.7-381 µm)	-0.17	0.92	<0.0001	0.05	1.00	<0.0001
Sand (<139.7 µm)	0.91	0.72	<0.0001	0.82	0.71	<0.0001
Potting Soil (139.7-381 µm)	-0.31	1.52	<0.0001	-0.39	1.27	<0.0001
Potting Soil (<139.7 µm)	0.70	1.08	<0.0001	0.69	0.96	<0.0001
Clay	3.49	0.30		3.55	0.29	

Note: transfer expressed as mg/8.97 cm^2 area, for parameter (i.e.: pressure, time, and soil type) effects on 'First Transfer' and 'Total Transfer' based upon Lognormal values.

Table 4 shows the adjusted means (exponentiated back to the original units) for adherence for sand, potting soil, and clay, where adherence of both soil sizes of sand and potting soil again showed significance over clay in the multivariate analysis ($p < 0.0001$). Table 4 also shows the highest soil mass transfer of 3.86 mg/8.97 cm^2 for the contact pressure of 50 kPa in the multivariate analysis. No significance in the effect of contact time on mass transfer was seen in the multivariate analysis. Relative humidity in the chamber ($p < 0.0215$) and soil on the surface before ($p < 0.006$) were inversely associated with mass transfer when the soil types were separated by sand, soil and clay in the model. However, only relative humidity ($p < 0.0001$) showed an effect when the soil particles sizes for sand and potting soil were separated in the model.

Table 4. Adjusted means

Effect	Mean for First Transfer over an 8.97 cm^2 area	p-value	Mean for Combined Transfer over an 8.97 cm^2 area	p-value
*Pressure (kPa)*20	2.03	0.0005	2.48	0.0139
30	2.22	0.0026	2.01	0.0002
40	2.23	0.0016	2.78	0.0624
50	3.86		3.71	
*Time in Seconds(s)*10	2.40	0.7660	2.33	0.2029
30	2.57	0.8993	2.97	0.6150
50	2.52		2.77	
Soil Groups Sand (139.7-381 µm)	0.64	<0.0001	0.82	<0.0001
Sand (<139.7 µm)	2.05	<0.0001	1.68	<0.0001
Potting Soil (139.7-381 µm)	0.47	<0.0001	0.52	<0.0001
Potting Soil (<139.7 µm)	1.84	<0.0001	1.70	<0.0001
Clay	85.04		112.29	

Note: transfer expressed as mg/8.97 cm^2 area, from multivariate regression model for ‘First Transfer’ and ‘Second Transfer’.

In summarizing First Transfer, the highest mass transfers were seen for clay particles of 33.86 mg/8.97 cm^2 compared to 2.21 mg/8.97 cm^2 for sand and 2.37 mg/8.97 cm^2 for potting soil before any adjustment or particle size comparison were made (Table 2). Therefore, the adherence for clay was significantly different at over 15 times that of sand and over 14 times that of potting soil. After model adjustment for skewness, (i.e., reflecting a high degree of variability in the adherence values) this difference rose to 85.68 mg/8.97 cm^2 (8.53 mg/cm^2) for clay, compared to 1.18 mg/8.97 cm^2 (0.13 mg/cm^2) for sand and 0.89 mg/8.97 cm^2 (0.10 mg/cm^2) for potting soil (Table 4). Therefore, the adherence for clay was over 73 times that of sand and over 114 times that of potting soil. Clay particles, with their softer texture and fine composition, likely compress and adhere to surface and to adjacent clay particles producing higher transfers. Although, adherence of the smaller particle sizes for sand and potting soil tend to be greater than the larger particle sizes, the adherence is still well below that of the clay particles (see Table 4). A previous paper from the authors summarized soil and soil adherence studies from other researchers (Ferguson et al., 2009c). Those past studies have reported values ranging from 0 to 21 mg/cm^2, with most values skewed in the 0 to 1 mg/cm^2 range and so clay transfer tends to be in the higher range. Again, other soil-skin adherence studies in the field show large variability in experimental conditions, measurement techniques, and soil type(s) used, thus complicating true comparisons of adherence values.

Table 5. Analyses for parameter effects

Effect	Mean Transfer	Standard Deviation	Minimum	Maximum	p-value
*Pressure (kPa)*20	1.33	0.69	-0.04	2.70	0.2814
30	-0.03	0.71	-1.43	1.37	0.7706
40	0.89	0.68	-0.45	2.24	0.5030
50	0.26	0.68	-1.07	1.59	
*Time in seconds(s)*10	0.35	0.59	-0.81	1.51	0.2932
30	0.25	0.58	-0.90	1.40	0.2367
50	1.23	0.59	0.08	2.39	
*Soil Groups*Sand (139.7-381 μm)	1.70	0.87	-0.01	3.41	0.6255
Sand (<139.7 μm)	-0.62	1.05	-2.70	1.44	0.1868
Potting Soil (139.7-381 μm)	-0.16	0.98	-2.10	1.78	0.2493
Potting Soil (<139.7 μm)	-0.55	0.86	-2.24	1.14	0.1465
Clay	2.69	1.77	-0.80	6.19	

Note: transfer expressed as mg/8.97 cm^2 area, on 'Second Transfer' for clay, sand, and potting soil transfers in the Mixed Procedure.

3.2 Second Transfer

Table 5 shows the results for the soil mass transfer amounts after the second contact. Second Transfer following the initial transfer is quite variable with a transfer of 0.64 mg/8.97 cm^2 (std. dev. 7.22 mg/8.97 cm^2) for sand, a transfer of -0.17 mg/8.97 cm^2 (std. dev. 2.12 mg/8.97 cm^2), and a transfer of 2.21 mg/8.97 cm^2 (std. dev. 4.15 mg/8.97 cm^2) for clay. Again, clay adherence is the largest on average for all pressures and times. No significant difference was observed in the mixed procedure for the mass transfer across the soil types for Second Transfer (Table 5). Pressure and time of contact was also not shown to have a significant effect on the mass transfer for the second contact (all $p > 0.05$). Likewise relative humidity and temperature did not significantly affect the amount of transfer for the Second Transfer.

3.3 Total Transfer

Table 1 also shows summaries for Total Transfer across all soil types for 236 combined mass transfer experiments. The mean total transfer (i.e., mass transfer of soil after two sequential contacts) to cloth samples before log transformation for adherence of all soil types combined from aluminum surface was 9.35 ± 14.8 mg/8.97 cm^2. After log transformation, log mass transfer for Total Transfer was on average 1.00 ± 2.61 mg/8.97 cm^2. Table 2, also shows a breakdown for Total Transfer before and after log transformation for the different soil types. In the univariate analysis for parameter effect on Total Transfer, adherence of both soil sizes of sand and potting soil showed significance over clay in the model ($p < 0.0001$) as seen in Table 3. Again, both types of soil show greater adherence, even after the second transfer for the smaller sized particles. Table 4 shows the adjusted means (exponentiated back to the original units) for adherence for sand, potting soil, and clay after the second contact (i.e., total transfer), where adherence of both soil sizes of sand and potting soil again showed significance over clay in the multivariate analysis ($p < 0.0001$).

The overall change after model adjustment from First Transfer to Total Transfer, following a second contact was a 32% increase for clay, an 18% decrease for the smaller sand particles, but an increase of 28% for the larger sand particles, and a 7.6% decrease for the smaller potting soil particles, but a 10.6% increase for the larger potting soil particles (Table 4). Negative transfer can occur during the second contact and is feasible in human contact scenarios wheresoil can transfer back to the surface upon contact. Table 4 also shows the highest total soil mass transfer of 3.71 mg/8.97 cm^2 occurred for the higher pressure of 50 kPa. In the multivariate analysis, the lower adherence of 2.48 mg/8.97 cm^2 at 20 kPa, and 2.01 mg/8.97 cm^2 at 30 kPa showed significant difference in the model to the total mass soil transfer at 50 kPa of 3.71 mg/8.97 cm^2 ($p = 0.0139$, 0.0002, respectively). There was only moderate significance for the transfer at 40 kPa of 2.78 mg 8.97 cm^2 over the transfer at 50 kPa ($p = 0.0624$). No significance in the effect of time of contact on adherence for Total Transfer was seen in the multivariate analysis.

Similar to First Transfer, relative humidity in the chamber ($p < 0.0019$) and soil on surface before ($p < 0.0004$) were inversely associated with mass transfer amount when the soil types were separated by sand, soil, and clay in the model for Total Transfer. Again, chamber relative humidity ($p < 0.0001$) still showed an inverse effect on mass transfer amount when the soil particles sizes for sand and potting soil were separated in the model. This is an unexpected result, as higher relative humidity levels were previously associated with higher mass transfers when sand and potting soils were analyzed in previous papers by this group. Clay particles may be affecting results by clumping together on surfaces resulting in lower transfer to the cloth. Additional experiments are needed to determine the relationship between the air humidity, measurements of soil moisture content, and its effect on adherence to skin.

This paper presents a statistical analysis of the mass transfer of three soil types from an aluminum surface to cloth samples for a limited and comparable dataset along with new results of multiple contacts for clay transfers. Previous papers presented transfer of soil and sand to human cadaver skin (i.e., a better representative of live skin) and demonstrated greater mass transfers compared to cloth (Ferguson et al., 2008; Ferguson et al., 2009 a, b, c). It is expected, therefore, that mass transfer of clay particles to human skin will also be greater. There is limited information in the literature concerning the adherence of clays to the human skin, and the information here can be used to expand the dataset for clay adherence for single and multiple contacts.

4. Conclusion

In general, this study found higher transfer of clays to cloth, compared to sand and soil. Results also showed higher transfer for the smaller sized particles of both sand and potting soil. Time was not shown to have an effect in this combined analysis, whereas pressure and humidity had some effect. Additional experiments using the adherence chamber have also been conducted to look at transfer from aluminum and carpet surfaces for soil and sand and from both cloth and human cadaver skin (Ferguson et al., 2009 a, c). Previous results showed that humidity did increase the adherence of those soils to human skin but not to cloth, and that there was less adherence from carpet than from an aluminum surface. Readers are encouraged to explore these papers to look at additional nuances and difference between soil types and surfaces. Additional conditions of adherence can be further explored including mixtures of soil types, moisture conditions within the soil, and contact dynamics (e.g., sliding and rubbing). Adherence data is important for children playing on various soil types in and around the house, for the clay artisans who handle mixes containing ball clay, and for other occupations and hobbies where dermal contact is likely (e.g., lawn care and gardening), and can be used in a modeling analysis to estimate loading on skin after relevant exposure events.

References

Butte, W., & Heinzow, B. (2002). Pollutants in house dust as indicators of indoor contamination. *Reviews of Environmental Contamination and Toxicology, 175*, 1-46.

Canales, R. A. (2004). *The cumulative and aggregate simulation of exposure framework.*Unpublished doctoral dissertation, Stanford University, Stanford, CA.

Centers for Disease Prevention and Control (1992). *Impact of lead contaminated soil on public health.* Retrieved from http://wonder.cdc.gov/wonder/prevguid/p0000015/p0000015.asp

Dunn, W. (1993). Grip strength of children aged 3 to 7 years using a modified sphygmomanometer: Comparison of typical children and children with rheumatic disorders. *American Journal of Occupational Therapy, 47*, 421-428. http://dx/doi.org/10.5015/ajot.47.5.421

Engle, C. F., Cogger, C. G., & Stevens, R. G. (1991). Role of soil in groundwater protection. *Cooperative Extension Publications of Washington State University.* Retrieved from http://cru.cahe.wsu.edu/CEPublications/eb1633/eb1633.html (Feb. 17, 2012).

Environmental Protection Agency (EPA). (1989). Risk assessment guidance for superfund (Volume I) *in Human Health Evaluation Manual (Part A).* Retrieved from http://www.epa.gov/oswer/riskassessment/ragsa/

Ferguson, A., Bursac, Z., Coleman, S., Biddle, D., & Johnson, W. (2008). Soil-skin adherence from carpet: Use of a mechanical chamber to control contact parameters. *The Journal of Environmental Health, 43*(12), 1451-1458. http://dx/doi.org/10.1080/10934520802232253

Ferguson, A., Bursac, Z., Coleman, S., Biddle, D., & Johnson, W. (2009a). Comparisons of computer controlled chamber measurements for soil-skin adherence from aluminum and carpet surfaces. *Environmental Research, 109*, 207-214. http://dx/doi.org/10.1016/j.envies.2008.12.011

Ferguson, A., Bursac, Z., Johnson, W., & Davis, J. (2012). Computer-controlled chamber measurement for clay adherence relevantfor potential dioxin exposure through skin. *Journal of Environmental Science and Health, Part A, 47*(3), 382-388. http://dx.doi.org/10.1080/10934529.2012.646098

Ferguson, A., Coleman, S., Biddle, D., Bursac, Z., & Johnson, W. (2009b). In-vitro soil adherence for dermal exposure using a controlled mechanical chamber. *Journal of Applied Sciences Research, 5*(2), 232-243.

Ferguson, A., Coleman, S., Bursac, Z., & Johnson, W. (2009c). Computer controlled chamber measurements for multiple contacts for soil-skin adherence from aluminum and carpet surfaces. *Human and Ecological Risk Assessment, 15*(4), 811-849. http://dx.doi.org/10.1080/10807030903051283

Ferrario, J., Byrne, C., &Schaum, J. (2004). An assessment of dioxin levels in processed ball clay from the United States. *Organohalogen Compounds, 66*, 1639-1644.

Guo, W., Park, J. S., Wang, Y., Gardner, S., Baek, C., Petreas, M., & Hooper, K. (2012). High polybrominated diphenylether levels in California house cats: House dust a Primary Source? *Environmental Toxicology and Chemistry, 31*(2), 301-306. http://dx.doi.org/10.1002/etc.1700

Gurram, R., Gouw, G. J., &Rakheja, S. (1993). Grip pressure distribution understatic and dynamic loading. *Experimental Mechanics, 33*(3), 169-173. http://dx.doi.org/10.1007/BF02322568

Hendryx, M., Fedorko, E., & Halverson, J. (2010). Pollution sources and mortality rates acrossrural-urban areas in the United States. *The Journal of Rural Health, 26*, 383-391. http://dx.doi.org/10.1111/j.1748-0361.2010.00305.x

Hwang, H. M., Park, E. K., Young, T. M., & Hammock, B. D. (2008). Occurrence of endocrine-disrupting chemicals in indoor dust.*Science of the Total Environment, 404*(1), 26-35. http://dx.doi.org/10.1016/j.scitotenv.2008.05.031

Isikli, B., Demir, T. A., Akar, T., Berber, A., Urer, S. M., Kalyoncu, C., & Canbek, M. (2012). Cadmium exposure from the cement dust emissions: A field study in a rural residence. *Chemosphere, 63*(9), 1546-1552. http://dx.doi.org/10.1016/j.chemosphere.2005.09.059

Jarup, L. (2003). Land contamination and renal dysfunction. *Occupational and Environmental Medicine, 60*, 461-462. http://dx.doi.org/10.1136/oem.60.7.461

Johnson, W. M., Ferguson, A., Hager, P., Sanou, A., &Shenoda, D. (2006). Development of a data acquisition system for the measurement of residue transfer coefficient presented at the annual American Society for Engineering Education Conference and Exposition in Chicago, IL, Paper No. 2006-2429.

Johnson, W. M., Ferguson, A., Hager, P., Sanou, A., & Shenoda, D. (2007). Calibration of a pneumatic system for an apparatus for measurement of surface residue transfer coefficient. *American Society of Mechanical Engineers Early Career Technical Journal, 6*(5.1), 5.7.

Kuo, C. Y., Wang, J. Y., Yeh, C. J., Kuo, C. W., & Chou, H. L. (2010). Metal exposure for residents neardieseltransport routes. *Journal of Environmental Science and Health, Environmental Carcinogenesis & Ecotoxicology Reviews, 28*(1), 22-38. http://dx.doi.org/10.1080/10590500903585374

Link, L., Lukens, S., & Bush, M. A. (1995). Spherical grip strength in children 3 to 6 years of age.*American Journal of Occupational Therapy, 49*, 318-326. http://dx.doi.org/10.5014/ajot.49.4.318

National Center for Environmental Assessment. (2008). Anexploratory study: An assessment of modeled dioxin exposure in ceramic art studios. *U.S. Environmental Protection Agency,* Washington, DC, EPA/600/R-06/044F.

Nawrot T., Plusquin, M., Hogervorst, J., Roels, H. A., Celis, H., Thijis, L., & Staessen, J. A. (2006). Environmental exposure to cadmium and risk of cancer: A prospective population-based study. *Lancet Oncology, 7*(2), 119-1126. http://dx.doi.org/10.1016/S1470-2045(06)70545-9

Paustenback, D. J., Fehling, K., Scott, P., Harris, M., & Kerger, B. D. (2006). Identifying soil cleanup criteria for dioxins in urban residential soils: How have 20 years of research and risk assessments experience affected the analysis. *Journal of Toxicology and Environmental Health, Part B, 9*, 87-145. http://dx.doi.org/10.1080/10937400500538482

Roberts, J. W., Wallance, L. A., Camann, D. E., Dickey, P., Gilbert, S. G., Lewis, R. G., & Takaro, T. K. (2009). Monitoring and reducing exposure of infants to pollutants in house dust. *Reviews of Environmental Contamination and Toxicology, 201*, 1-39. http://dx.doi.org/10.1007/978-1-4419-0032-6_1

Robertson, A., & Dietz, J. (1988). A description of grip strength in preschool children. *American Journal of Occupational Therapy, 42*, 647-652. http://dx.doi.org/10.5014/ajot.42.10.647

Thorne, P. S., Cohn, R. D., May D., Arbes, S. J., & Zeldin, D. C. (2009). Predictors of endotoxin levels in U.S. housing.*Environmental Health Perspectives, 117*(5), 763-771. http://dx.doi.org/10.1289/ehp.11759

U.S Environmental Protection Agency (EPA). (2007). Pilot survey of levels of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, and mercury in rural soils of the U.S. environmental protection agency. *United States Environmental Protection Agency,* Washington, DC, EPA/600/R-05/043F

Zartarian, V. G. (1996). *Dermal exposure reduction model* (Unpublished doctoral dissertation). Stanford University, Stanford, CA.

Zartarian, V. G., Ozkaynak, H., Burke, J. M., Zufall, M. J., Rigas, M. L., & Furtaw, E. J. (2000). A modeling framework for estimating children's residential exposure and dose to chlorpyrifos via dermal residue contact and non-dietary ingestion. *Environmental Health Perspectives, 108*(6), 505-514. http://dx.doi.org/10.1289/ehp.00108505

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Plankton Distribution and Diversity in Tropical Earthen Fish Ponds

Gabriel Ujong Ikpi[1], Benedict Obeten Offem[1] & Irom Bassey Okey[1]

[1] Department of Fisheries and Aquatic sciences, Cross River University of Technology, Obubra Campus, Cross River State, Nigeria

Correspondence: Benedict Obeten Offem, Department of Fisheries and Aquatic sciences, Cross River University of Technology, Obubra Campus, Cross River State, Nigeria. E-mail: benbeff06@yahoo.com

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Abstract

Our knowledge of the consumption of plankton by fish is still fragmentary. Trophic links between fish and plankton are often loose because plankton is a changing assemblage of pelagic organisms of valuable nutritional value. The objectives of the study were to determine the distribution and diversity of planktons in the fish ponds. Water samples for plankton analysis were collected monthly (January-December), along with water samples used for the determination of physico-chemical parameters in 250 ml bottles in Ponds 1, 2 and 5. The freshwater sample was mixed gently and pipetted into 5 ml plankton chamber containing 2 drops of Lugol's solution. The Lugol's solution which served as fixative also enhanced sedimentation of the organisms and was left overnight before analyzing plankton using Zeiss inverted plankton microscope and plankton determination keys. Data was analyzed using Shannon-Weaner diversity index, species richness, means and analysis of varians (ANOVA). The results showed that the highest species richness for dry season phytoplankton value of 27 was recorded in Pond 2, with the highest Shannon-Weaner diversity index value of 0.5401 in pond 5. *Merismopaedia elegans* with 3,434 in pond 2 was the most abundant phytoplankton species and chlorophyceae the most dominant taxa. The rainy season zooplankton species richness values (5 in pond 2) were higher than that of the dry season (3 in pond 3) with the highest Shannon-Weaner index value of 0.3501 recorded in pond 1. The most abundant zooplankton species *Asplanchna* species 57 was recorded in pond 2. Significant differences ($p < 0.05$) was observed for phytoplankton and zooplankton species between the ponds. Physico-chemical parameters had influence on the distribution and diversity of planktons in the fish ponds.

Keywords: plankton diversity, earthen fish ponds, tropical, physicochemical parameters

1. Introduction

The aquatic environment supports various communities of living organisms. They constitute the biotic load of a pond. Plankton designates the community of pelagic organisms, composed of various groups, which are in suspension in water and hence restricted mobility, often less than that of the water which carries (Declince, 1992). Plankton is divided into phytoplankton or photosynthetic organisms and zooplankton or heterotrophic organisms. The spectrum of phytoplankton found in ponds, consist of a large number of taxa. According to cell size, the following groups can be distinguished: picoplankton (from 0.02 to 0.2 μm) nanoplankton (from 0.2 to 2.0 μm) (Sieburth et al., 1978). Organisms over 20 μm in size are called netplankton. Picoplankton and nanoplankton, often ignored in plankton studies can contribute significantly to primary productivity, often in excess of what their biomass would suggest (Hardy et al., 1986; Stockner & Antia 1986; Stockner, 1988).

Zooplankton distribution within ponds is non-homogenous. In Dutch fish ponds, some species were mainly found in the littoral, while others selected limnetic waters (Verreth, 1990). Such distributions are probably related to food availability and avoidance of predators. Distribution varies with time, due in particular, to vertical migration, which is an almost universal phenomenon. The archetypical form of vertical migration is a swimming movement to deep water during the day and an active ascent towards the surface during the night (Declince, 1992). This diel movement from the warm, food-rich surface layers to the cold nutritionally poor deep waters has evolved in response to benefits to the community: (1) vertical migration results in predator avoidance by zooplankton; (2) fluctuations of the grazing pressure owing to vertical migration enables unimpeded growth of the algae during daytime. This results in higher rates of algal production, compared to those obtained in conditions where zooplanktons graze continuously on the same algal biomass. Zooplankton is subdivided, according to size, into microzooplankton, mesozooplankton and macrozooplankton. Microzooplankton are the smallest organisms, consisting of flagellates, ciliates and rotifers, mostly detritivorous and bactivorous, though rotifers also consume small algae. Mesozooplankton is comprised of cladocerans and macrozooplankton of copepods (or cladocerans and copepods). The dietary range of zooplankton organisms is often wider (Declince 1992). The objectives of the study is to determine the distribution and diversity of planktons in the fish ponds.

2. Materials and Methods

2.1 Description of Study Area

The Cross River University of Technology, Obubra Campus is located at Ovonum village of Obubra Local Government Area in the Central Senatorial District of Cross River State, Nigeria. The campus stands on a dissected Plateau which is bounded in the north by Ebonyi State, in the south by Ababene and Ofat Villages, in the east by Ofatura and Apiapum Villages and in the west by Ofodua Village all in Obubra Local Government Area.The latitude and longitude locations are 6^o and 8^o15'E approximately. It is almost equidistant between the north and southern ends of the state and 156km from Calabar the State capital.

2.2 Plankton Analysis

Water samples for plankton analysis were collected monthly (January to December), along with water samples used for the determination of physico-chemical properties, in a 250ml bottles, as in Ajani (2001). The water samples collected from ponds 1, 2 and 5 were taken to the Fisheries laboratory of Cross River University of Technology at Obubra Campus, for plankton analysis. Fresh water sample was mixed gently and pipetted into 5ml plankton chamber containing 2 drops of Lugol's solution. The Lugol's solution served as the fixative while also enhancing sedimentation of the organisms. The chamber was left overnight for complete sedimentation to take place. Plankton was analyzed using Zeiss inverted plankton microscope by the method described in UNESCO (1978), using plankton determination keys from Edmonton (1959), Prescott (1970), and Sharma (1986)

2.3 Data Analysis

Mean and standard deviation of each of the physico-chemical parameters were calculated. Analysis of variance (ANOVA) was used to test for statistical differences between the means of the physical and chemical parameters of the fish ponds. Diversity index which include Shannon-Weaner diversity index and species richness was used to determine the plankton species composition and their diversity across the different ponds sampled. Shannon-Weaner's Index (H) is commonly used to characterize species diversity in a community. Shannon-Weaner's Index accounts for both abundance and evenness of the species present. The proportion of species is relative to the total number of species (*Pi*) is calculated and then multiplied by the natural logarithm of this proportion (LnPi). The resulting product is summed across species and multiplied by Equation 1:

$$\mathrm{H} = -\sum_{i=1}^{s} Pi \mathrm{Ln} Pi \tag{1}$$

3. Results

There was no significant difference in the values of the physico-chemical parameters of the three sampling sites (Table 1).

Table 1. Mean (± SD) values of some physical and chemical characteristics of the three fishponds

Parameters	Pond 1	Pond 2	Pond 3
Surface Temperature (ºC)	27.7 ± 1.71 a	27.2 ± 2.11 a	27.5 ± 1.12 a
Turbidity(NTU)	34.2 ± 2.32 a	32.5 ± 3.12a	33.4 ± 7.23a
Ph	6.8 ± 0.66a	6.8 ± 0.12a	6.8 ± 1.81a
Dissolved Oxygen($mg.l^{-1}$)	4.8 ± 1.34a	4.9 ± 0.12a	4.8 ± 1.34a
Alkalinity ($mg.l^{-1}$)	41.2 ± 4.42a	40.8 ± 5.43a	41.5 ± 4.34a
Conductivity ($\mu S.cm^{-1}$)	32.4 ± 4.23a	31.4 ± 2.32a	31.7 ± 3.6a
BOD ($mg.l^{-1}$)	1.3 ± 0.66a	1.6 ± 0.84a	1.5 ± 0.62a
Phosphates (mg/l)	2.5 ± 0.01a	2.5 ± 0.04a	2.4 ± 0.12a

Calcium (Ca++)	10.4 ± 3.4a	9.3 ± 2.87a	10.4 ± 1.83a
Magnesium (Mg++)	1.6 ± 0.22a	1.1 ± 0.54a	1.2 ± 0.99a
Total iron	1.4 ± 0.56a	0.7 ± 0.43a	1.9 ± 0.36a
Silicon (SiO_2)	28.9 ± 1.89a	28.4 ± 5.34a	27.8 ± 9.65a
Sulphates (SO_4^{-2})	11.4 ± 1.23a	12.8 ± 2.88a	11.8 ± 3.31a
Nitrates (NO_3^-)	0.2 ± 0.18a	0.3 ± 0.14a	0.2 ± 0.16a
Chlorides (Cl^-)	10.6 ± 2.99a	11.2 ± 2.21a	11.0 ± 2.71a
Total hardness	55.5 ± 8.88a	54.3 ± 6.54a	56.0 ± 2.56a
Total solids (mg/l)	66.6 ± 5.34a	67.6 ± 3.76a	68.3 ± 5.30a
Total Dissolved solids (mg/l)	58.8 ± 7.54a	57..5 ± 4.66a	57.2 ± 6.41a

Values in the same rows with the same superscript are not significantly different ($p > 0.05$).

Seasonal variation was observed in the values of physico-chemical properties of the ponds with some chemical properties like TS, TDS, TH and DO, showing higher values during the wet months, while others like PO_4, NH_3 and BOD were higher during dry (Table 2).

Table 2. Plankton Diversity in Earthen Fish Ponds of Cross River University of Technology at Obubra Campus for Dry Season (December-April, 2008)

Fish Pond	POND 1	POND 2	POND 3
Plankton Species			
1. Phytoplankton			
a. Chlorophyceae			
Akinstrodesmus falcatus	151	162	75
Centritractus belanophorus	-	81	-
Ceriasteris irregular	-	70	-
Chlamydomonas ehrenbergii	128	23	34
Chlorella ellipsoidea	3,364	372	113
Euastrum pectinatum	-	186	25
Eudorina elegans	-	23	-
Micrasterias Americana	-	11	-
Oocystics species	-	81	27
Pediastrum simplex	-	11	31
Scenedesmus quadricuada	-	35	-
Scenedesmus species	-	220	-
Selenastrum gracile	-	58	17
Spinuclosterium curvatum	11	418	-
Staurastrum rotula	-	104	-
Tetraedron regulare	46	128	-
Ulothrix cylindricum	-	81	-
Volvox tertius	-	11	-
b. Bacillariophyceae			
Navicula pettersenii	-	58	5
Nitzchia species	-	35	-

c. Cyanophyceae			
Anabeana spiroids	-	23	-
Merismopeadia elegans	-	3,434	71
Oscillatoria species	-	35	5
d. Euglenophyceae			
Euglena rubra	70	255	12
Lepocynlis acuta	35	-	-
Trachelomonas volvocina	754	534	128
Trachelomonas ampula	23	-	37
Trachelomonas horida	11	-	-
e. Dinophyceae			
Gymnodinium palustre	-	116	46
f. Cryptophyceae			
Cryptomonas erosa	139	23	15
Species richness	11	27	15
Shannon Weaner diversity index	0.5401	0.2954	0.1156
2. Zooplankton			
a. Rotifera			
Chromogaster species	11	11	7
Euchlanis species	-	-	-
Asplanchna species	-	-	5
b. Ciliata			
Strombidium viride	23	11	-
Euplotes species	-		3
Species richness	2	2	3
Shannon Weaner diversity index (H)	0.5623	0.5	0.3689

3.1 Plankton

30 species of phytoplankton belonging to 6 families were recovered from the ponds during study period. The largest family was Chlorophyceae contributing 18 species, followed by Euglenophyceae with 5 species and the least were Dinophyceae and Cryptophyceae contributing one species each. The zooplankton had 5 species belonging to 2 families; Rotifera and Ciliata. The rotifers consist of *Chromogaster*, *Euchlanis* and *Asplanchna* species while the ciliates had *Strombidium viride* and *Euplotes*.

The occurrence of plankton species showed that values for phytoplankton were significantly higher during the dry season than the wet for most of the species (Figure 1) while the opposite was the case for zooplankton values (Figure 2).

3.2 Plankton Diversity in Dry Season

Phytoplankton richness index was highest in Pond 2 (27) and least in pond 1 (11) but Shannon-Weaner's diversity index showed highest values (0.5401) in Pond 1 and lowest in Pond 3 (Table 3). Richne index for zooplankton was low in all the ponds with no significant differences ($P > 0.05$) in Shannon-Weaner diversity values.

3.3 Plankton Diversity for Rainy Season

Wet season Phytoplankton richness (8, 24 and 13) and diversity values (0.4330, 0.3042 and 0.0943) were low in all ponds (1, 2, 3) respectively. On the other hand, zooplankton richness (4 for pond 1, 5 for pond 2 and 4 for pond 5) and Shannon-Weaner values for pond 1 (0.3501), Pond 2 (0.2409) and Pond 3 (0.2966) were significantly higher than dry.

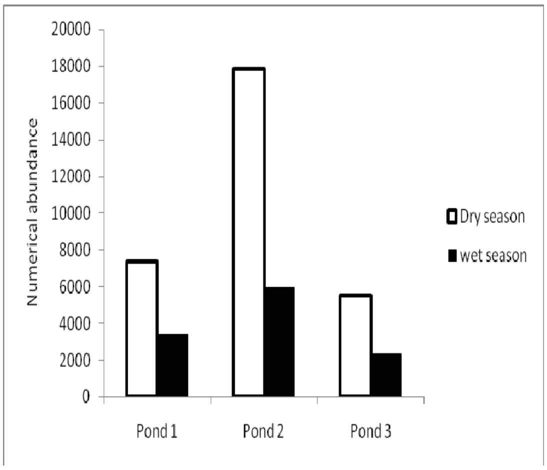

Figure 1. Abundance of phytoplankton during wet and dry seasons

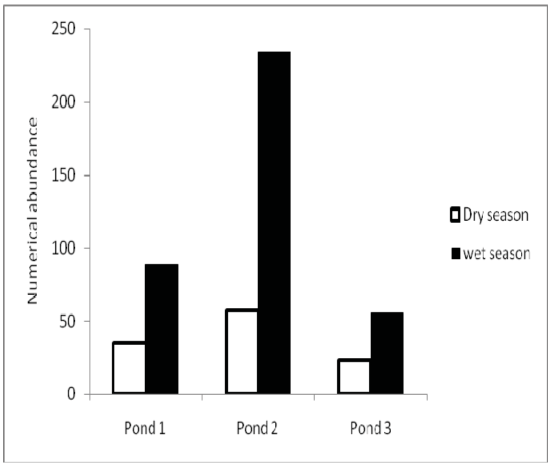

Figure 2. Numerical abundance of zooplankton during dry and wet season

4. Discussion

Physico-chemical parameters of water body serve as measure of water quality (Offem, 2008). Changes in the intensity of rainfall affects physico-chemical parameters of water which also affects biomass of aquatic organisms (Adebisi, 1981). The higher turbidity, salinity, TDS and most of the physico-chemical properties in the wet season had been explained to be as a result of flooding during heavy rainfall and run-off from nutrient rich agricultural lands increased (Ayodele & Ajani 1999; Offem, 2008). Lower dissolved oxygen values obtained during dry season could probably be due to higher temperatures during the season (Akpan & Offem, 1993a) while high levels of dissolved oxygen during the wet season could be due to lower water temperature and increased aeration due to agitation by wind in the wet season (Ochang et al., 2005). High organic enrichment of ponds during the dry season may be responsible for such low oxygen values that might have increased ionic substances such as nitrate, chloride and phosphate from fertilizers (Offem, 2008).

The Composition of phytoplankton community of the ponds agreed with reports that blue-green algae and green algae dominate most tropical water bodies (Adebisi, 1981; Ayodele & Ajani, 1999). Increased temperature, sunlight and tropholytic activities as a result of low water level coupled with frequent movement of water from the deep, nutrient-rich sediments into the tropholytic zone, increased the abundance of phytoplankton during dry season. Dominance of *Chlorophyceae* in the ponds in the dry season had been attributed to the presence of sunshine and extensive catchment area draining phosphate rich agriculture land (Uttah, 2008; Kurasawa & Shiraishi, 1954). Also flexibility in the Physiology and behaviour of Chlorophyceae can tolerate environmental changes better than other species (Silva, 2004). The observation in the ponds agree with findings by Utah (2008) that the phytoplankton biomass increase with increase in transparency, frequently associated with dry season, while the high turbidity frequent with the wet season results in a reduction of biomass. It was also reported in ponds in Asia, where phytoplankton scarcity can be observed during the wet months (Silva, 2004) and most of the ponds in India where three plankton pulses occur within the dry season. During the wet months flushing disturbs the standing crop of plankton. However, when the destabilising effect reduces, the accumulated nutrient input favours an increased plankton production during the dry.

The high population density of the zooplankton during the rainy season may be as a result of abundant food source from run-off through nutrient-rich agricultural lands. It has been reported that increase in primary production (phytoplankton), is accompanied by increase in zooplankton abundance (Rocha et al., 1999). Also, it had been observed that zooplankton abundance frequently reach their peak during the wet season in ponds (Muylaert et al., 2003). Besides food sources, low predation rate by fish during the wet season caused by their increased breeding activities could encouraged high population density of the zooplankton.

The zooplankton population dominated by rotifers in the wet season and ciliates in the dry season in all the ponds was similar to earlier findings (Egborge, 1981b). The alternation in abundance between rotifers and ciliates as shown in the seasonal distribution and abundance of zooplankton in the sampled ponds was regarded as a booster of all year round food for fish in the lake (Egborge, 1981b). This study showed that the seasonal variation in zooplankton concentration could largely be due to the Rotifera which normally constitute major diet items of larger zooplankton.

5. Conclusion

The study has shown that phytoplankton were more abundant during the dry season than the wet season, while occurrence zooplankton was more during the wet season than the dry. There was seasonal variation in the physico-chemical parameters of the pond water which in turn influenced the distribution and abundance of phytoplanktons and zooplanktons.

Reference

Adebisi, A. A. (1981). The physico-chemical hydrology of tropical seasonal river upper Ogun River. *Hydrobiologia, 79*, 157-165. http://dx.doi.org/10.1007/BF00006123

Ajani, E. K. (2001). Effects of biotic and abiotic components of the habitat on fish productivity in Lagos Lagoon, Nigeria Ph.D Thesis University of Ibadan. p. 277.

Akpan, E. R., & Offem, J. O. (1993a). Seasonal variation in water quality of the Cross River State Nigeria *Hydrobiologia, 26*, 95-103.

Ayodele, I. A., & Ajani, E. K. (1999). *Essentials of fish farming (aquaculture)*. Odufuwa publishers, Ibadan.

Declince, G. (1992). The ecology of the fish pond ecosystem. (pp. 127-187). Kluwer Academic Publishers. http://dx.doi.org/10.1007/978-94-017-3292-5_4

Egborge, A. B. M. (1981b). The composition, seasonal variation and the distribution of zooplankton in Lake Asejire, Nigeria. *Revue de Zoologie Africaine, 95*, 136-144.

Edmonton, W. T. (1959). *Freshwater biology* (2nd ed.) (p. 1248). New York: John Wiley and Sons.

Hardy, F. J. Shortreed, K. S., & Stockner, J. G. (1986). Bacterioplankton, phytoplankton and zooplankton communities in a British Columbia Coastal Lake before and after nutrient reduction. *Can J. Fish Aquat. Sci., 43*, 1504-1514. http://dx.doi.org/10.1139/f86-188

Kurasawa, H., & Shiraishi, Y. (1954). Studies on the biological production of Lake Suwa. *Research Institute of Natural Resources, 33*, 22-57.

Mustapha, M. K. (2009). Zooplankton assemblage of Oyun Reservoir, Offa, Nigeria. *Nig. J. Aquat. Sci., 9*, 1-14.

Muylaert, K. S., Declerck, V., Geenens, J. V., Wichelen, H., Deegans, J., Vandekerkhove, K. V., … Vyverman, W. (2003). Zooplankton, phytoplankton and the microbial food web in two turbid and two clear shallow lakes in Belgium. *Aquat. Ecol., 37*, 137-150. http://dx.doi.org/10.1023/A:1023988702926

Ochang, S. N., Ayotunde, E. O., & Okey, I. B. (2005). Some aspects of the physico-chemical and biological properties of Cross River, an inland water body in South-Eastern Nigeria. *Glob. J. Agric. Sci., 4*(2), 139-148.

Offem, B. O., Akegbejo- Samsons, Y., Omoniyi, I. T., & Ikpi, G. U. (2008). Dynamics of the limnological features and diversity of zooplankton populations of the Cross River System SE Nigeria. *Knowledge and Management of Aquatic Ecosystems, 393*, 2-19.

Prescott, G. W. (1970). *How to know freshwater algae* (p. 348). W.M.C. Brown Company Publishers.

Rocha, O., Matsumura-Tundisi, Y. T., Espindola, E. L. G., Roche, K. F., & Rietzler, A. C. (1999). Ecological theory applied to reservoir zooplankton, In J. G. Tundisi & M. Straskraba (Eds). *Theoretical reservoir ecology and its applications* (pp. 29-51). International Institute of Ecology, Brazilian Academy of Sciences. Backhugs publishers, Leiden, Holland.

Sharma, O. P. (1986). *Text book of algae* (p. 396). New Delhi: TATA Mcgrawhill publishing Comp. Ltd.

Sieburth, J., Mc, N., Smetacek, V., & Lenz, J. (1978). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol Oceanogr, 23*, 1256-1263. http://dx.doi.org/10.4319/lo.1978.23.6.1256

Silva, E. I. L. (2004). Phytoplankton Characteristics, Trophic Evolution and Nutrient Dynamics in an Urban Eutrophic Lake: Kandy Lake in Sri Lanka. In M. V. Reddy (Ed.), *Restoration and Management of Tropical Eutrophic Lakes* (pp. 219-260). New Delhi: Oxford and IBH Publishing.

Stockner, J. G. (1988). Phototrophic picoplankton: an overview from marine and freshwater ecosystems. *Limnol. Oceanogr, 33*, 765-775. http://dx.doi.org/10.4319/lo.1988.33.4_part_2.0765

Stockner, J. G., & Antia, N. J. (1986). Algal Picoplankton from marine and freshwater ecosystem: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci., 43*, 2472-2503. http://dx.doi.org/10.1139/f86-307

UNESCO. (1978). Phytoplankton manual (p. 337). Paris: UNESCO.

Uttah, E. C., Uttah, C., Akpan, P. A., Ikpeme, E. M., Ogbeche, J., Usip, L., & Asor, J. (2008). Bio-survey of plankton as indicators of water quality for recreational activities in Calabar River, Nigeria. *J. Appl. Sci. Environ. Manage, 12*(2), 35-42.

Verreth, J. (1990). The accuracy of population density estimates of a horizontally distributed zooplankton community in Dutch fish ponds. *Hydrobiologia, 203*, 53-61. http://dx.doi.org/10.1007/BF00005613

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Formation and Characterization of Paint Based on Alkyd Resin Derivative of *Ximenia americana* (Wild Olive) Seed Oil

Gabriel O. Oladipo[1], Ighodalo C. Eromosele[1] & Olujinmi M. Folarin[2]

[1] Department of Chemistry, Federal University of Agriculture, P. M. B. 2240, Abeokuta, Nigeria

[2] Department of Chemical Sciences, Ondo State University of Science and Technology, P. M. B. 353, Okitipupa, Nigeria

Correspondence: Olujinmi M. Folarin, Department of Chemical Sciences, Ondo State University of Science and Technology, P. M. B. 353, Okitipupa, Nigeria. Tel: 234-806-253-0595. E-mail: mofedara@yahoo.com

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Abstract

Alcoholysis-polyesterification method was used in the investigation of utilization of *Ximenia americana* seed oil in the formulation of alkyd resins. The extracted oilseed gave an oil yield of 45.7% (w/w) and the physicochemical characterization indicated it is semi-drying in nature with iodine value of 152.3 g I_2 100 g^{-1}. The fatty acid analysis of the oil carried out earlier revealed oleic and linolenic as dominant fatty acids, having values of 72.1 and 10.3% respectively. Four grades of alkyds were formulated at 40% (I), 50% (II), 60% (III) and 70% (IV) oil lengths. The reaction progression was followed by determining the acid value of the aliquots for the reaction mixture at various time intervals beside the volume of water evolved. Kinetic studies showed that the reaction was second-order with values of 3.12×10^{-4}, 2.44×10^{-4}, 2.3×10^{-4} and 2.28×10^{-4} g (mg KOH)$^{-1}$ min^{-1} for samples I, II, III and IV respectively. The extent of reaction (% P_{av}) determined at the initial stage of reaction ranged from 63.3 to 78.6%, indicating an appreciable degree of conversion. The corresponding average degree of polymerization (D_p) ranged from 2.7 to 4.7 and this indicated the formation of high molecular weight alkyd. Characterization of the alkyd gave iodine value of 70.1 $\pm$ 0.74, 76.1 $\pm$ 1.05, 81.2 $\pm$ 1.0 and 86.3 $\pm$ 0.8g$I_2$100g^{-1} for samples I, II, III and IV respectively. The corresponding saponification values were 297.5 $\pm$ 1.5, 332.5 $\pm$ 1.2, 378 $\pm$ 1.1 and 420.2 $\pm$ 1.3 mg KOH g^{-1}. The 40 and 50% oil length alkyd films were resistant to acid, brine and water with little resistance to alkali. Characterization of paint based on two alkyd resin derivatives, 40 and 50% oil lengths, gave values for surface drying and tack-free property of less than 2 and 5 hours respectively in respect of the 40% oil length.

Keywords: alkyd resin, polymer binder, condensation, polymerization, alcoholysis

1. Introduction

Paints could be described as colloidal mixture of chemical substances which when spread over a surface in a thin layer, form a solid, cohesive and adherent film. They are used in our daily life for decorative purposes as well as for protecting surfaces against various environmental effects like UV-radiation, chemical invasion and mechanical stresses. A conventional paint consists of binder, pigment, solvent and additives. The polymer-binding material (alkyd resin) with a large extent of variations is responsible for the formation of continuous film that adheres to the substrate and holds the other substances together (Wicks et al., 1994).

The preparation of alkyd resins is carried out by condensation polymerization of polyhydric alcohol (e.g. glycerol) and polyfunctional acid or polybasic acids (e.g. phthalic anhydride and isophthalic acid) modified by fatty acid or their triglyceride. The alkyd resins produced this way are referred to as oil-modified alkyd resins and contribute about 70% to the conventional binders used in surface coating (Bajpai & Seth, 2000). They determine the performance quality of surface coatings such as the rate of drying, gloss, durability of the dry film and resistance of the dry film to abrasion and chemicals. However, classification of alkyd resins is based on the oil length and oil type (Micciche, 2005). The vegetable oils used in oil-modified alkyd resins are usually extracted either by mechanical press or solvent extraction (Abulude et al., 2007). The natural oil in the oil-modified alkyds reacts with atmospheric oxygen leading to the formation of network of polymers cross-linked through the C = C bond. The oxidative drying of the oil brings about the formation of film that shows improved properties with drying time, hardness or water resistance (Akintayo, 2004). The oils used in surface coatings contain linolenic and conjugated acid groups, such oils include linseed, perilla and tung oils and possess pronounced drying abilities (Aigbodion et al., 2003).

There has been tremendous increase in the demand for alkyd resin production for use in the Nigerian surface coating industry due to the rapid growth of the economy. Consequently, large quantities of oil are needed for the production of alkyds. Hence, there is need to investigate indigenous oilseeds in order to find other sources of drying oils with comparable properties to those currently being imported for resin production for paint formulation; notably *Ximenia americana* seed oil (XASO).

Ximenia americana (Linn) is a shrubby tropical plant of the family Olacaceae. It grows in many tropical African countries such as Nigeria, Senegal, Sudan and Angola (Storrs, 1995) to a height of about 5m on the average. It produces a small, yellow plum fruits which are edible with oily seed kernel. It grows in the savannah region of Nigeria and has different local names: Hausa-Tsada; Fulani-Chabbuli; Tiv-Anomadze; Yoruba-Igo (Keay, 1989). In this work, *Ximenia americana* seed oil had been examined via physicochemical analyses for its practical application as alkyd resin base material for industrial paint formulation.

2. Materials and Methods

The seeds of *Ximenia americana* (Wild Olive) plant were collected in Yola, Adamawa State, Nigeria. The seeds were shelled by cracking to remove the embryos. The embryos were sun-dried for two weeks before milling into granular form. Oil was extracted from the seeds with petroleum ether (40-60 ºC) using soxhlet extraction method (Eromosele et al., 1994). The aliquot of the extract obtained was freed from petroleum ether by evaporation in a rotary evaporator to yield the oil. The physicochemical properties of *Ximenia americana* seed oil were assayed using the American Oils Chemists Society Methods (AOCS, 1996).

Table 3 shows the formulation of four grades of alkyd resin prepared with glycerol, phthalic anhydride and *Ximenia americana* seed oil. Monoglyceride of each grade was first prepared by heating a mixture of oil, glycerol and small quantity of CaO (Catalyst) in a 2-litre three necked round bottom flask fitted with a Dean and Stark having necessary accessories that will allow the reaction progress (Aigbodion & Okieimen, 1996). This mixture was heated by heating mantle to 200 ºC and maintained at this temperature for 2 hours after which a small quantity of the aliquot was taken to check for solubility in methanol indicating formation of the monoglyceride. At the onset of the second phase, the temperature was lowered to about 180 ºC and measured quantity of phthalic anhydride was added, followed by addition of xylene (10% of total weight charged) into the reaction mixture to remove the water of esterification by forming an azeotrope (Onukwli & Igbokwe, 2008; Ikhuoria et al., 2007). The temperature was increased to 230-250 ºC while the reaction lasted. Aliquots were withdrawn from the reaction mixture at intervals of 30 minutes to determine drop in acid value and volume of water evolved. The reaction was stopped when the acid value attained the value of about 10 mg KOH g^{-1} and the alkyd resin allowed to cool. The acid value of in-process samples taken at intervals were determined by titrating with a 0.1M KOH solution to the phenolphthalein end point after dissolution in a mixture of toluene and ethanol (1:1) (Bobalek et al., 1964). The acid value was related to the extent of the reaction P_{av} and the average degree of polymerization D_p thus:

$$P_{av} = \frac{(C_0 - C_t)}{C_0}, \quad D_p = (1 - P_{av})^{-1}$$

The rate equation for the reaction is expressed as: $(1 - P_{av})^{-1} = C_0(kt + 1)$

where C_o is the initial acid value, C_t is the acid value after time, t, of the reaction, k is the rate constant and t is the time of reaction.

Some physicochemical properties (colour, iodine value, saponification value and acid value) of the alkyd resins prepared were determined using standard method (AOCS, 1996). The alkyd samples were thinned to 50% with xylene and applied on thin glass panels at room temperature (Ikhuoria et al., 2007). The drying schedule of the sample in terms of time to set-to-touch and drying through was determined. The resistance of the dried films to different solvent media was also determined (ASTM, 1994).

The gloss paint formulation is shown in Table 10. The first six ingredients were mixed thoroughly using high speed shearing technique for about 30 min. The premix base was then subjected to a milling process to produce a refined dispersion. The remaining ingredients of the formulation were then added under controlled conditions to make up the overall volume (Essien et al., 2012). The paint produced was stored in a container.

3. Results and Discussion

The physicochemical properties of *Ximenia americana* seed oil is shown in Table 1. The acid value of 16.13 mg

KOH g^{-1} suggests low in-vivo lipolytic activities in the seed, and indicates a good non-degraded state of the oil and falls within limits for industrially useful oils (Oyedeji & Oderinde, 2006). However, it is high in comparison with the values for linseed oil and low comparing with rubber seed oil i.e. 1.0 mg KOH g^{-1} and 19.18 mg KOH g^{-1} respectively (Majumder, 1990; Ikhuoria & Okieimen, 2005). Williams (1966) reported that low acid values for oils are useful in the manufacture of paints and varnishes. The free fatty acid concentration of 8.07% for *Ximenia americana* is high, consistent with the high acid value observed. The saponification value of the XASO is 178.5 mg KOH g^{-1} and is lower in comparison with the corresponding values for rubber seed, castor seed and linseed oils i.e. 181.14, 182 and 185-194 mg KOH g^{-1} respectively (Onukwli & Igbokwe, 2008; Majumder, 1990; Ikhuoria & Okieimen, 2005). Eromosele et al. (1994) reported the saponification value of *Ximenia americana* seed oil as 182.3 mg KOH g^{-1}. This suggests that the seed oil may be suitable for preparation of soap. The iodine value of 152.28 g I_2 100 g^{-1} is comparable to the one for rubber seed 136.2 g I_2 100 g^{-1} (Ikhuoria & Okieimen, 2005). Also, Folarin et al. (2011) reported the value of *Ximenia americana* seed oil as 158.30. By this value, *Ximenia americana* seed oil is semi-drying although it has much lower value than that of linseed oil (188 g I_2 100 g^{-1}) (Majumder, 1990). Semi-drying oils can be used for synthesis of alkyd resins, formulation of liquid soap and hair shampoo while non-drying oil is suitable for use as plasticizer (Fasina, 1989). The seed oil appeared highly susceptible to oxidative rancidity, it has peroxide value of 31.25 mEq kg^{-1}. The viscosity of the seed oil is 900 cp, this indicates that the oil is highly viscous at ambient temperature. The specific gravity is 0.912 g cm^{-3}, it is the lowest in comparison with linseed oil, crude castor seed oil and rubber seed oil having values of 0.93-0.98, 0.93 and 0.93 g cm^{-3} respectively (Onukwli & Igbokwe, 2008; Ikhuoria et al., 2007; Majumder, 1990). The refractive index of the seed oil is 1.435, it is lower comparing with the range of refractive indices of drying oils which are comparatively narrow from 1.46 to 1.54 and this suggests that it can be used as a test for purity and as an aid to identification (BS 6900).

Table 1. Physicochemical properties of *Ximenia americana* seed oil (XASO) compared with castor seed oil, rubber seed oil and linseed oil

Properties	XASO	Castor seed oil*	Rubber seed oil**	Linseed oil***
Colour	Dark	-	Brown	Yellow
Oil yield (%)	45.7	44.69	-	-
Specific gravity (g cm^{-3}, 30 ºC)	0.912	0.93	0.93	0.931- 0.98
Refractive index (30 ºC)	1.467	-	1.477	1.479-1.48
Viscosity (cp, 30 ºC)	900	-	-	-
Saponification value (mg KOH g^{-1})	178.5	182.0	181.14	185.0-194.0
Iodine value (g $I_2$100 g^{-1})	152.28	82.8	136.2	188.0
Acid value (mg KOH g^{-1})	16.13	2.89	19.18	1.0
Free fatty acids (%)	8.07	1.45	9.54	-
Peroxide value (mEqkg^{-1})	31.25	6.7	-	-
Average molecular weight (g mol^{-1})	314.34	307.6	-	-

Onukwli and Igbokwe (2008)*, Ikhuoria & Okieimen (2005)**, Majumder (1990)***.

The fatty acid composition of *Ximenia americana* seed oil is shown in Table 2. Previous results (Eromosele & Eromosele, 2002; Folarin et al., 2011) indicate that the seed oil contains twelve fatty acids of which nine are unsaturated, yielding a total unsaturation of 92.92% and this is slightly higher than that of linseed oil (91.3%) (Tarandjiiska et al., 1996). The concentrations of the dominant fatty acids are 72.09 and 10.31% for oleic and linolenic acid respectively. The oil may have nutritional value due to the presence of essential fatty acids such as linoleic (1.34%), linolenic (10.31%) and arachidonic (0.60%). Linoleic acid in particular plays an important role in the biosynthesis of prostaglandins (Al-Jassir et al., 1995). The level of linolenic acid is significant, albeit a factor of five less than the value in linseed oil (Tarandjiiska et al., 1996). However, the presence of erucic acid in the oil makes toxicological analysis imperative to ascertain its edibility. From the level of unsaturation, its distribution and the presence of fatty acids with up to four double bonds, *Ximenia americana* seed oil appears to be a suitable base material for alkyd resin synthesis and paints formulation. Furthermore, the presence of stearic acid shows that the oil could be used for pharmaceutical preparations (Okieimen & Eromosele, 1999).

Table 2. Fatty acid composition of *Ximenia americana* seed oil

Fatty acid	Composition (%)
Caprylic	0.55
Palmitic	3.31
Stearic	3.47
Oleic	72.09
Linoleic	1.34
Linolenic	10.31
Eicosatrienoic	2.39
Gadoleic	1.58
Arachidonic	0.60
Erucic	2.46
Nervonic	1.23
Stearolic	0.92
Total unsaturation	92.92

Eromosele and Eromosele, 2002; Folarin et al., 2011.

Table 3. Recipe for the formation of alkyd samples

	Alkyd Samples			
Ingredient (100g)	I	II	III	IV
Ximenia americana seed oil (XASO) (%)	40	50	60	70
Phthalic anhydride (%)	36.31	30.2	24.09	17.98
Glycerol (%)	23.69	19.8	15.91	12.02
Mole Ratio (XASO : Glycerol)	1 : 2	1 : 1.4	1 : 0.9	1 : 1.06
Alkyd Constant (K)	1.02	1.02	1.02	1.02

Table 4. Variation in Acid Value (AV) of in-process samples (I, II, III and IV) with the corresponding extent of reaction (%P_{av})

Time (Min)	Alkyd Samples							
	I		II		III		IV	
	AV	%P_{av}	AV	%P_{av}	AV	%P_{av}	AV	%P_{av}
0	196.35	-	199.15	-	173.91	-	84.15	-
30	64.52	67.14	86.95	56.34	81.35	53.22	56.10	33.33
60	42.07	78.57	50.91	74.44	51.20	70.56	38.88	53.79
90	14.00	92.87	16.83	91.55	22.44	87.10	30.86	63.33
120	8.42	95.71	12.16	93.89	10.66	93.87	12.44	85.22
150	6.34	96.71	10.37	94.79	7.85	95.49	5.61	93.33
180	-	-	7.01	96.48	-	-	-	-

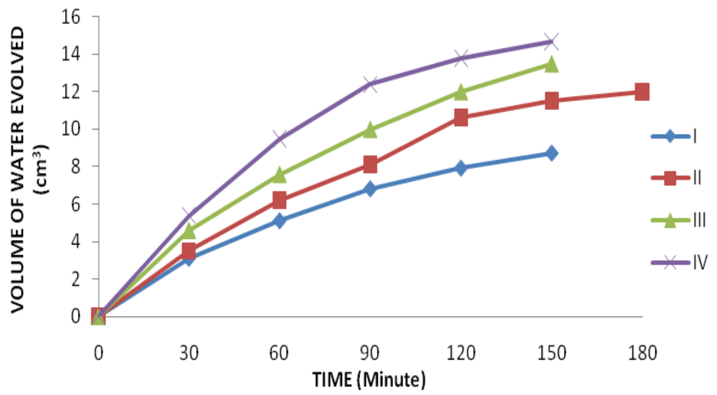

Figure 1. Plots of change in volume of water evolved versus reaction time for varying oil length alkyd resins. I (40%); II (50%); III (60%); IV (70%)

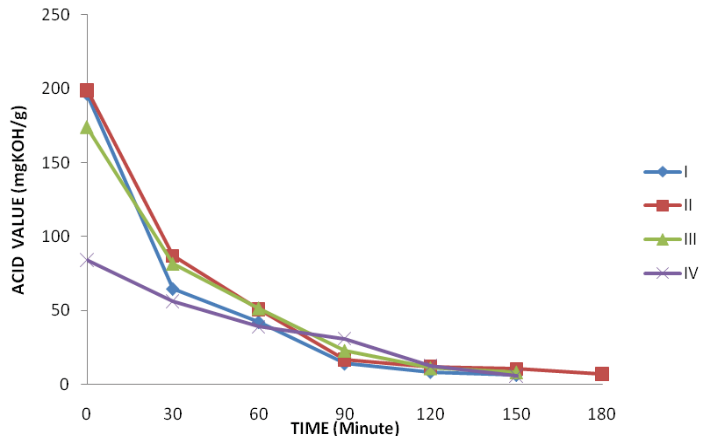

Figure 2. Plots of change in acid values versus reaction time for varying oil length alkyd resins. I (40%); II (50%); III (60%); IV (70%)

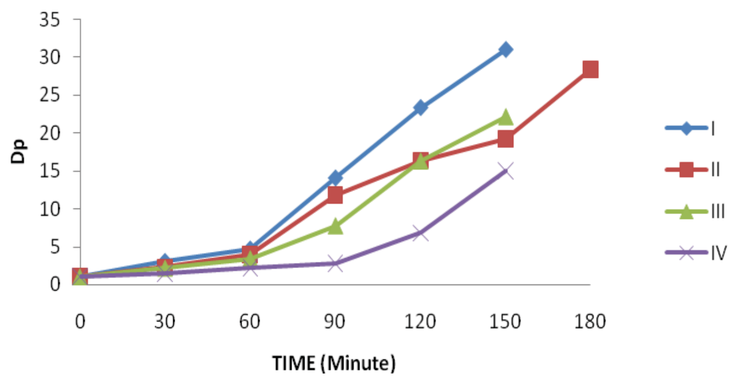

Figure 3. Plots of change in average degree of polymerization (D_p) versus reaction time for varying oil length alkyd resins. I (40%); II (50%); III (60%); IV (70%)

Four grades of alkyds were formulated at 40% (I), 50% (II), 60% (III) and 70% (IV) oil length with various amounts of phthalic anhydride and glycerol employing the two-stage alcoholysis-polyesterification method as shown in Table 3. The kinetics of polyesterification reaction was monitored by determining the acid value for the aliquot of the reaction mixture at various time intervals beside the volume of water evolved. The variation of volume of water evolved with reaction time for the alkyd samples is shown in Figure 1. It shows that at the early stages of reaction, the rate of water evolution was high and this is followed by a lower rate at longer periods of reaction. This may be due to high initial esterification reaction and the attendant reduction in the available functional groups for the condensation reactions. In addition, the increasing viscosity of the medium was reduced as the reaction progressed. It was observed that as the oil length increases, the total volume of water evolved for each alkyd sample increases. This is corroborated by the changes in acid value with reaction time as shown in Table 4 and in Figure 2. In the latter, it is revealed that there is an initial sharp decrease in acid value followed by a more gradual decrease. Similar observations had been reported (Onukwli & Igbokwe, 2008; Ikhuoria et al., 2007). The decrease in acid value is explained as arising from the desperate reactivities of primary and secondary hydroxyl groups of glycerol with carboxyl groups of phthalic anhydride. It is also attributable to incorporation of almost all reactants in the polymer chain at the initial stage of reaction, characteristics of step-growth polymerization. It has been reported that the primary hydroxyl groups of the monoglyceride reacts more rapidly than the secondary hydroxyl groups with carboxyl groups of phthalic anhydride. It is established that at a temperature of about 160 ºC, primary hydroxyl groups of glycerol react rapidly with carboxyl groups of phthalic anhydride, while secondary hydroxyl groups react at temperature above 230 ºC (Goldsmith, 1948). Thus, the initial marked decrease in acid value observed for all the alkyd samples may be due to increase in the rate of esterification accompanying the rapid increase in temperature to 230 ºC. In confirmation of the above, the acid value for all the samples decreased with increase in oil length, consistent with high amounts of di- and triglyceride present in the reaction mixture and the concentration of the acid anhydride (Onukwli & Igbokwe, 2008). However, in polyesterification reactions, the reactivity of the functional group does not depend on the size of molecule to which it is attached (Aigbodion & Okieimen, 1996).

The variation in the average degree of polymerization (D_p) with reaction time is shown in Figure 3. The first region of these plots shows the period when the primary hydroxyls of glycerol reacted with carboxyl groups of phthalic anhydride leading to formation of linear chains. Immediately the primary hydroxyl groups are esterified, there is an abrupt drop in acid value and the reaction slackens until a temperature is reached when the secondary hydroxyl groups begin to react (Aigbodion & Okieimen, 1996). Thus, the increasing D_p of the alkyd chains results in increasing viscosity of the reaction medium.

The average molecular weight (M_{av}) with respect to the acid value at different stages of the reaction was calculated using the equation:

$$M_{av} = W/ (k - P_{av})\ \ell_A$$ (Bobalek et al., 1964; Bobalek & Chiang, 1964)

where W is the total weight of all the reactants; k is the ratio of the total moles of all reactants to total equivalent of the acid (m_o/ℓ_A); and ℓ_A is the total equivalent of the acid. It was adduced that there is no reasonable increase in the molecular size of the reaction mixture at the earliest stages of the reaction. However, there is appreciable increase as the reaction progressed (Table 5). The low molecular weight observed at the beginning of the reaction is attributed to the low rate of polymerization. Increase in polymerization rate results in corresponding increase in molecular weight. This trend continues until the gelation point, at which structural changes begin to occur in the polymer size of the molecule in solution. The molecular weight of the alkyd decreases with increase in the oil lengths. This is consistent with earlier report by Aigbodion and Pallai (2001). An increase in oil length from 40-70% is accompanied by a decrease in the XASO: Glycerol ratio in the range 1: 2-1: 0.6. Consequently, the effective concentration of monoglyceride produced in-situ decreases resulting in lower D_p and molecular weight of alkyd resin. This is corroborated by the extent of reaction (P_{av}) calculated at the region of deviation from second-order as shown in Table 6.

The extent of reaction (%P_{av}) and average degree of polymerization (D_p) calculated for alkyd sample at the region of deviation from second order reaction is shown in Table 6. The result shows that the extent of reactions at this region varies from 63.33 to 78.57% for samples IV - I. Although these values are relatively lower than the range 75-80% obtained for reaction between phthalic anhydride and glycerol (Nagata, 1969; Carothers 1936), they indicate a significant degree of conversion. However, sample I (78.57%) falls within the range of the reactivity of phthalic anhydride and glycerol, with appreciable degree of conversion consistent with the ideal ratio of 1:2 for XASO: Glycerol for the alcoholysis reaction. The corresponding average degree of polymerization (D_p) ranges from 2.73 to 4.67 and this suggests the formation of high molecular weight species. The second-order rate constant, k, calculated from the linear portion of Figure 3 for the alkyd samples is represented in Table 7.

Table 5. Variation in Acid Value (AV) of in-process samples (I, II, III, and IV) with the corresponding average molecular weight (M_{av})

	Alkyd Samples							
	I		II		III		IV	
Time (Min)	(AV)	(M_{av})	(AV)	(M_{av})	(AV)	(M_{av})	(AV)	(M_{av})
0	196.35	-	199.15	-	173.91	-	84.15	-
30	64.52	464.5	86.95	386.3	81.35	361.7	56.1	312.8
60	42.07	691.1	50.91	640.1	51.2	561.2	38.88	445.6
90	14	1773.5	16.83	1688	22.44	1184	30.86	555.5
120	8.42	2574.5	12.16	2175.1	10.66	2170.1	12.44	749.3
150	6.34	3095.9	10.37	2446.6	7.85	2710	5.61	2477.8
180	-	-	7.01	3195.6	-	-	-	-

Table 6. Extent of reaction (% P_{av}) and average degree of polymerization (D_p) in the region of deviation from second order reaction

	Alkyd Samples			
Parameter	I	II	III	IV
Time (Min)	60	60	60	60
%P_{av}	78.57	74.44	70.56	63.33
D_p	4.67	3.91	3.40	2.73

Table 7. Value of second-order rate constant (k), calculated from the linear portion of plots (D_p) versus reaction time for the alkyd samples

Alkyd sample	Mole Ratio (XASO: Glycerol)	k x 10^4 g (mg KOH)$^{-1}$ min^{-1}
I	1: 2	3.12
II	1: 1.4	2.44
III	1: 0.9	2.30
IV	1: 0.6	2.28

The rate of esterification reaction is expressed as;

$$(1-P_{av})^{-1} = C_0(kt+1)$$

where C_o, k, t and P_{av} have been defined previously. From the slopes of the plots, the second-order rate constant is in range, 2.28-3.12 x 10^{-4} g (mg KOH)$^{-1}$min^{-1}, the highest value corresponding to sample I i.e. 40% oil length. It has been established that the rate constant for esterification reaction is dependent on the proportion of reactants, speed of agitation of the reaction mixture, changes in temperature and removal of water of esterification (Aigbodion et al., 2001; Akintayo & Adebowale, 2004). In addition, the rate constant for a second-order reaction depends on the initial concentration of the reactants monitored for the reaction. From the results above, the rate constants for all the samples are within the same order of magnitude, albeit higher for sample I. This is attributed to the mole ratio of XASO: Glycerol used in the formulations. The mole ratio of XASO: Glycerol of 1:2 for 40% oil length alkyd suggests higher initial concentration of α-monoglyceride formed in-situ and decreases as the oil length increases. Aigbodion and Okieimen (1996) reported that the rate constants for rubber seed oil range from 1.92-4.08 x 10^{-5}g (mg KOH)$^{-1}$min^{-1} for oil length of 20-60%. Similarly, for locust bean seed oil, k values were in the range, 5.98-12.51 x 10^{-5} g (mg KOH)$^{-1}$min^{-1} (Aigbodion et al., 2001). *Ximenia americana*-oil base alkyd resin has rate constants higher than the ones reported for locust bean oil and rubber seed oil. This may be due to difference in experimental conditions.

Table 8. Properties of the finished alkyds

	Alkyd Samples			
Property	I	II	III	IV
Colour	Dark brown	Dark brown	Dark brown	Dark brown
Acid Value (mgKOHg^{-1})	6.34$\pm$0.61	7.01 $\pm$0.72	7.85$\pm$1.00	5.61$\pm$1.90
Saponification Value (mgKOHg^{-1})	297.5$\pm$1.48	332.5$\pm$1.21	378.1 $\pm$1.10	420.2 $\pm$1.32
Iodine Value (gI$_2$100g^{-1})	70.06 $\pm$0.74	76.14 $\pm$1.05	81.22$\pm$0.97	86.30$\pm$0.81
Drying Time (hour)				
Surface Dry	<2	<2	<3	<4
Dry Through	Over night	Over night	Over night	Over night

Table 9. Resistance of the alkyd resin

Alkyd Samples	Medium			
	Distilled H_2O	5% NaCl	0.1M H_2SO_4	0.1M KOH
I	a	a	a	c
II	a	a	a	c
III	a	a	b	c
IV	a	a	b	c

a- no effect, b-wrinkle, c-film removed.

Table 10. A typical gloss paint formulation

Component	Amount (g)
Alkyd resin	200
Titanium dioxide (TiO_2)	160
Dispersant (Soya lecithin)	1.1
Kerosine	30
White spirit	20
Alkyd resin	400
Kerosine	70
White spirit	140
Anti-skin	1.1

Table 11. Properties of the paints

Parameter	Paint samples	
Oil Length	40%	50%
Colour	Cream	Cream
Specific Gravity (gcm^{-3})	1.01$\pm$ 0.14	1.07$\pm$ 0.12
Viscosity (cp)	130	110
Surface Dry (hour)	<2	<2
Tack Free (hour)	<5	<6
Dry Through (hour)	Overnight	Overnight

The properties of the finished alkyd resins are presented in Table 8. The dark colour of the alkyds is attributed to the colour of the oil. The iodine value is, as expected low, in the understanding that the reactive diol contains only one fatty acid moiety. However, as the oil length increases, the iodine value increases. The high saponification values of 297.5 $\pm$ 1.48, 332.5 $\pm$ 1.21, 378 $\pm$ 1.10 and 420 $\pm$ 1.32 mg KOH g^{-1} for alkyd samples I, II, III and IV respectively are expected since they are oligomeric and are composed of ester links. These results confirm earlier reports (Aigbodion & Pillai, 2001; Aigbodion et al., 2001). Another important property of alkyds, which of course, is the most critical to their application as binders, is the drying schedule. This is the ability of the alkyd to dry to a hard and durable film by the process of autoxidation. This is related to the amount of the double bonds present in the oil as measured by the iodine value (Muizebelt et al., 1998; Agbenyega et al., 1991).The results in Table 8 also show the effect of alkyd resin oil length on the drying time. It shows that the 40 and 50% oil lengths reached the surface-dry conditions faster at less than 2 hours while 60 and 70% oil length attained the same conditions less than 3 and 4 hours respectively. This is attributed to the decrease of the solubility of the alkyd in the solvent (xylene) as oil length decreases, increasing the amount of solvent embedded in the alkyd resin. It has been reported that higher oil content of long and medium oil alkyds gave slower initial drying due to more thermosetting of long oil alkyd as compared to short oil alkyd (Singh, 2009). Subsequently, gloss retention is more pronounced in long oil alkyd compared to the medium and short oil alkyd.

Table 9 presents the results of the resistance of the alkyds in different service media. It shows that the water and brine media have no effect on the alkyds, while they are affected to varying degrees by acid and alkali media. Thus, the 40 and 50% oil length alkyd films are highly resistant to acid, brine and water but fairly resistant to alkali. This may be due to the fact that alkyds are essentially polyesters that are susceptible to hydrolysis. The properties of the paints formulated is presented in Table 11. The cream colour of the paints is attributed to the initial colour of the alkyds and the amount of white pigment used in the formulation. The result shows that the 40 and 50% oil length alkyd paints gave values for surface drying and tack-free property of less than 2 and 5 hours respectively in respect of the 40% oil length, while it was less than 2 and 6 hours for 50% oil length.

4. Conclusion

The suitability of *Ximenia americana* seed oil in the preparation of alkyd resin and gloss paint formulation was investigated. The extracted oilseed gave an oil yield of 45.7% (w/w), this suggests the possibility of classifying *Ximenia americana* as oilseed plant. The physicochemical characterization of the oil indicated it is semi-drying in nature based on the iodine value and suitable for alkyd resin preparation. Four grades of alkyd resin were prepared via alcoholysis-polyesterification method and the kinetics of the reaction indicated it is second order. The extent of reaction and the corresponding average degree of polymerization determined revealed the formation of high molecular weight alkyd. The alkyd resins exhibited excellent resistance to acid, brine and water, but fairly resistant to alkali in respect of 40 and 50% oil length based on the degree of conversion of the reactant with the reaction time. Their resistance to different service media is consistent with observable resistance of oil-paint to these media when applied as surface coating. Characterization of paint based on alkyd resin derivatives gave values for surface drying and tack-free property of less than 2 and 5 hours respectively in respect of the 40% oil length. Thus, *Ximenia americana* seed oil-modified alkyd resin is a suitable base material for oil paint formulation.

References

Abulude, F. O., Ogunkoya, M. O., & Ogunleye, R. F. (2007). Storage properties of oils of two Nigeria oil seeds *Jatropha curcas* (physic nut) and *Helianthus annuus* (sunflower). *Amer. J. Food Tech., 2*, 207-211. http://dx.doi.org/10.3923/ajft.2007.207.211

Agbenyega, J. K., Claybourn, M., & Ellis, G. (1991). A study of the autoxidation of some unsaturated fatty acid methyl esters using fourier transform Raman spectroscopy. *Spectrochimica Acta, 47A*, 1375-1388. http://dx.doi.org/10.1016/0584-8539(91)80228-B

Aigbodion, A. I., & Okieimen, F. E. (1996). Kinetics of the preparation of rubber seed oil alkyds. *Eur. Polym. J., 32*, 1105-1108. http://dx.doi.org/10.1016/0014-3057(96)00053-5

Aigbodion, A. I., & Pillai, C. K. S. (2001). Synthesis and molecular weight characterization of rubber seed oil-modified alkyd resin. *J. Appl. Polym. Sci., 79*, 2431-2438. http://dx.doi.org/10.1002/1097-4628(20010328)79:13<2431::AID-APP1050>3.0.CO;2-A

Aigbodion, A. I., Pillai, C. K. S., & Bakare, I. O. (2001). Solution behaviour of rubber seed oil-modified alkyd resins. *Indian J. Natural Rubber Research, 14*, 48-58.

Aigbodion, A. I., Okiemen, F. E., Obazee, E. O., & Bakare, I. O. (2003). Utilization of malenized rubber seed oil and its alkyd resin as binders in water-borne coatings. *Prog. Org. Coat., 46*, 28-31. http://dx.doi.org/10.1016/S0300-9440(02)00181-9

Akintayo, C. O. (2004). Synthesis and Characterization of Albizia benth medium oil modification of alkyd resin and its chemically modified derivatives. PhD thesis. University of Ibadan, Ibadan, Nigeria.

Akintayo, C. O. & Adebowale, K. O. (2004). Synthesis and Characterization of acrylated *Albizia benth* medium oil alkyds. *Prog. Org. Coat., 50*, 207-212. http://dx.doi.org/10.1016/j.porgcoat.2003.09.017

Al-Jassir, M. S., Mustafa, A. I., & Nawawy, M. A. (1995). Studies on samh seeds (*Mesembryanthemum forsskalei* Hochst) growing in Saudi Arabia: 2: Chemical composition and microflora of samh seeds. *Plant Foods Hum. Nutr, 48*, 185-192. http://dx.doi.org/10.1007/BF01088439

AOCS. (1996). Sampling and analysis of commercial fat and triglycerides. Official methods of analysis of the American Triglyceride Chemist Society, pp. 801-855.

ASTM. (1994). Resistance of dried film of vanishes to water and alkali, Ann. Book of Amer. Soc. Testing and Material Standards, Philadephia, PA, 6, pp. 268-269.

Bajpai, M., & Seth, S. (2000). Use of unconventional oils in surface coating blends of alkyd resins with epoxy esters. *Pigment Resins Technol., 29*, 82-87. http://dx.doi.org/10.1108/03699420010317825

Bobalek, E. G., & Chiang, M. T. (1964). Synthesis and properties of some alkyds of more complex carboxyl functionality. *J. Appl. Polym. Sci., 8*, 1147-1168. http://dx.doi.org/10.1002/app.1964.070080310

Bobalek, E. G., Moore, E. R., Levy, S. S., & Lee, C. C. (1964). Some implications of the gel point concept to the chemistry of alkyd resins. *J. Appl. Poly.Sci., 8*, 625-657.

BS 6900. Specification for raw, refined and boiled linseed oil for paint and varnishes. British Standards Institution.

Carothers, W. H. (1936). Polymers and polyfunctionality. *Trans. Faraday Soc*., *32*, 39-49. http://dx.doi.org/10.1039/tf9363200039

Eromosele, C. O., & Eromosele, I. C. (2002). Fatty acid compositions of seed oil of *Haematostaphis barteri* and *Ximenia americana*. *Bioresource Technol., 80*, 303-304. http://dx.doi.org/10.1016/S0960-8524(01)00179-1

Eromosele, I. C., Eromosele, C. O., Akinloye, A. O., & Komolafe, F. O. (1994). Characterization of oils and chemical analyses of the seeds of wild plants. *Plant Foods Hum. Nutr., 46*, 361-365. http://dx.doi.org/10.1007/BF01088437

Essien, E. A., Umoren, S. A., Essien, E. E., & Udoh, A. P. (2012). Preparation and Evaluation of *Cucumeropsis mannii* Naud. Seed Oil Metallic Soaps as Driers in Gloss Paint. *J. Mater. Environ. Sci., 3*, 477-484.

Fasina, A. K. (1989). Chemistry and industry. Lead paper presented at the 14th Ann. National Conf. Chem. Soc. Nig. Petroleum Training Institute, Warri, Nigeria.

Folarin, O. M., Eromosele, I. C., & Eromosele, C. O. (2011). Relative thermal stability of metal soaps of *Ximenia americana* and *Balanites aegyptiaca* seed oils. *Sci. Res. Essays, 6*, 1922-1927.

Goldsmith, H. A. (1948). Alpha and beta hydroxyls of glycerol in preparation of alkyd resins. *Ind. Eng Chem, 40*, 1205-1211. http://dx.doi.org/10.1021/ie50463a009

Ikhuoria, E. U., Maliki, M., Okieimen, F. E., Aigbodion, A. I., Obazee, E. O., & Bakare, I. O. (2007). Synthesis and characterization of chlorinated rubber seed oil alkyd resins. *Prog. Org. Coat., 59*, 134-137. http://dx.doi.org/10.1016/j.porgcoat.2007.02.001

Ikhuoria, E. U., & Okieimen, F. E. (2005). Preparation and characterization of alkyd resins using crude and refined rubber seed oil. *J. Sci. Ind. Res., 48*, 68-73.

Keay, R. W. (1989). Trees of Nigeria, Clarendon Press, Oxford, 146, 311- 328.

Majumder, M. M. U. H. (1990). Studies in the physico-chemical properties of rubber *(Hevea brasiliensis)* seed oil and identification of different higher fatty acids of the oil and analysis of the seed cake. *Science, 14*, 31-36.

Micciche, F. (2005). The combination of ascorbic acid derivative/iron salts as catalyst for the oxidative drying of alky-paints: A Biomimetic approach. Technische Universiteit Eindhoven. http://alexandria.tue.nl/extra2/200512801.pdf

Muizebelt, W. J., Donkerbrook, J. J., Nlelen, M. W. F., Hussem, J. B., & Biemond, M. E. F. (1998). Oxidative crosslinking of alkyd resins studied with mass spectrometry and NMR using model compounds. *J. Coat. Tech., 70*, 83-93. http://dx.doi.org/10.1007/BF02720501

Nagata, T. (1969). Cooking schedule of alkyd resin preparation. Part 11. Effect of cooking schedule on molecular weight distribution of alkyd resin. *J. Appl. Polym. Sci., 13*, 2601-2619. http://dx.doi.org/10.1002/app.1969.070131208

Okieimen, F. E., & Eromosele, C. O. (1999). Fatty acid composition of seed oil of *Khaya senegalensis*. *Bioresource Technol., 69*, 155-161. http://dx.doi.org/10.1016/S0960-8524(98)00190-4

Onukwli, O. D., & Igbokwe, P. K. (2008). Production and characterization of castor oil- modified alkyd resins. *J. Eng. Appl. Sci., 3*, 161-165.

Oyedeji, F. O., & Oderinde, R. A. (2006). Characterization of isopropanol extracted vegetable oils. *J. Appl. Sci., 6*, 2510-2573. http://dx.doi.org/10.3923/jas.2006.2510.2513

Singh, S. (2009). Preparation and analysis of alkyd resin from deodourizer distillate. *J. Sci. Ind. Res., 68*, 807-811.

Storrs, A. E. G. (1995). Know your trees. Some of the common trees found in Zambia. Regional soil conservation unit, Nairobi, Kenya 51, 59-62.

Tarandjiiska, R. B., Marekov, I. N., Nikolova-Damyanova, B. M., & Amidzhin, B. S. (1996). Determination of triacylglycerol classes and molecular species in seed oils with high content of linoleic and linolenic fatty acids. *J. Sci. Food Agric., 72*, 403-410. http://dx.doi.org/10.1002/(SICI)1097-0010(199612)72:4<403::AID-JSFA671>3.3.CO;2-4

Wicks, Z. W., Jones, F. N., & Paappas, S. P. (1994). Organic coatings: Science and technology Vol. 1., John Wiley & Sons.

Williams, K. A. (1966). *Oils, fats and fatty food* (4th ed). New York: Elsevier Publishing Co.

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Effect of *Tithonia diversifolia* on Compost Pile Heat Built-Up and Physico-Chemical Quality Parameters of *Chimato* Compost

Angstone Noel J. Thembachako Mlangeni[1]

[1] Natural Resources College of Malawi, Malawi

Correspondence: Angstone Noel J. Thembachako Mlangeni, NR Department, Natural Resources College of Malawi, Malawi. E-mail: anjtmlangeni@yahoo.com

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Abstract

The study objective was to identify blending ratio of *T. diversifolia* and maize stalks that optimizes compost pile's heat built-up, precursor to compost maturity. In this study, 0%, 20%, 40%, 50%, 60%, 80% and 100% *of T. diversifolia* biomass were blended with maize stalks, and then composted using *chimato* composting technology. Temperatures were measured to determine heat built-up. TKN, nitrate-N and C/N ratios were determined using standard methods. Results showed high and more prolonged peak temperatures in compost piles with *T. diversifolia* content of 0%, 20% and 40% implying occurrence of more prolonged decomposition that generated prolonged heat. Resultant composts contained large amounts of non-compost materials (> 37%) and less dark colour indicating high level of immaturity. High but less prolonged peak temperatures were observed in compost piles with 50% and 60% *T. diversifolia* suggesting shortened active and rapid microbial activities that generated less heat. Their composts were very dark in colour and yielded least amounts of non-compost materials (< 17%) which indicated high level of maturity. Significantly large quantities of TKN and nitrate-N and low values of C/N ratios were obtained in chimato composts whose compost piles registered high but less prolonged peak temperatures. Optimal blending composition of 50% and 60% of *T. diversifolia* to maize stalks are judged to have significantly improved moisture content and porosity of feedstock which intensified and shortened active microbial activities leading to generation of optimal heat in chimato compost piles as well as retain optimal TKN, nitrate-N and C/N ratios in resultant composts.

Keywords: *T. diversifolia*, heat built-up, peak temperatures, maize stalks, chimato composts

1. Introduction

The need to apply well decomposed and nitrogen rich composts to improve soil fertility has recently received much attention as a comeback soil management technology (Nalivata, 2007) and as a mitigatory strategy to environmental challenges and concerns such as eutrophication that occur through run-off of chemical/inorganic fertilizers. High quality composts provide a good life cycle solution to poor soils, maintains soil nutrient and provides soil-building benefits that addresses environmental concerns of inorganic fertilizers management. High quality composts are well decomposed and are rich in nitrogen. However, quality of compost is a function of type and quality of vegetative organic resource materials (SAEM, 2008; Gachengo et al., 1999; Sanchez & Demchak, 2009). Quality ingredients determines the extent of microbial activity that break down chemical bonds of compounds thereby transforming chemical energy contained in the bonds to heat, which raises the temperature of the compost pile (WSU, 2010; Biddlestone & Gray, 1987). In turn, temperature ensures availability of microorganisms that work at different temperatures in the compost pile that multiply and change at a rapid rate, and generate heat that adds to heating up process thereby initiating a self-accelerated process (Agromisa, 1990). However, too high temperatures deactivate mesophilic microbes whereas a longer duration of high temperatures accelerates nitrogen loss as ammonia through volatilization (WSU, 2010; WERL, 2005). Temperatures higher than 70 ºC, however, deactivate even most of the thermophilic bacteria (Tsutsuki, 2009).

Traditionally chimato composts are made using maize stalks and grass, which are poor organic resource materials (C/N ratio >60) (Agromisa, 1990; WERL, 2005). However, maize stalks are less bulky and their compost piles possess enormous spaces -high porosity- thereby providing adequate air circulation to the pile structure. Most organic resource materials available for *chimato* composting including maize stalks do not meet the recommended C/N ratios and porosity such that it is mandatory that different organic resource materials be blended in attempt to obtain optimum C/N ratios and porosity. Blending of more porous and low quality carbon rich and less porous and high quality nitrogen rich would ensure that composting temperatures are optimum for active and rapid microbial activities. This research proposes blending *T. diversifolia* (high quality organic resource materials (Olabode et al., 2007; Jama et al., 2000) with maize stalks (poor quality organic resources (Lewis, 2008; Agromisa, 1990; Biddlestone & Gray, 1987)) to identify blending composition of *T. diversifolia* and maize stalks that optimizes compost pile's heat built-up and temperature progression.

2. Materials and Methods

2.1 Study Site

The study was carried out at the Natural Resource College (NRC) farm in Lilongwe District. NRC lies on 13º 85' S 33º 38' E, at an altitude of 1146 m above sea level, experiences a mean annual temperature of 20 ºC, a mean annual relative humidity of 68% and receives an annual mean rainfall of 892 mm of which 85% falls between November and March (DARETS, 2002).

2.2 Chimato Composting Experiments

Seven *chimato* composting experiments Td0, Td20, Td40, Td50, Td60, Td80 and Td100 in which 0%, 20%, 40%, 50%, 60%, 80%, and 100% of chopped tender *T. diversifolia* were blended with chopped dry maize stalks respectively. *T. diversifolia* plant materials and maize stalks were cut into pieces ranging from 5.0 cm to 10.0 cm. The small sizes were chosen to increase surface area of the organic resource materials and enhance efficient diffusion of air throughout the entire pile for microorganisms to efficiently decompose the organic resource materials (Nalivata, 2007; Michel et al., 2010). Composting experiments were carried out between December and March under iron sheet roofed shade to prevent leaching of water-soluble nutrients especially nitrates.

2.3 Determination of Effect of T. diversifolia on Heat Built-Up

All treatments were closely monitored to make sure that they are exposed to the same conditions. Temperatures readings were taken from all the treatments of *chimato* composts on a daily basis at 12:30 pm for the first ten days. The tenth day was an approximate time for temperatures stabilizing in the mesophilic phase (Tani, 2009). Thereafter, decomposition proceeded without taking temperature readings

2.4 Determination of Effect of T. diversifolia on Physical Characteristics of the Chimato Compost

Intensities of colours of all *chimato* composts were compared to determine which *chimato* compost was more darkish than the other was. To affirm results from colour comparison, chemical colour tests were also carried out by dissolving a 10 g sample of chimato composts in 0.5 M of NaOH and then compare the intensity of the colour that developed (Swift et al., 1979). The colour intensity of the solution showed how well humified organic matter was. Manure and non-manure components of *chimato* compost were determined by crushing the *chimato* compost and then passed through a ½-inch manure screen (Darlington, 2010) and manure materials were expected to pass through the screen. Quantity of each component (manure and non-manure) was measured using digital mass balance.

2.5 Determination of Nitrate-N in Chimato Compost Samples

Standard chimato compost sample solutions were prepared using standard methods. The prepared samples were then analyzed for available nitrogen (nitrate) using a Jenway model No.: 6405 digital UV- Visible Spectro-photometer at wavelength of 410 nm. The values were read out and reported in mg/L in Figure 2.

2.6 Determination of Compost Component and Non-Compost Component

Chimato compost samples were weighed using digital mass balance, homogeneously mixed and crushed to produce representative sample. Compost components and non-compost components were determined by passing the crushed composts through a ½-inch manure screen (Darlington, 2010). The results were reported in Figure 3.

2.7 Determination of TKN and Organic Carbon

The fine *Chimato* compost samples were tested for TKN and total carbon using the Kjeldahl apparatus (Jeffery et al., 1989). The resulted were reported in Table 1.

2.8 Determination of C/N Ratio of Product Chimato Compost

C/N ratio of each type of product chimato compost was estimated by dividing chimato compost's total carbon by TKN (WSU, 2010) as follows:

$$C \ / \ N \ = \frac{\text{Total} \quad \% \ \text{Carbon}}{\text{Total} \quad \% \ \text{Nitrogen}} \tag{1}$$

2.9 Data Analyses

The collected data were subjected to analyses of variance (ANOVA), to compare treatment effects on total-N and nitrate-N. Treatment differences were evaluated using the least significance difference (LSD) at $P < 0.05$.

3. Results and Discussion

3.1 Effect of T. diversifolia Content on Heat Built Up

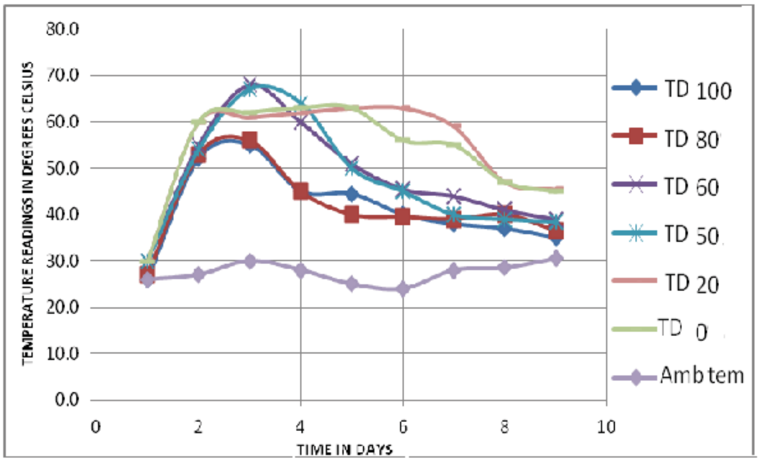

Figure 1. Effect of amount of T. diversifolia on Heat built up of compost product piles

Temperature of Td80 and Td100 rapidly increased to high thermophilic peak temperature (54.5 and 55 ºC respectively) then rapidly dropped to mesophilic phase, with Td100 reaching its peak temperature first followed by Td80. Prolonged high thermophilic temperatures were observed in chimato composts Td0, Td20 and Td40 that rose to peak temperatures (64, 65 and 70 ºC respectively) before dropping to lower mesophilic temperatures. The prolonged temperatures indicate that quantity of heat built-up in Td0, Td20 and Td40 was larger than in Td80 and Td100. Peak temperatures of Td0, Td20 and Td40 were significantly different from those of Td80 and Td100.

The significant differences are attributed to quantity of *T. diversifolia* used to make these chimato compost piles which significantly affected porosity, initial C/N ratio and microbial activities (Biddlestone, 1987; WSU, 2009) in chimato compost piles that also affected substrate digestibility. The blending composition probably provided the organic resources of Td0, Td20 and Td40 with large initial C/N ratio (67:1, 41:1, 37:1) and organic resources of Td80 and Td100 with lower initial C/N ratio (15:1, 10:1) such that Td0, Td20 and Td40 organic resources possessed large quantities of carbon atoms without corresponding provision of nitrogen atoms for microbes to use as source of energy and as building materials of their structures respectively. As such, microorganisms slowly digested the abundant carbon atoms in the organic resource by using and/or reusing their stored limited nitrogen to burn large quantities of carbon atoms for a longer period supporting findings by Petrus et al. (2009) and Tani (2009) that slow and passive microbial activities are experienced in compost piles with initial C/N ratio of greater than 30:1. Thus, the process prolonged exothermic decomposition reaction that induced the observed high-prolonged temperatures (heat built-up) as well as broad and greater range of compost heat built-up in chimato composts Td0, Td20 and Td40.

The observed rapid increase in temperature in Td80 and Td100 could possibly be attributed to rapid and active initial mesophilic microbial activities (WSU, 2009; ICRAF, 1998) that enhanced fastest though short-lived exothermic decomposition reaction leading to rapid heat built-up in the chimato compost piles. Blending that included 100% and 80% of *T. diversifolia* (in Td80 and Td100) lowered initial C/N ratios of the compost piles to fall below the recommended range of 20-30:1 or lower (Petrus et al., 2009; Tani, 2009). Thus, Td100 and Td80 possibly provided microorganisms with enormous amounts of nitrogen, easy to digest substrate, for building their cell structure during microbial activities. Since rapid and active microbial activities are experienced in compost piles with initial C/N ratio of lower than 30:1 because they possess large quantities of easy to digest substrate as stated above microorganism digested and decomposed the organic resource materials of Td100 and Td80 rapidly, leading to fast generation of heat. However, it was observed that temperatures in Td100 and Td80 rapidly dropped to lower mesophilic temperatures. This could possibly be associated with slowing down of growth and respiration of microorganisms activities after the limited quantities of carbon atoms were digested (Petrus et al., 2009; Tani, 2009) and used up, which further limited exothermic reaction process of decomposition (Biddlestone, 1987; WSU, 2010). This limitation probably led to limited generation of heat, hence the observed low temperatures. Thus, decomposition processes in Td100 and Td80 occurred rapidly and heat built-up short lived in the thermophilic phase due to limited supply of carbon even though nitrogen was in abundance.

The observed prolonged broad and greater range of thermophilic temperatures in chimato compost piles Td0, Td20 and Td40 are attributable to their high initial C/N ratios (C/N ratio 66:1, 41:1 and 37:1 respectively; C/N> 31:1) of the blend that induced slow microbial decomposition process because their substrate possessed abundant carbon in agreement to reports by WSU (2010), Petrus et al. (2009) and Tani (2009). It is suggested that maize stalks provided the large quantities of high carbon content cellulosic materials (lignin) whose oxidation and digestion generate large amounts of heat that made Td0, Td20 and Td40 to experience prolonged high temperatures. Since high prolonged heat built-up are symptomatic of loss of nitrogen and immaturity compost, chimato composts Td0, Td20 and Td40 could probably yield less nitrogen and show physical appearances of immaturity as discussed in subsequent sections.

3.2 Effect of 50% and 60% T. diversifolia Content on Heat Built Up

High peak temperatures of *chimato* compost piles of Td50 (67 ºC) were not significantly different from Td60 (66 ºC) (p= 0.201; α= 0.05). The temperatures were less prolonged moderately progressed without over heating the chimato composts piles. The observations suggest that blending *of T. diversifolia* and maize stalks in chimato compost piles Td50 and Td60 seemed to have provided a balanced proportion of carbon atoms - adequate amount of energy source- and nitrogen atoms - adequate amount of cell building materials. Microorganism efficiently used carbon atoms in converting available nitrogen into protein to building their cell structure. The moderate short stay of built-up heat in *chimato* compost piles Td50 and Td60 is attributable to absence of hard to digest materials such lignin, cellulose, and poly-phenols, which required thermophilic microbes' action as already discussed. Based on temperature progression, optimum-blending ratios probably occurred in chimato compost with *T. diversifolia* content of 50% and 60% that enhanced optimum (rapid and active) microbial activities that generated optimum heat for sustainable decomposition process. Thus, optimum microbial activities induced heat built-up that was neither too low nor too high; induced heat that neither dropped too soon nor prolonged for longer period for microbes to enhance sustainable decomposition process.

3.3 Effect of Heat Built up on TKN, Nitrate-N, C/N Ratio

Table 1. Effect of chimato compost pile heat built-up on mean TKN, Nitrate-N & C/N ratio

Chimato Compost Treatment	**Mean TKN %**	**Std. error**	**Mean Nitrate-N %**	**Std error**	**Estimated initial C/N Ratio**	**Mean Final C/N Ratio of resultant compost**	**Std error**
Td0	1.17	±0.13	0.078	±0.002	66:1	38:1	±2
Td20	1.37	±0.08	0.23	±0.02	41:1	27:1	±2
Td40	1.67	±0.13	0.80	±0.01	28:1	18:1	±1
Td50	2.33	±0.10	1.04	±0.03	24:1	13:1	±1
Td60	2.47	±0.10	0.98	±0.02	20:1	10:1	±1
Td80	2.02	±0.26	0.57	±0.01	13:1	14:1	±1
Td100	2.06	±0.21	0.70	±0.02	10:1	11:1	±1

As shown in Table 1, *chimato* composts analysis showed that Td0, Td20 and Td40 whose heat built-up were significantly prolonged possessed low TKN, low Nitrate-N and large final C:N ratios whereas Td50, Td60, Td80 and Td100 whose heat built-up was short-lived possessed high TKN, high Nitrate-N and small C:N ratios. This revelation suggests that composts piles of Td0, Td20 and Td40 were heated for a long time while those of Td50, Td60, Td80 and Td100 were not sufficiently heated of which both affected TKN, Nitrate-N and C/N ratios negatively. Ingredients of Td0, Td20 and Td40 piles possessed low initial nitrogen (high C/N ratio), optimum aeration and low moisture content. Therefore, microbial activity probably prolonged because there was insufficient amounts of nitrogen for microbes to use in digesting the feedstock with speed. As such, microbes reused the insufficient nitrogen to build up their structures (Agromisa, 1990; Ngeze, 1993; SAEM, 2008; WERL, 2005) in digesting the ingredients which prolonged the digestion process to take a little longer as observed. In addition, since significant nitrogen losses are certain to occur in such high temperatures prolonged microbial activities due to ammonia volatilization, it resulted in significant TKN and nitrate-N losses hence the low observed TKN and nitrate-N.

Ingredients of Td50, Td60, Td80 and Td100 piles possessed high initial nitrogen (low C/N ratio), poor aeration and high moisture content. Microbial activity was short-lived in Td50, Td60, Td80 and Td100 piles because microbes had probably plenty nitrogen atoms to use to digest available little carbon atoms, which was accomplished with greater speed and significantly short-lived heat built-up of compost piles. However, Td80 and Td100 piles experienced poor aeration and poor moisture content, since the green secreted additional which made Td80 and Td100 piles more wet than Td50 and Td60 piles. This wet and poor porosity condition probably nitrogen in piles of Td80 and Td100 to enhance faster aerobic decomposition which probably lead to Nitrates formation that characterizes anaerobic decomposition. Nitrates were leached out of the compost piles as leachate or as slurry (Manahan, 2008) that collected under the piles. Hence, the short-lived heat built-up in Td80 and Td100 is associated to decreased recorded amounts of TKN and nitrate-N while the less prolonged were recorded in Td80 and Td100 while high amounts were recorded in. Td50 and Td60 did not experience prolonged heat built-up that forced microbes to reuse the available nitrogen to build up their structures thereby causing significant nitrogen losses nor did they experience short-lived heat built-up which was wet and induced favorable conditions for anaerobic decomposition and significant nitrates losses. As a result, microbes made full use of available carbon atoms to get rid of excess nitrogen as ammonia (Petrus et al., 2009; Onwueme & Sinha, 1991). Since ammonia volatilization is certain to occur in compost piles with high nitrogen content, Td80 and Td100 were certain to experience to reductions of both TKN and nitrate-N. Therefore, it can be concluded that, optimum porosity and optimum amounts of initial nitrogen and carbon atoms occurred in Td50 and Td60 induced active and rapid aerobic decomposition that determined the optimum heat built-up.

3.4 Effect of T. diversifolia Percentage on Physical Appearance of Chimato Compost

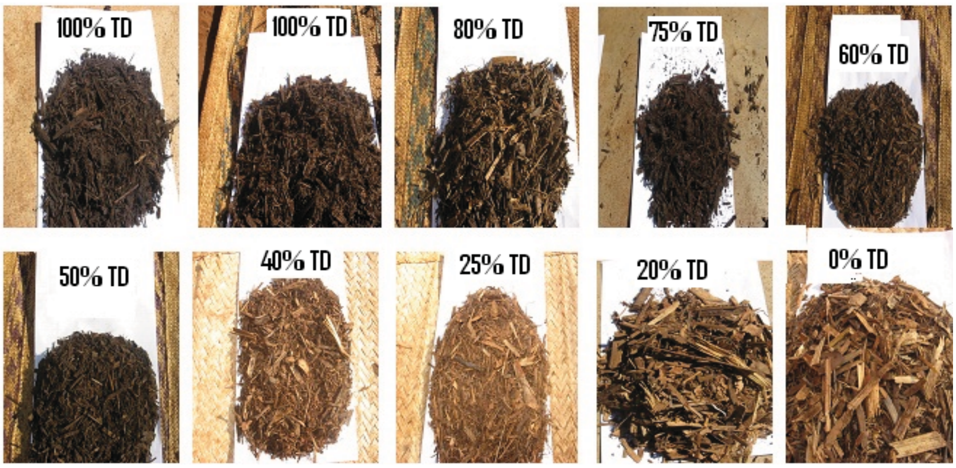

Figure 2. Effect of amount of *T. diversifolia* and heat built up on appearance of compost product after 50 days

As shown in Figure 2, colour intensities of *chimato* composts Td0, Td20, Td40, Td50 Td60 and Td100 were observed increasing in that order with Td0 being least dark whereas Td60 and Td100 were most dark in colour. Even though colour intensity of Td50 was less dark than that of Td60. they were not significantly different from each other. Colour intensity of Td100 was not significantly different from that of Td60. Generally, colour intensities of the *chimato* composts were observed increasing in increasing order of quantities of *T. diversifolia* in the compost piles (up to 60% of *T. diversifolia* in blending composition). Beyond 60% of *T. diversifolia*, other variables seem to have taken a leading influence on decomposition process and on intensity of colour of compost product. Most dark colours in composts indicate high level of maturity and how well-finished the composting processes (SAEM, 2008; Swift et al., 1979; WERL, 2005). Therefore, the results indicate that *chimato* composts Td50, Td60 and Td100 were probably well finished and more matured while Td0, Td20 and Td40 were immature with poorly finished composting process. The results indicated occurrence of minimal decomposition likely to yield least amounts of compost. *Chimato* compost Td80 was less dark than Td60. Thus, besides being made up of higher percentages of *T. diversifolia* content, *chimato* compost Td80 was observed lighter (less dark) in colour than the preceding ones. The observation could be attributable to high level of water content of secreted by leafy *T. diversifolia* (the greens) that characterized their *chimato* compost piles that reduced amount of air spaces in the pile. The porosity reduction rendered microbes with insufficient amounts of oxygen that probably induced anaerobic respiration (rotting) that made the ingredients to rot rather than decompose (ICP, 2004; Michel, et al, 2010; SAEM, 2008; WERL, 2005), hence the *chimato* compost made using *T. diversifolia* content of 80%, was less mature and less dark than the preceding ones.

3.5 Colour Test Results of Chimato Compost Products

When 10.0 g of *chimato* compost were dissolved in 0.5 M of NaOH, study results showed that chimato compost Td50 and Td60 formed most blackish solutions comparable to *chimato* compost Td100. *Chimato* compost Td0, the control, formed a solution with a colour interpreted too light to be a compost (Johnson, 2007; Swift et al., 1979). The intensity of the colour was most dark in *chimato* compost Td100 (with lowest percentages of *T. diversifolia*) followed by Td60 and Td50 then Td80, Td40, Td20 descending in that order down to *chimato* compost Td0 with lowest percentages of *T. diversifolia*. This observation also confirms the possibility of formation of larger amounts of humus in chimato compost made using larger proportion of *T. diversifolia* than those made using lower percentages of *T. diversifolia*. Since humus as well as composts turn black in a solution of NaOH and high intensity of black colour indicates high levels of humus and composts, the most dark colour observed in Td50, Td60, Td80, and Td100 suggests that these composts were either well-humified organic matter (more humus) or well composted (Swift et al., 1979). However, humus is a product of anaerobic respiration (rotting) while compost is a product of aerobic respiration (decomposition) (Michel, et al., 2010). The dark brown colours in chimato compost made using T. diversifolia content of larger than 80% could be due to humus since these chimato compost piles were too wet indicating high moisture content that probably characterized them with anaerobic respiration. *Chimato* compost made using 0% *T. diversifolia* content produced very light in colour compost that indicated occurrence of minimal decomposition likely to yield least amounts of compost.

3.6 Amount of Compost and Non-Compost Components of the Chimato Compost

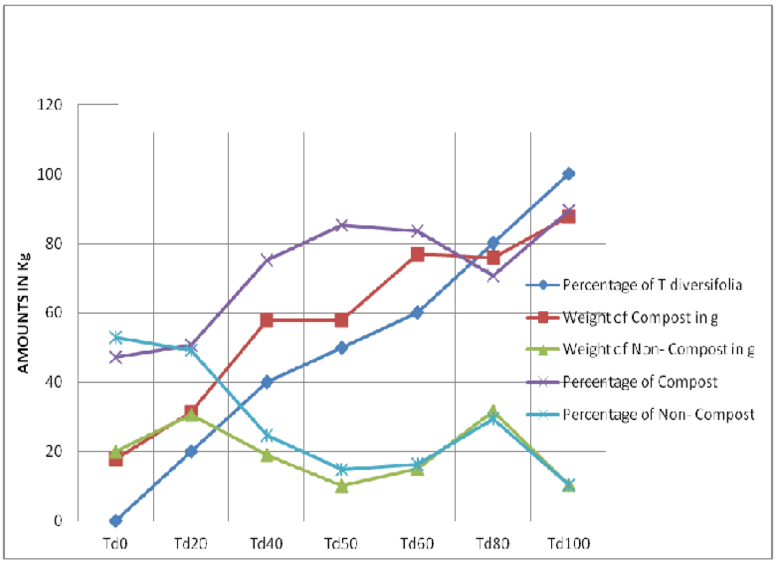

Figure 3. Effect of *T. diversifolia* on amount of compost components and non-compost components

Generally, quantity of non-compost components increased in chimato compost with increase of quantity of *T. diversifolia* used in making chimato compost piles. As shown in Figure 3, *chimato* compost Td100 yielded least quantity of non-compost components whereas Td0 yielded greatest quantity of non-compost components.

The non-compost components in *chimato* compost increased in the following order of *T. diversifolia* content: 100% = 50% < 60% < 40% < 80% < 20% < 0%. Most of ingredients in *chimato* compost piles made using *T. diversifolia* of 100% contained greater proportion of easily degradable materials and minimal amounts of hard to decompose substances such that they were completely decomposed by the 40th day. Ingredients consisting of greater composition of nitrogen rich ingredients (which are low in lignin and phenol content and possess soft leaves), such as *T. diversifolia* have high level of degradability (SAEM, 2008; WERL, 2005; ICRAF, 2008; Biddlestone & Gray, 1987). *Chimato* compost Td50 then Td0, Td20 and Td40 (being made of greater percentage of maize stalks) possessed large proportions of difficult to degrade ingredients that limited degradability of the composite ingredients. This limitation resulted in prolonged, slow, and less active microbial activities, which yielded more non-compost (non-manure) components. Even though *T. diversifolia* content was lower in chimato composts Td50 and Td60 than in chimato composts Td80 and Td100, comparable percentage of compost and non-compost components were found suggesting that both attained greater degradability.

Greater degradability in *chimato* compost Td50 and Td60 could also be attributable to balanced provision of nitrogen and carbon that microbes used to build up their cell structure and as energy source respectively (WERL, 2005) that in turn provided greater microbial activities for active and rapid sustainable decomposition process (SAEM, 2008; Biddlestone & Gray, 1987). As previously discussed, greater degradability could also be attributable to availability of sufficient air spaces by the less bulky maize stalks (50% and 40% of maize stalks). This probably enhanced aerobic respiration that supported sustainable active and rapid composting process as well (SAEM, 2008; Biddlestone & Gray, 1987; Lewis et al., 2008; *WSU*, 2010). This is in agreement to the heat built-up trend already discussed which supports formation of well-finished compost.

4. Conclusion

The results have shown that blending ratios of 50% and 60% of *T. diversifolia* biomass with maize stalks were optimum and enhanced rapid and active microbial activities that generated optimum heat for sustainable decomposition process. The resultant composts were more matured and possessed large quantities of nitrate-N and TKN and their C/N ratios were within the recommended ranges of mature composts. Smallholder farmers should be encouraged to compost *T. diversifolia* blended with maize stalks using these blending ratios in order to utilize large amounts of nitrogen contained in *T. diversifolia*. Farmers will find application of composts made from *T. diversifolia* more rewarding than application of raw *T. diversifolia* due to reduction of bulk mass that significantly reduces number of trips carried to the field and the associated cost of transportation.

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References

Agromisa. (1990). Preparation and use of compost. (Agrodox series 8). Wageningen. AGROMISA.

Biddlestone, A. J., & Gray, K. R. (1987). Production of Organic Fertilizers by Composting, In D. J. Moriarty & R. S. V. Pullin (Eds.), *Detritus and Microbial Ecology in aquaculture: ICLARM Conference proceeding* (pp.151-180). Philippines: International Centre for living aquatic resources management.

DARETS (Department of Agriculture Research Extension and Technical Services). (2002). About Chitedze.

Darlington, W. (2010). Compost- A guide for evaluating and using compost material as Soilamendments. *Soil and Plant Laboratory, Inc. 714*(282), 8777-8788.

Gachengo, C. N., Palm, C. A., Jama, B., & Othieno, C. (1999). Tithonia and Senna green manures and inorganic fertilizers as phosphorus sources for maize in Western Kenya. *Agro-forestry Systems, 44*(1), 21- 36. http://dx.doi.org/10.1023/A:1006123404071

International Centre for Research in Agro-forestry (ICRAF). (1998). ICRAF Annual report for 1997. Nairobi: ICRAF.

Intervale Compost Products (ICP). (2004). Testing for five key compost characteristics. Burlington: ICP.

Jama, B., Palm, C. A., Buresh, R. J., Niang, A., Gachengo, C., Nziguheba, G., & Amadalo, B. (2000). *Tithonia diversifolia* as a green manure for soil improvement in Western Kenya: A Review. *Agro-forestry Systems, 49*(1), 201-221. http://dx.doi.org/10.1023/A:1006339025728

Jeffery, G. H., Bassett, J., Mendham, J., & Denney, R. C. (1989). *Vogel's textbook of quantitative chemical analysis* (5th ed.). New York: Longman.

Lewis, E. (2000). *Composting fundamentals*. Maryland: Maryland University (College park).

Manahan, S. E. (2000). *Fundamentals of environmental chemistry* (2nd ed.). New York: Taylor and Francis Group. http://dx.doi.org/10.1201/9781420056716

Michel, F. C., Heimlich, J. E., & Hoitink, H. A. (2010). Composting at home. (Horticultural and Crop Sciences Composting Series). Ohio: Ohio State University Extension.

Nalivata, P. C. (2007). Evaluation of factors affecting quality of compost made by smallholder farmers in Malawi. Unpublished doctoral thesis, Cranfield University (UK), National Soil Resources Institute.

Ngeze, P. B. (1993). *Artificial fertilizers and how to use them*. Nairobi (Kenya) Stantex publishers.

Olabode, O. S., Ogunyeni, S., Akanbi, W. B., Adesina, G. O., & Babajide, P. A. (2007). Evaluation of *Tithonia Diversifolia* (Hemsl): A gray for soil improvement. *World Journal of Agricultural Sciences, 3*(4), 503-507.

Onwueme, I. C., & Sinha, T. D. (1991). Field crop production in Tropical Africa principles and practice. Ede, Netherlands: Technical Centre for Agricultural and Rural Co-operation (CTA).

Petrus, A. C., Ahmed, O. H., Muhamad, A. M. N., Nasir, H. M., Jiwan, M., & Banta, M. G. (2009). Chemical Characteristics of Compost and Humic Acid from Sago Waste. *American Journal of Applied Sciences, 6*(11), 1880-1884. http://dx.doi.org/10.3844/ajassp.2009.1880.1884

Sanchez, E., & Demchak, K. (2004). The organic way- use of compost and manure in small fruit production. *New York Berry News, 1*(12), 1-5.

Sustainable Agriculture Extension Manual (SAEM). (2008). Using organic matter. *Sustainable Agriculture Extension Manual for Eastern and Southern Africa, 1*(1), 124-130.

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). *Decomposition in terrestrial Ecosystem*. Oxford: Blackwell Scientific Publication.

Tani, M. (2009). *Analysis of Total Carbon and Nitrogen in Composts and Manures*. Obihiro: Obihiro University of Agriculture and Veterinary Medicine.

Tsutsuki, K. (2009). *Fundamentals in compost preparation and Utilization*. Obihiro: Obihiro University of Agriculture and Veterinary Medicine.

Washington State University (WSU). (2010). *Fundamentals of composting: Why compost, material and methods to ensure quality compost* (Whatcom Extension Research Report). Washington: Washington State University.

Woods Ends Research Laboratory (WERL). (2005). Interpreting Waste and Compost Tests. *Journal of the Woods End Research Laboratory, 2*, 1-10.

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Agrosteppe Method

D. Dzybov[1]

[1] Stavropol Research institute Agriculture, Mikhailovsk, Russia

Correspondence: D. Dzybov, Stavropol Research institute Agriculture, Mikhailovsk, Russia. E-mail: agrosteppe@ mail.ru

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Abstract

Steppes, pusztas, prairies, savannas, the Pampas and other natural ecosystems function on one biological principle: they are multicomponent, self sustaining within a certain flora (and fauna) biodiversity, vertical structure, zone associations, dominants, bioproductivity (yield) and erosion resistance. Fully degraded steppes and the analogues slowly reestablish over succession stages- within 80-100 and more years.

Keywords: agrosteppe, competition for light, wild plants

1. Introduction

Man's active intervention in natural ecosystems started since the Paleolithic-about 10(12)000 years ago. Anthropogenic stress increased 6(7)000 years later with the introduction of animals herding and farming. Only 32% of the world's land area is undisturbed wild lands (Grigoryev & Kondratyev, 1994). The rest area is subjected to digressive processes such as desertification and weakening of zonal dominant flora, vegetation types, and animal life against the background of climate warming (Mabbut, 1981; Lavrov, 1989; Lazukov, 1989; Koronovsky, Khain, & Yasamanov, 2008; Rooth, 1992; Salem, 1993). Science suggests various protection measures as an alternative: reservations and national parks establishment, anthropogenic stress reduction by 30-60% (Schmid & Thomet, 1986; Odum, 1986). These measures are of great value and are used by countries where primary vegetation is preserved. But they do not solve the problem of eroded and desertified land rehabilitation on wide areas. The remaining soil and vegetation continue to degrade. This is a burning issue in the regions with mean annual rainfall of 200-500 mm; these are steppes, prairies, savannas, the Pampas, etc.

The first prairie restoration experiment was held by Curtis in Wisconsin University (USA) in 1935. He transplanted small blocks of initial prairie sod (Green & Curtis, 1953; Cottam Grant, & Wilson, 1966). The other methods included harvesting seeds of certain plants (Stipa spartia and other) further introduced at plain segments, transplantation of mature plants, and hay application. Curtis stated that sod method gave good results but it was expensive and labour-consuming. Sodding was used in Russia for meadow steppe restoration in the North Caucasus at Stavropol Botanical Garden. Restoration process took 8-17 years (Skripchinsky, 1973 and other). Wisconsin and Stavropol studies were of great importance, but they stayed within the limits of science experiment and failed to lay the foundation for a large-scale application. The author of this article has also conducted experiments with sod at Stavropol Botanical Garden and stated that spaced sod planting initiated a competition between sod blocks' mature plants and seedlings of the next generation. This was the reason for delayed steppe restoration by the abovementioned method, besides it turned out to be anti-ecological as sod-making areas became weeded for a long time and there were very few natural steppes left (Dzybov, 1980, 2008 and other).

2. Methods

The author of the project used another method. A plough land was sown with complex steppe seeds mix. The major difficulty of this method-different ripening time of rich steppe flora-was overcome by combine harvesting of equal adjacent areas of seed donor in two or three stages at 25-30 days intervals. Seed mixes of different harvest time were then combined into one mix representing a "portrait" model of initial steppe. The final mix was not cleared of impurities and weeds to preserve in the future steppe relative abundance of steppe plants species: dominants, assectators and other accompanying species. Some plants give few seeds (Carex humilis, Cleistogenes bulgarica), others have week germination capacity or spring up very slowly (Adonis vernalis, Paeonia tenuifolia). These species were scarcely represented in the agrosteppe. In some cases we transplanted some mature plants (Adonis vernalis, Paeonia tenuifolia) manually. Such species account for about 3% of the agrosteppe flora.

The first natural seed mix sowing experiment was conducted at Stavropol Botanical Garden in 1975 (Dzybov, 1979). Soil-chernozem, humus-3%, annual rainfall-623 mm, seed bearing plot (donor)-meadow steppe. Steppe seed mix was harvested at two stages – on July 17, and on August 21, 1975. Seed bearing plot (donor) comprised 59 plant species per 100 m^2. Association: Brachypodium rupestre, Carex humilis, Galium ruthenicum, Phleum phleoides. Sowing was carried out with surface applicator on September 10, 1975, seed rate-4 g of mix per 1 m^2 (40 kg/ha). The seeds were twice rolled over. The project's name is "Agrosteppe method"; agricultural practices applied were as follows: plowing, combine harvesting, etc.; reconstructed phytocenoses were similar to original steppes in all basic characteristics (Dzybov, 2001 and other).

3. Results and Discussion

After wintering on May 6, 1976 there were recorded the following steppe plants seedlings: Anthyllis macrocephala, Betonica officinalis, Brachypodium rupestre, Bromopsis riparia, Echium maculatum, Festuca rupicola, Filipendula vulgaris, Medicago romanica, Phleum phleoides, Galium ruthenicum, Poterium polygamum and other. Weeds grew quicker than steppe plants and germinated mainly from soil seed reserve, among them were the following: Amaranthus blitoides, Amaranthus retroflexus, Ambrosia artemisiifolia, Bromus japonicas, Capsella bursa-pastoris, Lactuca serriola and some other. During its first summer the test plot resembled one of the primary stages of layland demutation. In order to reduce weeds competition for water, nutrients and light young phytocenosis was twice cut on May 6, and on June 28, 1976. Cutting did not damage steppe plants due to their short height. Before the next winter 1976-1977 grasses finished tillering, the other groups of plants had good vitality.

The second summer (1977) agrosteppe was very bright (Figure 1) and looked like initial steppe: lots of virgin land plants were blossoming against the background of green vegetative organs and had high occurrence (%):

Brachypodium rupestre	80	Medicago romanica	100
Echium maculatum	100	Phleum phleoides	100
Festuca pratensis	80	Silene densiflora	80
Festuca rupicola	80	Stachys atherocalyx	70
Galium ruthenicum	100	Stipa pulcherrima	80
Linum nervosum	100	Thymus marschallianus	80

Occurrence of the rest species in the agrosteppe varied from 20 to 60%.

Figure 1. The first agrosteppe method science experiment. Professors V. V. Skripchinsky and V. G. Tanfilyev (1975)

Experimental plot comprised 42 species, including 8 weeds from soil seed reserve: Ambrosia artemisiifolia, Lamium amplexicaule, Setaria pumila, Sinapis arvensis and some other annuals. Over 82% of agrosteppe flora was in generative phase and gave seeds. Ground vegetation was dense, degree of soil coverage reached 70%. Hay yield equaled 468 g/m^2 exceeding the yield of reference standard by 35.6 gram. The reason for this was low competition of roots in sod horizon of the young agrosteppe. Specific features of experimental agrosteppe development in different years are shown in Table 1.

Table 1. Agrosteppe development dynamics within 10 years

Phytocenosis, years	Quantity of species per 100 m²	Weeds		Botanical groups					
				Grasses		Legumes		Mixed herbs	
		QTY	%	QTY	%	QTY	%	QTY	%
Reference standard (1975)	83	13	16	16	19	9	11	58	70
Agrosteppe, 1977	60	19	31	12	20	6	10	42	70
1978	86	15	17	13	15	12	14	61	71
1979	74	10	14	16	22	13	18	45	60
1980	65	6	9	17	26	13	20	35	54
1981	65	6	9	16	25	12	18	35	57
1982	81	10	12	14	18	11	13	46	57
1983	83	13	16	13	16	14	17	43	51
1984	94	18	19	17	18	18	19	41	44
1985	83	11	13	15	18	14	17	43	52
1986	84	13	15	18	21	14	17	39	47
Agrosteppe average (1977-1986)	78	12,1	15,5	15,1	19,9	13,6	16,3	43,0	56,3

Table 1 data shows the following: 1) species composition per 100 m^2 increased in 10 years time from 42 to 78; 2) high index of diversity preserved; 3) flora groups ratio (grasses, legumes, and mixed herbs) stabilized and equaled approximately 1:1:4 as in reference standard; 4) biological hay yield varied from 224 to 511 g/m^2 within 10 years.

The second agrosteppe was started in 1980 on 10 ha of sandy soil (humus-0.9-1.0 %, annual rainfall-450 mm). Association: Festuca valesiaca+Koeleria cristata+mixed herbs, flora diversity of seed bearing plot (donor) comprised 62 species per 100 m^2: grasses and sedges-21.0%, legumes-11.3%, mixed herbs-67.0%, annuals-1.6%, perennials-98.4% (Table 2).

Table 2. Agrosteppe dynamics over the period from 1981 to 1991 compared to data registered at 17 and 32 years

Agrosteppe age, years	Species per 100 m^2	Floral groups, %			Life cycles, %		
		Grasses+sedges	Legumes	Mixed herbs	Annuals	Biennials	Perennials
2	47	17.0	2.1	80.9	30.0	4.3	65.7
3	55	22.0	3.5	74.5	9.1	16.4	74.5
4	30	26.7	3.3	70.0	16.6	6.7	76.7
5	58	15.5	3.4	81.0	10.3	10.3	79.4
6	61	18.0	18.0	64.0	0.0	9.8	90.2
7	60	11.7	11.7	76.6	3.3	5.0	91.7
8	65	10.8	17.0	72.2	1.5	10.8	87.7
9	54	13.0	11.1	75.9	0.0	11.1	88.9
10	62	16.0	13.0	71.0	1.7	8.1	90.3
11	68	13.2	16.2	70.6	4.4	10.3	83.3
Agrosteppe average over 10 years	56	16.4	9.9	73.7	7.7	9.3	82.8
17 years	56	14.3	19.6	66.1	7.1	5.4	87.5
32 years	66	21.0	14.0	65.0	0.0	3.0	97.0

Fluctuation of agrosteppe basic characteristics within 32 years was insignificant and approached 10 and 17 years' average. By 2012 legumes and mixed herbs ratio slightly decreased. A natural result of reestablished steppe ageing was the sharp fall of therophytes. The number of perennials reached its maximum-97% of the flora on the experimental plot of 100 m^2. There was recorded stabilization of agrosteppe composition and vertical structure whereas weeds invasion resistance increased.

Agrosteppes can serve as a reliable way of desertified lands ecological reestablishment (Figures 2 and 3), a source of pasture forage and hay, and a real means of saving floral biodiversity, including rare protected species (Gorbunov, Dzybov, Kuzmin, & Smirnov, 2008) (Figure 4). Table 3 shows various multiple-aged agrosteppes associations. The data suggests the following: within 1-32 years' time span agrosteppes preserved high degree of biodiversity-floral density fluctuations were within 39-93 species per 100 m^2 of test area; grasses (the Poaceae), legumes (the Fabaceae), and other families were rich in species diversity. Perennials dominated steppe flora. Jaccard similarity index of agrosteppe and its donor might vary from 42 to 80. It reached its minimum at one-time seed donor harvesting and rose to the maximum at harvesting in 3-4 stages. The hay yield of multiple-aged agrosteppes with different composition varied from 178 to 497 g/m^2 (Figure 5).

Figure 2. Ecological reestablishment of desertified stony land with agrosteppe method. Dominants: Festuca valesiaca, Bromopsis riparia, Medicago romanica. In aspect: Galium ruthenicum, Centaurea orientalis. 73 species per 100 m^2

Figure 3. Ecological reestablishment of sandy soil with agrosteppe method. 100 m^2 comprise 68 steppe plants species (Stipa pulcherrima, Dianthus ruprechtii. Festuca valesiaca, Lotus caucasicus, Onobrychis arenatia, and other)

Figure 4. Anemone sylvestris-rare protected plant in a thirty-year agrosteppe

Figure 5. Haymaking in a two-year agrosteppe

At the age of two years agrosteppe becomes a donor of new seed mixes since 87-98% of steppe plants start to blossom and give seeds. Seed mix collected from 1 ha of steppe (agrosteppe) can restore 7-10 ha of deserted area. This gives the opportunity to carry out lands reestablishment exponentially within a short period of time (Dzybov, 2010). Seed harvesting and sowing should be conducted within the limits of the same geographical area. Biological principles of multispecies natural ecosystems are valid for agrosteppes as well. This makes the agrosteppe method applicable throughout the world.

Table 3. Biodiversity and yield of agrosteppes different in composition and age

Association	Area. ha	Age (year of sowing)	Species per 100 m^2	Floral groups, %			Life cycles, %			Hay yield. g/m^2
				Grasses+sedges	Legumes	Mixed herbs	Annuals	Biennials	Perennials	
Festuca valesiaca+ Festuca pratensis+ mixed herbs	1.0	1 (2011)	59	32.0	19.0	49.0	5.0	10.0	85.0	409.1
Festuca valesiaca+ Trifolium pratense+ Festuca pratensis	1.3	1 (2011)	51	31.4	13.7	54.9	15.7	9.8	74.5	390.7
Lolium perenne+ Dactylis glomerata+ Poteriym polygamum	5.0	1 (2011)	41	27.0	22.0	51.0	27.0	7.0	66.0	225.9
Festuca valesiaca+ Bromopsis riparia+ Poteriym polygamum	50	4 (2009)	41	22.0	19.5	58.5	7.3	12.2	80.5	436.0
Festuca valesiaca+ Bromopsis inermis+ Poterium polygamum	50	4 (2009)	39	33.0	13.0	54.0	0.0	8.0	92.0	496.8
Festuca valesiaca+ Bromopsis inermis+ Poterium polygamum	2.5	16 (1995)	84	20.0	13.0	67.0	13.0	11.0	76.0	221.2
Festuca valesiaca+ Koeleria cristata+ Poterium polygamum	3.0	16 (1995)	63	20.3	11.7	68.0	11.0	11.0	78.0	178.1
Festuca valesiaca+ Bromopsis riparia+ Galium ruthenicum	4.0	26 (1986)	71	23.0	13.0	64.0	4.2	4.2	91.6	226.7
Bromopsis riparia+ Festuca valesiaca+ Bromopsis inermis	4.0	26 (1986)	93	16.0	16.0	68.0	5.4	5.4	89.2	248.4
Filipendula vulgaris+ Galium ruthenicum+ Medicago romanica	100.0	29 (1983)	88	19.0	16.0	65.0	3.0	6.0	91.0	206.9
Festuca valesiaca+ Koeleria cristata+ Filipendula vulgaris	10.0	32 (1980)	74	22.0	16.0	62.0	2.0	2.0	96.0	300.0

References

Cottam, G., & Cameron, W. H. (1996). Community Dynamics on an Artificial Prairie. *Ecology, 47*(1), 88-96.

Dzybov, D. S. (1979). Method of accelerated reestablishment of grass biogeocenoses. *Experimental biogeocenology and agrocenosis, Moscow*, 129-131.

Dzybov, D. S. (1980). Sod planting method of hayland formation. Stavropol steppes and meadows. *SNIISH proceedings, 43*, 68-82.

Dzybov, D. S. (2008). Phytocenoses inter-age competition and its experimental study at permanent steppe exposition of botanical garden. Ecological aspects of plant associations in botanical gardens of the South Federal District. Science conference, Krasnodar, 49-63.

Dzybov, D. S. (2001). Method of agrosteppes. Accelerated reclamation of the natural vegetation (p. 40). Guidance manual, Saratov.

Dzybov, D. S. (2010). Agrosteppes. Monograph (p. 256). Stavropol.

Gorbunov, Yu. N., Dzybov, D.S ., Kuzmin, Z. E., & Smirnov, I. A. (2008). *Methodological Recommendations for Botanic Gardens on the Reintroduction of Rare and Threatened Plants* (p. 52). Moscow.

Greene, H. C., & Curtis, J. T. (1953). The re-establishment of prairie in the University of Wisconsin Arboretum, 29, 77-88.

Grigoryev, Al. A., & Kondratyev, K. Ya. (1994). *Modern* state of the global *environment* and natural resources. *Russian Geographical Society Bulletin, 126*(3), l2-26.

Koronovsky, N. V., Khain, V. E., & Yasamanov, N. A. (2008). Historical Geology. Natural sciences (p. 448), MGU, Moscow.

Lavrov, L. I. (1989). Role of natural-geographic factors in the history of Caucasian nations. *Caucasus ethnographic works, 5*(4), 5-9.

Lazukov, G. I. (1989). The Pleistocene in the USSR territory (p. 319). Moscow, Vysshaya shkola.

Mabbut, J. A. (1981). Climate cycles and landscape variability as desert advancing environmental factors. Desertification control by complex development. UNEP Complex Development Committee of the USSR, VINITI, Tashkent, 20-22.

Odum, Y. P. (1986). Ecology. *Moscow, 5*(1), 328.

Salem, B. B. (1993). *Detection of Temporal Environmental* Changes in *Arid Land by Remote Sensing. Case study*: North Coastal desert of Egypt. Proceeding of the 25th International Symposium. Remote Sensing and Global Environmental Change, Graz, Austria.

Schmid, W., & Thomet, P. (l986). Unsere Naturwiesen im Spannungsfeld zwischen Landwirtschaft und Naturschutz. Schweiz. *langn.-Mh., 64*(12), 325-331.

Skripchinsky, V. V. (1973). Artificial reestablishment of destroyed phytocenoses experience. Higher School North Caucasus Science Center Bulletin. *Natural Sciences, 3*, 17-20.

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Characteristics of Vegetation Growing in Indeterminate Fallows in South-Central Zimbabwe

Simbarashe Mudyazhezha[1], Emmanuel Manzungu[1], Tawanda Chimombe[1], Linda Mtali[2], Bruce Tavirimirwa[3] & Sharai Ncube[3]

[1] Department of Soil Science and Agricultural Engineering, University of Zimbabwe, PO Box MP 167, Mt Pleasant, Harare, Zimbabwe

[2] University of Zimbabwe, Marondera College of Agricultural Science and Technology

[3] Department of Animal Science, University of Zimbabwe, PO Box MP 167, Mt Pleasant, Harare, Zimbabwe

Correspondence: Simbarashe Mudyazhezha, Department of Soil Science and Agricultural Engineering, University of Zimbabwe, PO Box MP 167, Mt Pleasant, Harare, Zimbabwe. E-mail: simmudy@hotmail.com

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Abstract

Despite widespread food insecurity in Zimbabwe, there is an increasing amount of agricultural land being left fallow for indeterminate periods of time. The objective of the study was to assess the characteristics of vegetation growing in indeterminate fallows in Chivi district in south-central Zimbabwe. One metre by one metre quadrats were used to assess the species composition of herbaceous species while 8 m × 8 m quadrats were used for woody species. Attributes that were assessed included frequency, abundance, density of different plant species from which were computed diversity indices (Shannon-Weiner index and Shannon evenness index). The density, Shannon-Weiner index and Shannon Evenness index), and species richness of the fallow land sites were significantly lower than those of the uncultivated land. However, there were no significant differences among fallow treatments. Species richness, Shannon Index, and Shannon's Evenness Index showed a weak and non significant correlation with length of the fallow period. Woody species which were cleared during land preparation and repeated weeded during the cultivation years were absent in all fallows regardless of the fallow period.

Keywords: agricultural land, indeterminate fallows, vegetation, species composition, species diversity

1. Introduction

In Zimbabwe, rural areas that are under communal land tenure locally known as communal areas, occupy 16.4 million ha that is equivalent to 42% of the national land and accommodate 70% of the national population (Muir-Leresche, 2004). The livelihoods of the majority of the approximately 6-7 million people that are resident in these areas are agriculture-dependent (Moyo, 2006). However, agriculture is severely constrained by, among other factors, soils that are of inherent poor physical and chemical fertility and low organic matter content (Nyamadzawo et al., 2003). Consequently crop production is severely limited by nutrient deficiencies (Zingore et al., 2007) since most farmers cannot afford to buy the needed inorganic fertilizers (Manzungu & Mtali, 2012). The situation is made worse by low and erratic rainfall with 75% of the area receiving less than 600 mm of rainfall per year (Muir-Leresche, 2006). This is why, on the basis of agro-ecological conditions that are based on rainfall patterns, most of the country's communal lands are designated as only suitable for livestock production (Whitlow, 1985). However, because of the country's colonial history, which has left the majority of the 1 million smallholder farming households without many options, farming continues to be undertaken, albeit with a high failure rate (Muir-Leresche, 2006). For every five agricultural seasons, farmers in communal areas only get good harvest in two seasons.

The poor prospects for successful crop production have resulted in a situation where an increasing proportion of agricultural land in Zimbabwe is being left fallow (Mapfumo et al., 2005). This is a paradox given the widespread food insecurity (Manzungu & Mtali, 2012). To date interventions in Zimbabwe to address the problem of increasing fallow has focused on ways and means of increasing the crop production (Mapfumo et al., 2005). Despite the availability of such measures the proportion of fallow land does not seem to be decreasing. A recent study showed that as much as 50% of the land that was cultivated since the 1980s is fallow for indeterminate periods of time (Manzungu & Mtali, 2012). This phenomenon of agricultural land being left fallow for indeterminate periods of time with no prospect of being re-cultivated again has been reported to be on the increase (Nyoka et al., 2004). Such indeterminate fallows are being used for grazing areas to supplement the generally inadequate grazing areas in terms of quantity and quality which is worsened by disproportionate large livestock numbers (Sibanda & Khombe, 2006). In other countries, fallows have been reported to be sources of fuelwood and timber (Wadsworth, 1997), edible and medicinal fruits (Voeks, 1996) and providers of environmental services such as protection from erosion and atmospheric carbon fixation (Fearnside & Guimaraes, 1996).

Despite the increasing use of indeterminate fallows for grazing in Zimbabwe, there are no known studies that have investigated the characteristic of secondary vegetation as well as its grazing quality. Such a study is critical since most of the information on the subject of vegetation recovery originates from wet subtropical areas (Colon & Lugo, 2006). This paper reports on a study whose objective was to assess the characteristics of the secondary vegetation growing in indeterminate periods of fallow land as well as the forage quality of the grasses found therein. The study was based in an area in south central Zimbabwe that typifies many communal lands of Zimbabwe. The area is characterised by low and erratic rainfall and poor soils, and increasing fallow land that is used for grazing (Manzungu & Mtali, 2012).

2. Materials and Methods

2.1 Site Description

The study was carried out in Chivi district that is located in south central Zimbabwe some 400 km away from the capital city of Harare . It extends from 20° 14' S to 20° 24' S and lies between 30° 13' E and 30° 57' E (Mapanda & Mavengahama, 2011). Chivi district falls in agro-ecological region 5 that receives an annual rainfall of 400-500 mm and is one of the driest in the country (Nyamadzawo et al., 2003). Land pressure in the district is high, with an average size of arable land holding of about 2 ha per household (Mavedzenge et al., 1999).

As a consequence of low and erratic rainfall and poor soil fertility, crop production of even drought tolerant crops, is a challenge. Due to the poor crop yields that are attained, people in the district rely on food aid from the government and non-governmental agencies (Mapanda & Mavengahama, 2011). The research was carried out in ward 28, which in many ways is representative of the district. Field work was conducted in four out of the 19 villages. The total number of households in the four villages amounted to 130.

2.2 Vegetation Characteristics

The study was undertaken in plots of different fallow periods, which constituted the treatments (Table 1). A completely randomized experimental design with three replications was used. A nearby stretch of land that was classified as non-arable (Manzungu & Mtali, 2012) and had not been cleared for agriculture was used as the control.

Table 1. Plots used to study vegetation characteristics in ward 28, Chivi district in south central Zimbabwe

Plot	Length of fallow period (years)
1	0-2
2	3-5
3	6-10
4	11-15
5	16-35
6	Control

Source: Manzungu and Mtali (2012).

Samples of vegetation, that included grasses, herbaceous species, shrubs and woody tree species, were collected in February 2011. A 1m x 1m quadrat was randomly thrown three times in each plot in order to assess grass and herbaceous species. In each plot the number of grasses and herbs species were counted and recorded. An 8 m X 8 m subplot randomly placed was used for the enumeration of shrubs and trees. Across all vegetation types effort was made to identify the species in the field. Samples of those species that could not be identified in the field were preserved and taken to the National Herbarium in Harare for identification. Grass and herbaceous species were uprooted using a knife and entire plants preserved in plant pressers to maintain their colour, roots and flowers. For trees and shrubs, small branches with leaves and fruits, and the bark were cut from trees and preserved in brown envelopes for identification at the National Herbarium.

Species richness (S) was determined by counting the number of species in a plot while diversity was determined by calculating Shannon-Weiner index. The Shannon-Wiener index (H') was calculated using the formula:

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

where s = number of species, and pi = proportion of individuals per species in the community made up of s species with known proportions p1, p2, p3, …., ps. To quantify the evenness component of diversity evenness index or Shannon-evenness index 1 (E1= H'/ ln S) was computed.

Density was calculated as number of individuals divided by area sampled while relative density was determined as (density for a species)/(total density for all species)*100. Normality tests were performed on species richness, Shannon Weiner index, evenness index, frequency and density. After meeting the normality tests, one way Analysis of Variance (ANOVA) was used to test for statistical differences in the above parameters across the various treatments. Hierarchical Cluster Analysis (HCA) using the average linkage method was performed to determine the similarities amongst the treatments with regards to species composition.

3. Results

3.1 Vegetation Characteristics

3.1.1 Species Richness, Diversity and Dominance

A total of 68 plant species were collected and identified from the fallow fields and the woodland of which 15 species were grasses, 33 herbs, 11 shrubs and 8 trees (Table 2). Fallow fields were dominated mainly by grasses and herbs, with sparse tree species around the edges of fields near water-ways while the non-arable control was dominated by trees.

Table 2. The mean densities of grasses, herbs and trees in different plots in Chivi district, south central Zimbabwe

Fallow period (years)	Grasses (per m^2)	Herbs (per m^2)	Trees (per ha)
0-2	248±47.31	97±23.87	0
3-5	235±69.05	51±17.43	0
6-10	483±73.71	32±9.14	0
11-15	145 ±55.82	99±29.65	0
16-35	189 ±44.56	23±7.61	0
Control	62±14.74	47±11.02	307±61.89

The total number of grass species showed an increase with fallow period up to 6-10 years where a peak value of 483 grasses per m^2 was observed. This value was slightly more than double compared to that of 0-2-year fallow period and almost eight times that of the control. There were significant differences in the number of herb species among the six treatments ($P < 0.05$). There were more herb species in the control than in the fallows. All fallow stands did not have any shrub or tree. A total of 11 shrubs and 9 tree species were found in the control.

Table 3. Mean densities of individual grass species (m^{-2}) across the six plots (5 fallow periods and control) in Chivi district in south central Zimbabwe

	Fallow period (Years)					
Species	0-2	3-5	6-10	11-15	16-35	Control
Perotis paterns	57±19.67	29±20.98	49±23.93	64±38.71	51±30.37	0
Cynodon dactylon	163±117.08	187±91.12	7±7.65	13±7.55	31±16.68	9±3.35
Digitaria eriantha	20±8.67	8±5.16	14±8.09	40±23.93	20±13.63	0
Digitaria longiflora	5±5.73	5±5.73	7±6.84	1±1.23	1±1.73	13±6.58
Brachiaria	1±1.34	0	54±42.55	0	61±24.17	0
Pogonarthria squarossa	0	4±3.94	6±4.01	6±4.21	1±1.73	0
Hyperthelia dissoluta	0	0	12±7.98	20±21.80	20±24.99	0
Panicum maximum	0	0	0	0	0	20±5.32
Mariscus macer	0	0	0	0	0	16±11.05
Urochloa mosambiausis	0	0	0	0	0	2±2.5

Table 3 shows that *Panicum maximum, Mariscus macer and Urochloa mosambiausis* were absent in all fallow lands. *Panicum maximum* was found to be the most dominant species in the control while *Cynodon dactylon* was the most dominant grass species in the 0-2 year and 3-5 year fallows with densities of 163 and 187 individual stems per m^2 respectively. *C. dactylon* was also present in the control with a density of 3 stems per m^2. *Pogonarthria squarossa* only appeared after 2 years of fallow while *Hyperthelia dissoluta* was only present from 6 years of cessation of cultivation. Both were absent in the control. *Perotis paterns*, was the second most dominant grass species in the 0-2 yr and 3-5 year fallow, the most abundant in the 6 year fallow onwards and absent in the control (Table 3). The densities of grasses found in the fallow did not show any pattern with fallow period.*Richardia scabra* was the most abundant non grass herb in the 0-2 yr and 3-5 year fallows. It was also present in the control with 3 individuals per m^2. *Zonia gluchidiata* dominated the 6-10 yr and 11-15 year fallows. *Phyllanthus fracternus, Acanthacea fimbriata, Acanthacea spp* and *Malvacea species* were only present in the control. *Rubiacea species* and *Bergia decumbens* were found in fallow periods of 6 years upwards but were absent in the control. *Triumfetta rhomboidea* was present in fallows younger than 6 years. It was the non-grass herb with the highest density in the control with 16 individuals per m^2. *Zonia gluchidiata* was the most abundant species in the fallows and the least abundant in the control with 2 stems per m^2. *Indigofera astragalina* was the second most densely populated in the fallows while *Chamecrista absis* was the fourth most abundant species in the fallows. Both *I. astragalina* and *C. absis* were absent in the control (Table 4).

Table 4. Mean densities of non grass herbaceous species (m^{-2}) found across the six plots in Chivi district in south-central Zimbabwe

	Fallow period (Years)					
Species	0-2	3-5	6-10	11-15	16-35	Control
Richardia scabra	23±14.96	31±17.44	2±1.57	6±4.65	7±5.41	3±3.36
Chamecrista absus	19±16.59	0	0±0.74	1±0.84	3±2.65	0
Indigofera astragalina	28±22.19	4±2.49	2±2.57	13±14.96	9±9.09	0
Ocimum americanum	2±2.58	3±3.76	1±1.34	0	0	0
Zonia gluchidiata	22±17.53	2±1.68	16±11.51	68±43.67	1±1.73	2±2.88
Triumfetta rhomboidea	1±1.34	1±1.34	0	0	0	16±9.24
Rubiacea spp	0	0	1±1.34	5±2.68	1±1.5	0
Bergia decumbens	0	0	2±1.3	3±2.83	1±1.5	0
Phyllanthus fracternus	0	0	0	0	0	2±1.89
Acanthacea fimbriata	0	0	0	0	0	4±3.73
Acanthacea spp	0	0	0	0	0	4±4.04
Malvacea species	0	0	0	0	0	4±3.51

Table 5 shows the species richness, diversity and dominance of different species across the treatments. The control was found to have significantly higher species richness (S) than the fallows lands ($P < 0.05$). This was because in addition to having grasses and herbs, it was the only piece of land which had woody species. The control was also found to have significantly higher ($P < 0.05$) density-based Shannon-Wiener diversity index values (H') and density-based evenness index values (Table 5). The different fallow periods did not show significant variation in dominance ($F=2.27$, $P = 0.085$). There were, however, no significant differences in grass species numbers among the six treatments ($P < 0.05$). Shannon-Weiner index and Shannon evenness index also did not show any significant variation among the fallow lands of different ages.

Table 5. Means of species richness (S), Shannon-Weiner index (H'); Shannon Evenness Index (e^H/S) in different plots in ward 28 of Chivi district, south-eastern Zimbabwe

Period (yrs)	S	H'	e^H/S
0-2	15	1.24	0.6
3-5	19	0.99	0.4
6-10	20	1.42	0.71
11-15	15	1.18	0.57
15-35	16	1.49	0.6
Undisturbed	41	2.42	0.56

Hierarchical Cluster analysis could only separate the treatments into two clusters. Cluster one had all the sites from the fallow treatments while cluster two exclusively contained the sites from the control. The control treatments separated from the fallow treatments because of the presence of the woody species which were non-existent in the fallows. There were no differences among the treatments in relation to species composition among the fallows of different ages.

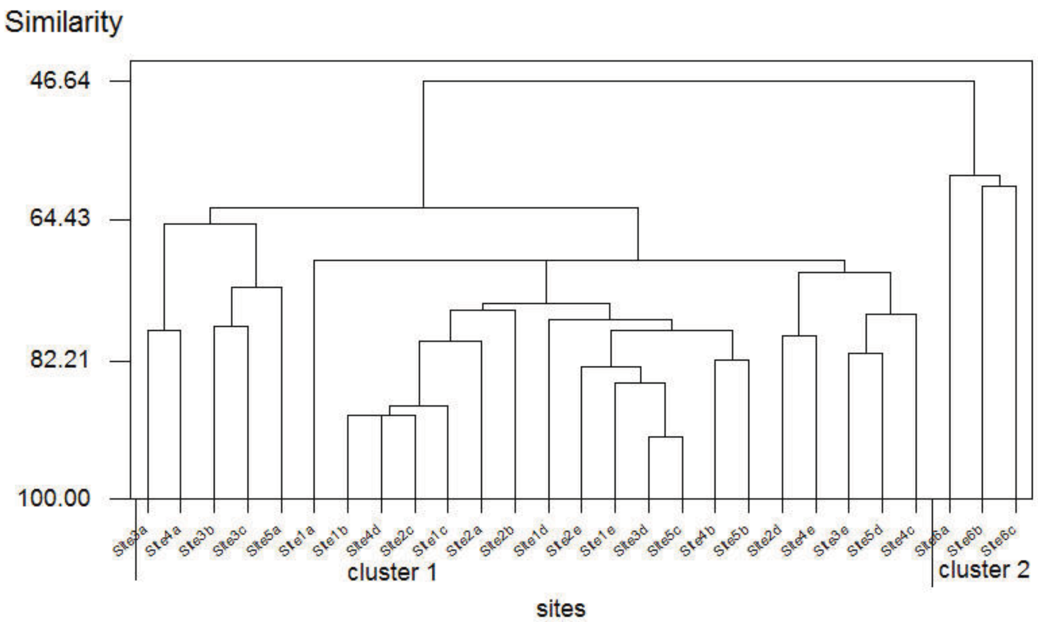

Figure 1. Dendogram of Hierarchical Cluster Analysis (HCA) based on presence/absence data for vegetation growing in different fallows in Chivi District, south cental Zimbabwe

Regression analyses of the diversity indices (Species Richness (S), Shannon Index, Simpson Index, and Shannon's Evenness Index) along the developmental stages indicated weak correlations which were not significant. Species richness was found to have a weak negative correlation with age of the fallow ($R^2 = 0.056$, P= 0.70). Shannon Index and the Shannon Evenness Index showed weak and non significant positive correlation with length of the fallow period (Shannon Index: R^2 = 0.39, P = 0.259; Evenness Index: R^2 = 0.047, P = 0.726). Simpson Index had a more positive correlation. However, the relationship with lengthy of the fallow period was not significant (R^2 = 0.69, P = 0.082) (Figure 2).

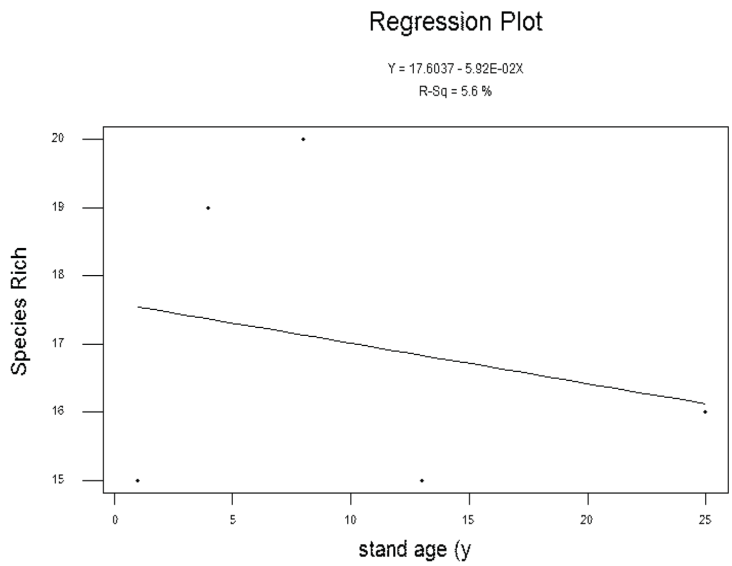

Figure 2. Correlation between fallow period and species richness for vegetation growing in different fallows in Chivi District, south cental Zimbabwe

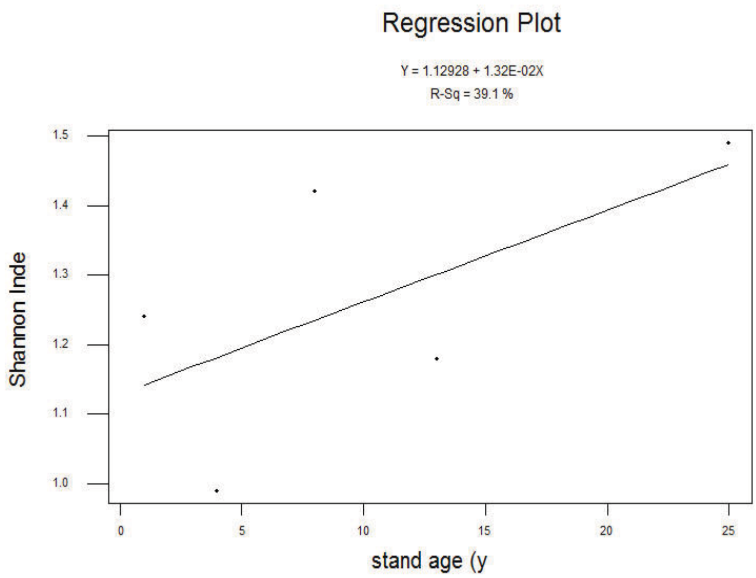

Figure 3. Correlation between fallow period and Shannon index for vegetation growing in different fallows in Chivi District, south cental Zimbabwe

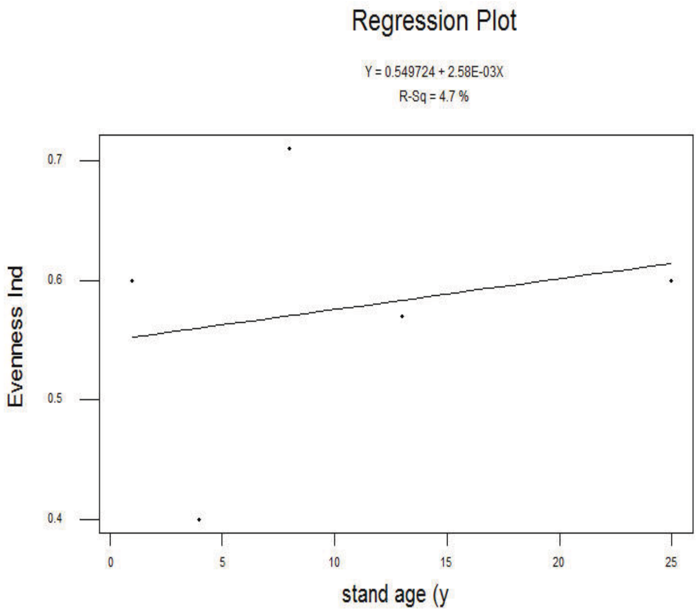

Figure 4. Correlation between fallow period and Shannon's Evenness index for vegetation growing in different fallows in Chivi District, south cental Zimbabwe

4. Discussion

4.1 Vegetation Characteristics

The structure and composition of fallow vegetation is influenced by factors such as: the method of clearing; length; intensity of the preceding cultivation period (which among other things determine the number and vigour of active stubs); frequency of fire; availability of seed sources and dispersers; the length of the fallow period; the soil (texture, structure, drainage and fertility) as well as the grazing, browsing and trampling intensity (Ohler, 1985). These interacting factors lead to different vegetation types, dominated (apart from stand-overs) by annual and/or perennial grasses and/or thorny or thornless shrubs or trees (Guariguata & Ostertag, 2001). However, these factors are site-specific (Guariguata & Ostertag, 2001), hence the need for detailed local assessment as was carried out in this study. This explains why patterns of vegetation observed in this study were quite different from other fallow recovery studies. Most natural fallows in high rainfall areas manage to develop secondary vegetation which includes shrubs and trees as years of fallow progress (Ngobo, 2004; Hooper et al., 2005). This was however, hindered in fallows found in Chivi district because of the low and erratic rainfall, poor soil fertility and over grazing by livestock.

The fact that more species were found in the control than in the fallows and there were neither trees nor shrubs in the fallow fields can be attributed to complete removal of coppicing trees in preparation for cultivation. After cutting, burning and weeding have largely eliminated mechanisms of on-site regeneration, the only way for woody species to re-establish is by seed dispersal. This dependence on seed dispersal seriously slows succession because forest species are not readily dispersed to fallow lands. According to Uhl (1987) 54% of the 192 Ocotea costulata diaspores placed in the farm study site were removed (presumably by animals) within four days, and all had disappeared after one month. *Jessenia bataua* also showed a similar trend, with all but one of 192 diaspores being removed within one month of placement in the farm site. Uhl (1987) concluded that many more woody individuals could have been packed onto the main study site if propagules were available.

In addition to poor seed dispersal, competition with herbaceous vegetation (Holle et al., 2000), soil compaction and low soil nutrients (Reiners et al., 1994), or photoinhibition can inhibit the initial establishment of woody species in many tropical sites (Aide et al., 2000). Forbs and grasses are able to germinate, flower and set seed in the interval between weedings and, therefore, can build up high densities and large seed banks in fallows. In contrast, the woody pioneer species, which establish from the seeds surviving the burn, are weeded from the site before they have had time to produce seeds (Uhl, 1987). This could possibly explain the absence of wood species since they were weeded out annually during the cultivation period before they could produce seeds.

Soil water availability is generally considered critical factor for determining the development of woody vegetation (Frensham & Holman, 1999). Classical models suggest that water is the key resource in determining tree densities although other important variables like fire and grazing can maintain the population below their climatic equilibrium (van Wijk & Rodriguez-Iturbe, 2002). According to the savannah literature, grasses utilize the topsoil water while tree roots have exclusive access to deeper water, creating a clear niche separation (Weltzin & Coughenour, 1990). This allows grasses and trees to co-exist without one competitively excluding the other for soil moisture. The high numbers of grasses observed in the control supports this water resource partitioning theory.

The presence of more herbs in the woodland could have been a result of the presence of long rooted trees and shrubs. These have the capacity to pump leached nutrients making them available (through decomposition of their leaf litter and other organic components) to other plants growing on the surface and nitrogen fixing species increasing the amount of nitrogen available for the herb species (Nepstad et al., 1996). The varied growth forms of herb species, which are upright, trailing or creeping also allows them to colonize woodlands with more competitive shrubs and trees as they can compete well for light and nutrients (McClanahan & Wolfe, 1993).

The higher species richness in the control compared to fallow lands can be explained by the suitable conditions for regeneration in the former. The remaining tree species created favourable conditions. Under canopies germination and establishment of different (tree) species is higher since seedlings are protected from high temperatures and moisture stress as well as being provided with nutrients through falling and decomposing leaves (Holl et al., 2000). The presence of higher organic matter content in the control due to decaying litter could also have facilitated the establishment of herbs and trees. Organic matter plays an important role in nutrient and moisture retention and favours the establishment of wide leaved herbaceous plants which are more sensitive to low moisture levels compared to grasses (Holl et al., 2000). The lack of significant differences in species richness and diversity (Shannon-Weiner Index) among the fallows showed that species richness was not necessarily a function of time only but may be affected by other environmental conditions such as soil fertility and continuous disturbance such as grazing.

The dominance of grasses in fallow fields such as *Cynodon dactylon* and *Perotis paten* showed that they were adapted to sandy soils with low nutrient levels. *Cynodon dactylon* is a grass which is adapted over a broad range of soil pH (4.5-8.5) (http:/www.tropicalforages.info/key/Forages/Media/Html/Cynodon-dactylon.htm, May 2011). This explains its presence in acidic soils of Chivi district. *C. dactylon* is a rhizomatous perennial grass that propagates mainly vegetatively, through stolon and rhizome fragmentation (Fenandez, 2003). It is also a drought and salt tolerant species. A major component of the drought resistance is the development and maintenance of a deep, extensive and viable root system and proline content (Marcum et al., 1995; Carrow, 1996, Huang et al., 1997b). This enables it to grow it Chivi, which is a drought prone area with annual rainfall erratic and usually below 650 mm. In addition to the above characteristics, *C. dactylon* is extremely tolerant of heavy grazing, thus explaining its abundance in the overgrazed areas of Chivi.

C. dactylon is a low-growing C4 weed that is highly sensitive to shading (Whittaker, 2001). Plants with a C_4 metabolism have been shown to have physiological advantages in productivity over C_3 species under conditions of higher light intensities, high leaf temperatures and reduced stomatal conductances (Chapin et al., 1987). However, *C. dactylon* lacks some specific attributes to compete against tall crops since it is a low growing herb. This species therefore shows severe biomass reductions and highly plastic morphological changes in response to resource availability. Severe biomass reduction of *C. dactylon* was observed as a result of light competition by maize even when nitrogen and water were not limited (Guglielmini & Satorre, 2002). This shade intolerance could possibly explain its absence in the control area where canopy cover from the trees causes severe shading. Tillage has been mentioned as important factor enhancing colonization of *C. dactylon* in the pampas cropping systems (Guglielmini & Satorre, 2002). This could be the result why the fallow lands are infested by *C. dactylon* since they were once under tillage.

Perotis paten was also dominant in the fallows due to its ability to produce a large proportion of viable seeds which are well adapted to dry land conditions. It has also thick stems just above the roots which concentrate nutrients and protect the meristems when conditions are too dry, which allows the grass to re-sprout quickly when favorable conditions are restored (Whittaker, 2001). It is a perennial weed native to South America and prevalent in disturbed soils of southern coastal plains of the U.S., Mexico, South Africa, Indonesia and Hawaii. (Chandran & Singh, 2003). This explains its prevalence in the fallows and absence in the control where disturbance was minimum.

Grasses like *Panicum maximum* and *Urochloa mosambioausis*, which were not found in the fallows and least abundant in the control because they are less adapted to low rainfall conditions and poor fertile soils. These grasses were present in the control area where the conditions were more moist and fertile than those in the fallows. Such conditions are required to reach the reproductive stage (Whittaker, 2001). *Pacimum maximum* (guinea grass) grows on a variety of well drained soils of good fertility and in areas receiving more than 900 mm rainfall (Aganga & Tshwenyane, 2004). Aphelandra aurantiaca is an understorey herb (Calvo-Irabien & Islas-Luna, 1999). This explains why it is absent in the fallows where there are no trees and yet present in the control where there are trees to provide cover. Malvaceae is a polymorphic perennial herb of open moist, sunny to partly shaded riverine habitats (Spooner et al., 1985), hence its presence in the control which is an open and moist woodland.

Higher grass densities were found in the fallows than in the control. Although the effect is not consistent, the most common effect of trees in the tropical grasslands is to reduce the herbaceous yield beneath the canopy (Mordelet & Manaut, 1995). While there is substantial evidence that trees augment nutrient levels in the soil beneath their canopies, increases in herbaceous yield do not necessarily result when there is nutrient enrichment. If available light is insufficient for photosynthesis, no positive response can be expected (Mordelet & Manaut, 1995). Therefore, while in the control, there is likely to be high nutrient levels, inadequate light reduces the grass biomass. The interaction between trees and grasses is affected by a variety of factors. These factors include rainfall (Belskey et al., 1993a) and grazing (Belsky et al., 1993b). Differences in species composition under and away from savanna trees are more distinct in low than in high rainfall zones suggesting that environmental gradients are stronger in habitats where effects of radiant energy regime or root competition have a greater influence in species interactions (Whittaker, 2001). This may explain why some species are found in the fallows which are open grassland and not in the control which is a wooded savanna. Grazing and browsing pressure may also alter patterning of herbaceous vegetation in savannas. In heavily grazed savannas, few differences between tree-crown and grassland zones may occur (Whittaker, 2001).

5. Conclusions

Results of the this study indicated that in the dry region of Chivi district, a fallow period of up to 30 years had no effect on vegetation characteristics (richness and diversity). This suggests that regeneration of vegetation is not only a function of time but may be due to other environmental conditions such as poor rainfall and low fertility as well as continuous grazing, which resulted in trees and shrubs failed to establish in Chivi fallow fields over a period of 30 years.

References

Aganga, A. A., & Tshwenyane, S. O. (2004). Potentials of guinea grass (*Panicum maximum*) as forage crop in livestock production. *Pakistan Journal of Nutrition, 3*(1), 1-4. http://dx.doi.org/10.3923/pjn.2004.1.4

Aide, T. M., & Cavelier, J. (1994). Barriers to tropical lowland forest restoration in the Sierra Nevada de Santa Marta, Colombia. *Restoration Ecology, 2*, 219-229. http://dx.doi.org/10.1111/j.1526-100X.1994.tb00054.x

Aide, T. M, Zimmerman, J. K., Pascarella, J. B., Rivera, L., & Marcano-Vega, H. (2000). Forest regeneration in chronosequecnce of Tropical Abandoned pasture: Implications for Restoration Ecology. *Restoration Ecol., 8*, 328-338. http://dx.doi.org/10.1046/j.1526-100x.2000.80048.x

Amundson, R. G., Ali A. R., & Belsky, A. J. (1995). Stomatal responsiveness to changing light intensity increases rain-use efficiency of below-crown vegetation in tropical savannas. *J. Arid. Environ., 29*, 139-53. http://dx.doi.org/10.1016/S0140-1963(05)80085-0

Belsky, A. J., Mwonga, S. M., Amundson, R. G., Dux-bury, J. M., & Ali, A. R. (1993a). Comparative effects of iso-lated trees on their undercanopy environments in high- and low-rainfall savannas. *Journal of Applied Ecology, 30*, 143-155. http://dx.doi.org/10.2307/2404278

Belsky, A. J., Mwonga, S. M., & Duxbury, J. M. (1993b). Effects of widely spaced trees and livestock grazing on understory environments in tropical savannas. *Agroforestry Systems, 24*, 1-20. http://dx.doi.org/10.1007/BF00705265

Calvo-Irabien, M. L., & Islas-Luna, A. (1999). Predispersal Predation of an Understory Rainforest Herb Aphelandra aurantiaca (Acanthaceae) in Gaps and Mature Forest. *American Journal of Botany, 86*(8), 1108-1113. http://dx.doi.org/10.2307/2656973

Carrow, R. N. (1996). Drought resistance aspects of turfgrasses in the southeast: Root–shoot responses. *Crop Sci., 36*, 687-94. http://dx.doi.org/10.2135/cropsci1996.0011183X003600030028x

Chandran, R. S., & Singh, M. (2003). Survey and control of Brazil pulsey (Richardia Brasiliensis) in Florida Citrus. *Proc Fla State Hort Soc., 116*, 211-214.

Chapin, F. S., Bloom, A. J., Field, C. B., & Waring, R. H. (1987). Plant Responses to Multiple Environmental Factors. *Bioscience, 37*(1), 49-57. http://dx.doi.org/10.2307/1310177

Colón, S. M., & Lugo, A. E. (2006). Recovery of a Subtropical Dry Forest After Abandonment of Different Land Uses. *Biotropica, 38*(3), 354-364. http://dx.doi.org/10.1111/j.1744-7429.2006.00159.x

Fearnside, P. M., & Guimara˜ es. W. M. (1996). Carbon uptake by secondary forests in Brazilian Amazonia. *Forest Ecology and Management, 80*, 35-46. http://dx.doi.org/10.1016/0378-1127(95)03648-2

Fensham, R. J., & Holman, J. E. (1999). Temporal and spatial patterns in drought related tree dieback in Australian savanna. *J. Appl. Ecol., 36*, 1035-1050. http://dx.doi.org/10.1046/j.1365-2664.1999.00460.x

Fernandez. O. N. (2003). Establishment of Cynodon dactylon from stolon and rhizome fragments. *Weed Research, 43*, 130-138. http://dx.doi.org/10.1046/j.1365-3180.2003.00324.x

Guariguata MR, Ostertag R (2001). Neotropical secondary succession: Changes in structural and functional characteristic. *Forest Ecol. Manage., 148*, 185-206. http://dx.doi.org/10.1016/S0378-1127(00)00535-1

Guglielmini, A. C., & Satorre, E. H. (2004). The effect of non-inversion tillage and light availability on dispersal and spatial growth of Cynodon dactylon. *Weed Research, 44*, 366-374. http://dx.doi.org/10.1111/j.1365-3180.2004.00409.x

Holl, K., Loik, M., Lin, E., & Samuels, I. (2000). Tropical montane forest restoration in Costa Rica: Overcoming barriers to dispersal and establishment. *Restoration Ecology, 8*, 339-349. http://dx.doi.org/10.1046/j.1526-100x.2000.80049.x

Hooper, E., Legendre, P., & Condit, R. (2005). Barriers to forest regeneration of deforested and abandoned land in Panama. *Journal of Applied Ecology, 42*, 1165-1174. http://dx.doi.org/10.1111/j.1365-2664.2005.01106.x

Huang, R., Duncan, R., & Carrow, R. N. (1997b). Root spatial distribution and activity of four turfgrass species in response to localized drought stress. *Int. Turf. Soc. Res. J., 8*, 681-9.

Manzungu. E., & Mtali, L. (2012). An Investigation into the Spatial and Temporal Distribution of Fallow Land and the Underlying Causes in Southcentral Zimbabwe. *Journal of Geography and Geology, 4*(4), 62-75. http://dx.doi.org/10.5539/jgg.v4n4p62

Mapanda, F., & Mavengahama, S. (2011). Assessment of selected soil nutrients and irrigation water quality in the dryland area of Chivi District, Zimbabwe. *Scientific research and Essays, 6*(14), 2918-2927.

Mapfumo, P., Mtambanengwe, F., Giller, K. E., & Mpepereki, S. (2005). Tapping indigenous herbaceous legumes for soil fertility management by resource-poor farmer in Zimbabwe. *Agriculture Ecosystem and Environment, 109*, 221-233. http://dx.doi.org/10.1016/j.agee.2005.03.015

Marcum, L. B., Engelke, M. C., Morton, S. J., &White, R. H. (1995). Rooting characteristics and associated drought resistance of zoysia grass. *Agron. J., 87*, 534-8. http://dx.doi.org/10.2134/agronj1995.00021962008700030023x

Mavedzenge, B. Z., Murimbarimba, F., & Mudzivi, C. (1999). Experiences of farmer participation in soil fertility research in southern Zimbabwe. Manag. *Afr. Soils, 5*, 19.

McClanahan, T., & Wolfe, R. (1993). Accelerating forest succession in a fragmented landscape: the role of birds and perches. *Conservation Biology, 7*(2), 279-288. http://dx.doi.org/10.1046/j.1523-1739.1993.07020279.x

Mordelet, P., & Menaut, J. C. (1995). Influence of trees on above-ground production dynamics of grasses in a humid savanna. *J. Veg. Sci., 6*, 223-28. http://dx.doi.org/10.2307/3236217

Moyo, S. (2006). The evolution of Zimbabwe's land acquisition. In M. Rukuni, P. Tawonezvi, C. Eicher, M. Munyuki-Hungwe & P. Matondi (Eds.), *Zimbabwe's agriculture revolution revisited* (pp. 144-160). Harare: University of Zimbabwe Publications.

Mpofu, I. D. T. (2005). Nutritional biochemistry laboratory manual, Department of Animal science, University of Zimbabwe

Muir-Leresche, K. (2006). Agriculture in Zimbabwe. In M. Rukuni, P. Tawonezvi, C. Eicher, Munyuki-Hungwe & P. Matondi (Eds.), *Zimbabwe's agricultural revolution revisited* (pp. 94-114). Harare: University of Zimbabwe Publications.

Muir-Leresche, K. (2004). Agriculture in Zimbabwe. In M. Rukuni, P. Tawonezvi & E. Eicher (Eds.), *Zimbabwe agricultural revolution,* Harare, University of Zimbabwe Publications.

Nepstad, D. C., Uhl, C., Pereira, C. A., & Cardosa da Silva, J. M. (1996). A comparative study of tree establishment in pasture and mature forest of Eastern Amozonia. *Oikos*, 76. http://dx.doi.org/10.2307/3545745

Ngobo, Martine, P., Weise, Stephan, F., & Mcdonald, Morag A. (2004). Revisiting the Performance of Natural Fallows in Central Africa. *Scandinavian Journal of Forest Research, 19*, S4, 14-24. http://dx.doi.org/10.1080/14004080410034029

Nyamadzawo, G., Nyamugafata, P., Chokowo, R., & Giller, K. E. (2003). Partioning of simulated rainfall in a kaolinitic soil under improved fallow-maize rotation in Zimbabwe. *Agroforestry Systems, 59*, 207-214. http://dx.doi.org/10.1023/B:AGFO.0000005221.67367.fd

Nyoka, R., Chikumba, N., Chakoma, I., Mazaiwana, P., Mukombe, N., & Magwenzi, N. (2004). Evaluation and screening of forage legumes for sustainable integration into Crop-Livestock farming systems of Wedza District. In A. Whitbread & B. C. Pengelly (Eds.), *Tropical Legumes for Sustainable Farming Systems in Southern Africa and Australia* (pp. 58-64). Australian Centre for International Research (ACIAR) Proceedings No. 115.

Reiners, W. A., Bouwman, A. F., Parsons, W. F. J., & Keller, M. (1994). Tropical rain forest conversion to pastures: changes in vegetation and soil properties. *Ecological Applications, 4*, 363-377. http://dx.doi.org/10.2307/1941940

Sibanda, S., & Khombe, C. (2006). Livestock research and development. In M. Rukuni, P. Tawonezvi, Eicher, C., Munyuki-Hungwe, & P. Matondi (Eds.), *Zimbabwe's agricultural revolution revisited* (pp. 439-458). Harare: University of Zimbabwe Publications.

Spooner, D. M, Cusick, A. W., Hall, G. F., & Baskin, J. M. (1985). Observations on the distribution and ecology of Sida Hermaphrodita (1) Rusby (Malvaceae) *SIDA* 11(2), 215-225.

Uhl, C. (1987). Factors controlling succession following slash and- burn agriculture in Amazonia. *Journal of Ecology, 60*, 675-695.

Van Wijk, M. T., & Rodriguez-Iturbe, I. (2002). Tree-grass competition in space and time: insights from a simple cellular automata model based on ecohydrological dynamics. *Water Resource Research, 38*, 18.11-18.15.

Vincent, V., & Thomas, R. G. (1961). An Agricultural survey of Southern Rhodesia. Part I. The Agro-ecological survey. Government Printer. Salisbury, Federation of Rhodesia and Nyasaland, p. 147.

Voeks, R. A. (1996). Tropical Forest Healers and Habitat Preference. *Economic Botany, 50*(4), 381-400. http://dx.doi.org/10.1007/BF02866520

Wadsworth, F. H. (1997). Forest production for tropical America. Agriculture Handbook 710. U.S. Department of Agriculture, Forest Service, Washington, D.C.

Weltzin, J. F., & Coughenour, M. B. (1990). Savanna tree influence on understorey vegetation and soil nutrients in north-western Kenya. *J. Veg. Sci., 1*, 325-334. http://dx.doi.org/10.2307/3235707

Whitlow, R. (1985). Conflicts in Land Use in Zimbabwe: Political, Economic and Environmental Perspectives. *Land Use Policy, 2*(4), 309-322. http://dx.doi.org/10.1016/0264-8377(85)90029-8

Whittaker, J. B. (2001). Insects and plants in a changing atmosphere. *Journal of Ecology, 89*(4), 507-518. http://dx.doi.org/10.1046/j.0022-0477.2001.00582.x

Zingore, S., Murwira, H. K., Delve, R. J., & Giller, K. E. (2007). Influence of nutrient management strategies on variability of soil fertility, crop yields and nutrient balances on smallholder farms in Zimbabwe. *Agriculture, Ecosystems and Environment, 119*, 112-126. http://dx.doi.org/10.1016/j.agee.2006.06.019

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Prediction of Remediation Rates of Microbes in Polluted Crude Oil Soil Samples

B. C. Okoro[1], O. A. Nwadike[1] & J. C. Agunwamba[2]

[1] Department of Civil Engineering, Federal University of Technology Owerri, PMB 1526, Owerri, Nigeria

[2] Department of Civil Engineering, University of Nigeria, Nsukka, Nigeria

Correspondence: B. C. Okoro, Department of Civil Engineering, SEET, Federal University of Technology Owerri, PMB 1526, Imo State, Nigeria. Tel: 234-083-508-6235. E-mail: bc1okoro@yahoo.com

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Abstract

The rate of removal of Total Petroleum Hydrocarbon Content (TPHC) of a crude oil polluted land was investigated using field experimental data generated from the Research Farm soil at the Federal University of Technology Owerri, Imo State, Nigeria. The soil was artificially polluted in the laboratory with crude oil - bonny light - with specific gravity of 0.8323. Petroleum contaminant present in the soil was 230 mg per kilogram of soil. The treatment variables used include: inorganic fertilizer (NPK 20:10:10), poultry manure, cow dung and a mixture of the three in equal proportion. A natural treatment was allowed to occur as the control experiment. fungi as well as bacteria played an important role in the degradation of petroleum hydrocarbon. The identified crude oil degrading Fungi are *Penicillium notatum*, *Mucor spp*, *Rhizopus stolonifer* and *Penicillum caseicolum* with *P. notatum* and *P. caseicolum* (*penicillum spp*) as the strongest fungi degraders. The identified degrading Bacteria are *Pseudomonas putida* and *Bacillus subtilis*. These can therefore be isolated and cultured and then employed on remediation sites either as indigenous or foreign degrading microbes in the engineering of bioremediation of crude oil polluted soil using the best engineering techniques. The treatment with mixture of treatment variables proved to be a better option from the results obtained with 82.38 mg/kg after 9 weeks of remediation followed by fertilizer, 83.13 mg/kg and 86.75 mg/kg for poultry manure. Cow dung had 105.5 mg/kg and the control had least with 204.50 mg/kg.

Keywords: total petroleum hydrocarbon content, bioremediation, microbial analysis, organic species, polluted soil

1. Introduction

Globally, there is a growing concern over environmental pollution and it's management. The three major areas of environmental pollution include: water, air and land. One of the major causes of this environmental pollution in Nigeria is as a result of hydrocarbon exploitation and exploration (Okwuosha, 2000). This has led to the degradation of farmlands, pollution of surface and ground waters as well as air due to gas flaring. The natural recovery of crude oil polluted land is slow. Communities affected are denied meaningful and economic use of their lands a long time. Hence remediation was brought about. Remediation has been defined as "the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health or the environment which can also lead to quick recovery of the affected land" (Dodman, 1994; Ebuehi et al., 2005).

Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms. A more expansive definition from the Joint Research Council Review of Bioremediation Research in the UK published in February 1999, defined bioremediation as being: "The elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, to minimize the risk to human health and the environment". The replacement of 'microorganisms' with 'biological processes' reflects the inclusion of the use of plants to include phytoremediation processes. Bioremediation processes enhance the activities of indigenous microbes, such as bacteria, via the addition of oxygen and nutrients to degrade hydrocarbon to water and harmless gases like carbon dioxide. To remediate petroleum contaminated sites, we need a low-cost, low input treatment alternative to use in conjunction with existing methods. In general, indigenous soil microbiota can degrade petroleum compounds. When soils fail to bioremediate at optimum rates, it is often a function of the water solubility of the compound and environmental limitations imposed on the microbes. Major limitations to the microbiota are temperatures that are too high or too low, excess or deficient water, insufficient or excessive nutrients, insufficient carbon in a form that microorganisms can use, poor mixing or distribution of the petroleum in the soil, and, for aerobic microorganisms, lack of oxygen (O_2). The relative effectiveness of different treatment systems will vary over time. For example, lack of oxygen has been believed to be the primary limitation at depth; thus, air-injection technologies are commonly employed to overcome this. However, it is now well established that subsurface (relatively deep) microbial activity is common, and anaerobic biodegradation of water-soluble petroleum takes place without the need to inject air if alternate electron acceptors, such as oxidized species of iron or nitrogen (such as nitrate), are available.

Bioremediation treatments are successful when limitations are overcome. The key problem, however, is identifying and implementing the most cost-effective means of doing this at sites. Two important aspects in comparing low cost to more costly alternatives are time constraints and monitoring difficulties. In comparing treatments, it is important to measure effectiveness over time.

The pollution of soil, upland pollution, directly affects the quality of water due to runoff washing pollutants from soil surface into natural channels. Runoff carrying hydrocarbons and other contaminants from polluted land enters into natural channels thereby polluting the water. Treatment of such water for municipal and any local use becomes more expensive and challenging. Even the use of the water for ordinary domestic, agricultural, recreational and industrial uses may be seriously hindered. The prevention of such occurrence, therefore, is most advocated than the cleansing or expensive treatment of polluted water. Quick remediation of oil polluted land is therefore very relevant in maintaining pollution free surface water in the oil producing and oil polluted environments. Allowing crude oil polluted soil to recover naturally is dangerous to shallow water tables and open wells. Seepage down the soil though may be slow, can contaminate a whole source of water for a household or a whole community.

2. Material Studied

This study was done by carrying out a field study on bioremediation to ascertain the degrading elements in the bioremediation technology and comparing the field data generated with other similar works for the purpose of comparative analysis of the probable time of petroleum contaminant removal from a polluted site using different treatment variables.

2.1 Experimental Design

The study was done for a period of eighteen (18) weeks. Polluted crude oil soil samples were placed into five (5) different containers, of similar size and geometry, dimensioning 17 cm (height) and 18.5 cm (diameter). The 5th container, was used for the control experiment (CT). 5 kg of polluted soil was placed in each of the containers and were all exposed to the same atmospheric and environmental conditions.

(CD)	(PM)	(FZ)	(MX)	(CT)
Cow Dung	Poultry Waste	Inorganic fertilizer	Synergy /Mixture	Control Experiment

Figure 1. Layout of the experimental design

2.2 Soil Collection and Pollution

The soil used in the study was collected from the FUTO Research Farm from 15 cm to 20 cm depth with shovel. The soil was collected into containers and was taken to the site for treatment (greenhouse treatment). The soil was air dried for four days and 25 kg of soil was polluted with 1litre of crude oil (Bonny light) with specific gravity of 0.8343 leaving about 230 mg/kg of soil. The crude oil was allowed to cover the surface of the soil completely. This was to simulate a natural field condition of major spill. The pollution is equivalent of 73,800 litres per hectare and 200 cm^{-3} per 5 kg of soil.

3. Area Descriptions

The experiment was carried out as an ex-situ treatment of polluted soil obtained from the Research Farm of Federal University of Technology Owerri (FUTO), Imo State, Nigeria. The study area is located in Owerri, Imo state and lies between latitude 5º 22' 51.5" N and longitude 6º 59' 39.3" E, with an elevation of 61 m. It is a humid tropical environment with average annual rainfall of 2400 mm and 3 distinct months of dryness (December to February). The mean daily temperature is about 27 ºC. The soils are derived from coastal plain sands called acid sands - Benin formation (Orajaka, 1975).

4. Methods

4.1 Soil Treatment Procedure

The polluted sample was allowed to stay 14 days before the start of treatment. The amendments (treatment variables) used included: cow-dung CD, poultry waste (manure) PM, and inorganic fertilizer (NPK 20 10 10) FZ, and Synergy (mixture) MX, of the above three (ie CD, PM and FZ) in the appropriate proportion. The polluted sample was thoroughly mixed to ensure even distribution of pollutant. The various amendments were then added to 5kg of soil each and thoroughly mixed except for the control sample. The samples were thoroughly mixed twice a week with the addition of moisture to provide a conducive environment for the degrading microbes.

Quantities of amendments used:

a) Poultry Waste (manure) - PM: 55 g of poultry manure per 5 kg of soil. This is equivalent to 20 tons/ha as recommended by Amadi and Bari (1992).

b) Cow dung - CD: 55 g of cow-dung per 5 kg of soil as recommended by Amadi and Bari (1992).

c) Inorganic fertilizer - FZ: 25 g of NPK per 5 kg of soil was used which is equivalent to 8.2 ton/ha. This was based on the recommendation of 4.7 - 12.5tons/ha by Ogaji, Ayotamuno, Kogbara, and Probert (2005).

d) Synergy - MX: mixture of 25 g of inorganic fertilizer, 25 g of cow dung and 25 g of poultry manure.

Experimental Soil Sampling: The experimental soil was analyzed in the laboratory at intervals. The soil was taken to the laboratory before pollution and two weeks after pollution before the start of treatment. The rest of samplings were during treatment. The samples were thoroughly mixed and homogenized before collection into neat and well labeled polythene bags free from contamination. The soil samples were immediately taken to the laboratory for analysis.

4.2 Laboratory Investigation

Both microbial and Total Petroleum Hydrocarbon (TPH) content analysis was done in the laboratory.

4.2.1 Microbial Analysis

Preparation of diluents: diluents used for the dilution of the samples were prepared by dispensing 9 ml of distilled water into bijou bottles. This was sterilized by autoclaving at 121 ºC for 15minutes and allowed to cool before use (Cheesbrough, 2000).

Preparation of media: nutrient agar (NA) and potato dextrose agar (PDA) were prepared according to manufacturer's specification described by Cheesbrough (2000). Mineral based petroleum agar (PA) was prepared according to the method adopted by the Institute of Petroleum Studies (IPS), Rivers State University of Science and Technology, Port Harcourt. The recipe used includes:

NH_4Cl 0.5 g; K_2HCO4 0.5 g; $NaHPO_4$ 2.5 g; diesel/oil 0.5%; Agar 15.0 g in 1litre of distilled water.

Inoculation of Samples: One gram (1 g) quantity of the sample was dispersed into 9 ml of sterile distilled water to obtain 10^{-1} dilution. Further dilutions were made by transferring 1ml of the previous solution until 10^{-6} was obtained. One-tenth nullilitre (0.1 nil) was collected from 10^{-6} and inoculated into freshly prepared surface dried nutrient agar in duplicates. The same quantity was collected from 10^{-4} dilution into potato dextrose agar and mineral based petroleum agar (International Commission on Microbiological Specification in Foods [ICMSF], 1978; Beishir, 1987; Cheesbrough, 2000). The inoculum was spread evenly with a sterile hockey stick like glass rod.

4.2.2 Enumeration of Microbial Population

This was done manually by dividing the Petri-dish into four quadrants at the reverse side of the culture plates. Total colony count was expressed in colony forming units per gram (CFU/g). The mathematical expression was adopted from Harrigan and McCauce (1990).

$$\frac{CFU}{g} = \frac{N}{V} x \frac{D}{1} \tag{1}$$

Where

N is number of colonies counted

V is volume of inoculums transferred to the plates

D is the dilution factor

4.2.3 Characterization of Microbial Isolates

Colonial, microscopic and biochemical characteristics of the microbial isolates was done according to Cheesbrough (2000), Harrigan and McCauce (1990) and Beishir (1987).

4.2.4 Identification of Microbial Isolates

This was done with reference to standard bacteriological and mycological manual cited in Buchanan and Gibbons (1974) and Barnet and Hunter (1987) respectively.

4.2.5 Total Petroleum Hydrocarbon (TPH)

TPH is a term used for any mixture of hydrocarbons found in crude oil. There are several hundred of these compounds, but not all occur in any one sample. Because there are so many different chemicals in crude oil and in other petroleum products, it is not practical to measure each one separately. However, it is useful to measure the total amount of TPH at a site.

Procedure: 2 g of soil sample was weighed into a 100 ml flask and 50 ml of chloroform was added into it. After shaking vigorously for 3minutes, the liquid phase was extracted and measured using a UV-Visible Spectrophotometer. Standard curve of the absorbance of different known concentrations of petroleum hydrocarbons in the extract was derived using fresh crude oil appropriately diluted with the solvent and was used to read off petroleum hydrocarbon content. Mathematically, Petroleum hydrocarbon concentration in soil was then calculated after reading the absorbance of the petroleum hydrocarbons in the extract from the spectrophotometer. The Total hydrocarbon content (THC) was obtained as described below:

$$\text{THC}\left(\frac{\text{mg}}{\text{kg}}\text{soil}\right) = \frac{\text{Absorbance x DF X 50}}{\text{Weight of soil used}} \tag{2}$$

Where DF is dilution factor

50 is the initial extraction volume

5. Results

Analysis of the laboratory results' changes in microbial count is almost directly proportional to changes in TPHC and hence the % reduction. The sharp increase in the microbial load of hydrocarbon degrading fungi and bacteria within the 6th and 9th weeks resulted in sharp reduction in TPHC which is seen in the increase in the % reduction. This increase continued as can be seen in the 12th week with Synergy having 1.65×10^7 degrading bacteria and 3.4×10^6 degrading fungi, followed by Poultry manure, 1.21×10^7 and 3.0×10^5 respectively. Fertilizer followed with 6.2×10^6 and $1.0\ 10^6$, then Cow dung 6.1×10^6 and 1.6×10^5 respectively. Table 1 provides microbial count before and after pollution.

Table 1a. Total microbial count before pollution (cfu/g)

THBC	THCBC	%Degraders	THFC	THCFC	%Degraders
1.2×10^{10}	6.9×10^6	0.03	1.2×10^7	1.4×10^6	11.67

Table 1b. Total microbial count 2 weeks after pollution (cfu/g)

THBC	THCBC	%Degraders	THFC	THCFC	%Degraders
1.28×10^{10}	9.1×10^6	0.07	3.6×10^7	2.8×10^6	7.78

Table 1c. Total microbial count during remediation (cfu/g)

TRT	THBC	THCBC	%Degraders	THFC	THCFC	%Degraders
1week of Remediation						
CD	7.2×10^{9}	4.1×10^{5}	0.006	1.2×10^{7}	1.0×10^{5}	0.83
PM	4.9×10^{10}	3.9×10^{6}	0.008	3.6×10^{7}	1.2×10^{6}	3.3
FZ	2.8×10^{9}	1.2×10^{5}	0.004	1.1×10^{7}	5.0×10^{6}	45.45
MX	9.8×10^{10}	5.1×10^{6}	0.005	4.1×10^{7}	2.1×10^{6}	5.12
CT	4.9×10^{9}	1.6×10^{5}	0.003	2.8×10^{7}	1.0×10^{5}	0.36
3 weeks of Remediation						
CD	1.3×10^{10}	3.0×10^{6}	0.02	4.0×10^{7}	$1.0X10^{5}$	0.25
PM	6.9×10^{9}	9.0×10^{6}	0.15	5.0×10^{7}	NG	-
FZ	3.3×10^{9}	3.0×10^{5}	0.01	2.0×10^{7}	NG	-
MX	1.7×10^{10}	7.9×10^{6}	0.05	6.0×10^{7}	NG	-
CT	2.3×10^{10}	3.0×10^{5}	0.001	1.0×10^{7}	1.0×10^{6}	10
6 weeks of Remediation						
CD	2.48×10^{10}	5.0×10^{6}	0.02	1.8×10^{6}	1.0×10^{5}	5.56
PM	3.4×10^{9}	9.6×10^{6}	0.28	3.4×10^{6}	1.0×10^{5}	2.9
FZ	2.7×10^{9}	4.0×10^{5}	0.01	NG	1.0×10^{5}	-
MX	2.61×10^{10}	1.1×10^{7}	0.04	1.69×10^{9}	2.0×10^{6}	0.11
CT	1.21×10^{10}	3.0×10^{5}	0.002	NG	1.0×10^{5}	-
9 weeks of Remediation						
CD	1.96×10^{10}	5.2×10^{6}	0.03	2.8×10^{6}	1.0×10^{5}	3.57
PM	4.2×10^{9}	9.0×10^{6}	0.21	4.7×10^{6}	1.0×10^{5}	2.13
FZ	2.1×10^{9}	4.5×10^{5}	0.02	NG	1.0×10^{5}	-
MX	2.72×10^{10}	9.5×10^{6}	0.03	1.81×10^{9}	1.0×10^{6}	0.06
CT	9.6×10^{9}	4.2×10^{5}	0.004	NG	1.0×10^{5}	-
12 weeks of Remediation						
CD	2.95×10^{10}	6.1×10^{6}	0.02	1.11×10^{7}	1.6×10^{6}	14.41
PM	7.2×10^{9}	1.21×10^{7}	0.17	6.5×10^{6}	3.0×10^{5}	4.62
FZ	6.4×10^{9}	6.2×10^{5}	0.1	-	1.0×10^{6}	-
MX	2.61×10^{11}	1.65×10^{7}	0.002	1.69×10^{7}	3.4×10^{6}	20.12
CT	1.65×10^{10}	4.2×10^{6}	0.02	-	1.0×10^{5}	-

THBC, Total Heterotrophic Bacteria Count; THCBC, Total Hydrocarbon Bacteria Count; THFC, Total Heterotrophic Fungi Count; THCFC, Total Hydrocarbon Fungi Count; TRT, Treatment; NG, No Growth; CD, cow-dung; PM, poultry waste(manure); FZ, fertilizer; MX, Synergy(mixture); CT, control.

TPHC Values: In Table 2, the TPHC values during treatment for 9 weeks are stated. The TPHC (mg/kg) before pollution is 1.6 and TPHC (mg/kg) 2 weeks after pollution is 230

Table 2. TPHC Values during treatment

Week 1 of Remediation		
Variables	TPHC mg/kg	% Reduction
Cow dung	209.5	8.91
Poultry manure	208	9.56
Fertilizer	209.25	9.02
Synergy	217.63	5.38
Control	223	3.04
Week 3 of Remediation		
Cow dung	156.25	32.06
Poultry manure	163.5	28.91
Fertilizer	146.63	36.25
Synergy	158.5	31.08
Control	221.13	3.85
Week 6 of Remediation		
Cow dung	117.63	48.85
Poultry manure	111.13	51.68
Fertilizer	101.75	55.76
Synergy	142.88	37.88
Control	204.4	11.13
Week 9 of Remediation		
Cow dung	105.5	54.13
Poultry manure	86.75	62.28
Fertilizer	83.13	63.85
Synergy	82.38	64.18
Control	204.4	11.13

Figures 2a to 2e show the graphs of the TPHC with time for the different treatment variables. Figures 2a and c showed a pretty gradual reduction in TPHC with time with R^2 as 0.889 and 0.906 respectively. The regression equations are also shown, y = 207.1-12.61x and y = 207.6-15.26×respectively. Figure 2b also showed a steady decrease in TPHC with time with R^2 as 0.962 and the regression equation y = 214.5-15.19x. However, Figure 2d shows that the removal of TPHC from the soil medium was initially slow but suddenly increased sharply around the 7th week of treatment. This is as a result of the sharp increase in the activities of the petroleum contaminant degrading micro-organisms which probably were acclimatizing before now. The R^2 is 0.936 and the regression equation given by y = 223.3-15.36x. Then, the Figure 2e which shows a curve quite different from the rest is the control. The reduction of TPHC was slow. R^2 = 0.850 and y = 226-2.694x. ‘y’ =TPHC and “x’ = time (duration) covered during treatment. The higher the value of x, the lower the value of y. From the prediction equations, the rates of remediation for the different treatments could be ascertained at any time x, till maximum treatment is achieved.

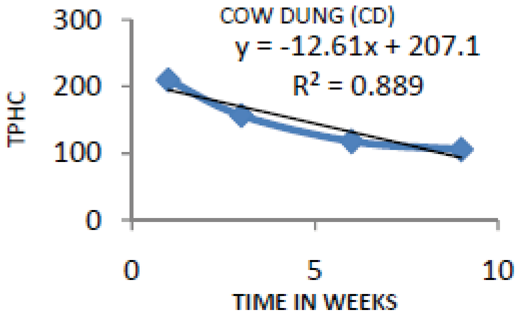

Figure 2a. TPHC for cow-dung treatment

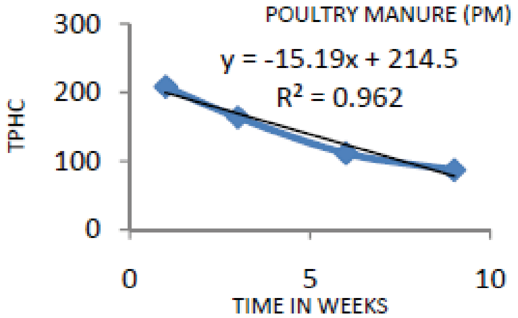

Figure 2b. TPHC for poultry manure treatment

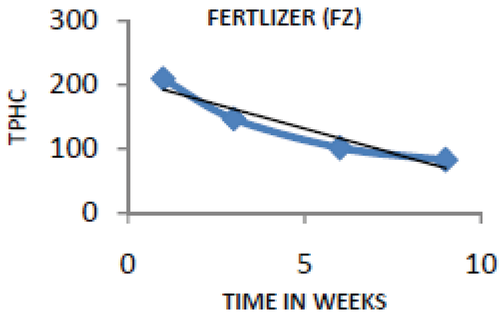

Figure 2c. TPHC for NPK fertilizer treatment

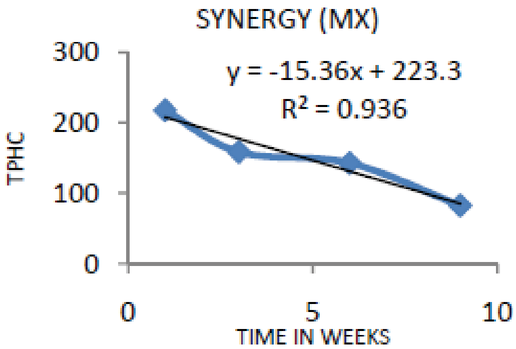

Figure 2d. TPHC for synergy treatment

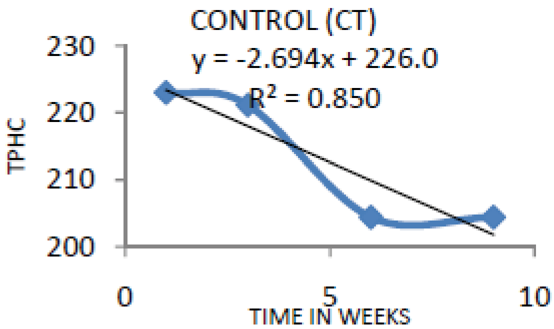

Figure 2e. TPHC for control experiment

6. Discussion

From the laboratory results and analysis, it can be seen that the percentage (%) degraders are higher with fungi than bacteria. THBC is higher than THFC but % of THCFC is higher than %THCBC. The % degraders from Table 1 are a reflection of the degrading microbial counts. The Synergy remediated fastest with time. Initially the remediation was faster with fertilizer within the first 6 weeks of treatment. From the % reduction at the 6^{th} week, Fertilizer had 55.76 while Synergy had 37.88 but after the 6^{th} week, there was a sharp increase in the microbial activities of the Synergy and the percentage reduction increased to 64.18 while Fertilizer stood at 63.85 followed by Poultry manure, 62.28. Cow-dung had 54.13 while the control remained constant at 11.13. The identification of degrading microbes is a very important aspect of the results. Identified microbes can be isolated, whether as indigenous or non-indigenous and can be cultured and then introduced into a polluted site for hydrocarbon degradation. The identified microbes that survived and multiplied (cultivable) throughout the period of remediation are suitable for the remediation. This is because they could eat up and use the hydrocarbon, thereby degrading the contaminant. Many microbes both indigenous and non-indigenous (non cultivable) phased out with time and so are not suitable for bioremediation of crude oil. The following microbes have therefore been seen to be good and suitable fungi and bacteria for the bioremediation of crude oil polluted land: Fungi:

Penicillium notatum, Mucor spp, *Rhizopus stolonifer* and *Penicillum caseicolum* with *P.notatum* and *P.caseicolum* (*penicillum spp*) as the strongest fungi degraders. Bacteria: *Pseudomonas putida* and *Bacillus subtilis*. These fungi and bacteria (cultivable) can therefore be isolated and cultured in the laboratory and then introduced to a polluted site with the best engineering method as a less laborious alternative to the use of inorganic fertilizers, solid poultry or animal wastes or other input variables where the variables are not readily available or are nor in large quantities. The curves also revealed the rates of the microbial degradations with time

7. Conclusion

It is obvious from the forgoing that it is most appropriate to induce and facilitate remediation of crude oil polluted land by use of organic or inorganic amendments than to allow the polluted land natural recovery. Natural recovery most likely will lead to economic loss of land. Organic variable can remediate in record time and is environment friendly. These species of fungi and bacteria, namely: *Penicillium notatum*, *Mucor spp, Rhizopus stolonifer* and *Penicillum caseicolum* and *Pseudomonas putida* and *Bacillus subtilis* can therefore be isolated and cultured (cultivable) and can be directly employed in the remediation of the polluted land using the best engineering and aeration techniques. This process has no pollution effects on land, air and water. It will be less cumbersome and laborious than the application of inorganic fertilizers, animal wastes and municipal wastes which are normally introduced to stimulate the action of the microbial population in a polluted site. Government, oil servicing and producing companies and environmental protection agencies should endeavor to work together to ensure minimum occurrence of oil spills in the environment both on land and in the water. Offenders should be sanctioned and made to face the responsibility of fast recovery of crude oil contaminated land and surface water. Above all, the sustainability of whatever strategy employed in the remediation, in terms of environmental impacts, should be seriously taken into consideration. This is because remediation of contaminated land is only an integral part of sustainable development.

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References

Amadi, A., & Bari, Y. U. (1992). Use of poultry manure for the amendment of oil polluted soils in relation to growth of maize. *Environment, 18*, 521-527.

Barnet, H. I., & Hunter, B. B. (1987). *Illustrated Genera of Imperfecti Fungi* (4th ed.). Macmillan Publishing Company New York, USA, pp. 106, 130.

Beishir, I. (1987). *Microbiology in Practice. A self instructions laboratory course* (4th ed.) (pp. 96-111, 120-130, 238-272). New York: Harper and Row Publisher.

Buchanan, R. E., & Gibbons, N. E. (1974). *Bergey's Manual of Determinative Bacteriology* (pp. 522-568) Williams and Wilkins Co. Baltimore, USA.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropic Countries Part 2* (pp. 35-38, 62-69). Cambridge University Press.

Dodman, P. (1994). European Perspectives of Field Research on Bioremediation Special Attention the Netherlands (p. 15). Invited paper for the International congress of soil Science, Acapulco, Mexico.

Ebuehi, O. A. T., Abibo, I. B., Shekwolo, P. D., Sigismund, K. I., Adoki, A., & Okoro, I. C. (2005). Remediation of Crude Oil Contaminated Soil by Enhanced Natural Altenuation Technique. *Journal of Applied. Science & Environmental Management, 9*(1), 103-106. http://hdl.handle.net/1807/6428

Harrigan, W. F., & McCance, M. E. (1990). *Laboratory Method in Food and Diary microbiology* (8th ed.) (pp. 7-23, 286-303). London: Academic Press Inc.

International Commission on Microbiological Specification in Foods [ICMSF]. (1978). Microorganisms in Foods 1: Their Significance and Methods of Enumeration (pp 100-107.), Toronto Canada: University of Toronto Press.

Ogaji, S. O. T., Ayotamuno, M. J., Kogbara, R. B., & Probert, S. D. (2006). Bioremediation of a Crude oil Polluted Agricultural Soil at Port Harcourt, Nigeria. *Applied Energy, 83*(11), 1249-1257. http://dx.doi.org/10.1016/j.apenergy.2006.01.003

Okwuosha, S. C. (2000). Physio-chemical Characterization of Soil under the Influence of Gas Flaring. A thesis submitted to the department of crop and soil technology (pp. 74-88). Federal University of Technology Owerri. Imo State, Nigeria.

Orajaka, S. O. (1975). Geology in Ofomata, G. E. K. (Ed.), *Nigeria in Maps: Eastern states* (pp. 5-7). Benin City: Ethiope Publishing House.

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Acetylcholine Deficiency in *Caenorhabditis elegans* Induced by Hyperthermia Can Be Compensated by ACh-esterase Inhibition or Activation of GAR-3 mAChRs

Tatyana B. Kalinnikova[1], Rifgat R. Shagidullin[1], Rufina R. Kolsanova[1], Eugenia B. Osipova[1], Sergey V. Zakharov[2] & Marat Kh. Gainutdinov[1]

[1] Research Institute for Problems of Ecology and Mineral Wealth Use of Tatarstan Academy of Sciences, Kazan, Russia

[2] New Scientific Technologies Ltd, Moscow, Russia

Correspondence: Tatyana B. Kalinnikova, Research Institute for Problems of Ecology and Mineral Wealth Use of Tatarstan Academy of Sciences, Kazan, Russia. E-mail: tbkalinnikova@gmail.com

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Abstract

The nervous system is a target of hyperthermic failure of animal behavior. *Caenorhabditis elegans* can be used as an excellent model organism to investigate mechanisms underlying thermotolerance of nervous system. Inhibition of ACh-esterase by neostigmine produces rise in thermotolerance of *C. elegans* swimming induced by mechanical stimulus at constant temperature 36 °C. Protection of *C. elegans* behavior against heat stress by neostigmine indicates that hyperthermia induces ACh deficiency in the *C. elegans* nervous system which is one of the causes of hyperthermic failure of behavior. Activation of mAChRs by pilocarpine or oxotremorine M elevates behavior thermotolerance similarly with neostigmine while inhibition of these receptors by atropine has opposite effect. These results suggest that ACh protects *C. elegans* behavior against hyperthermia by binding with mAChRs. It is known that three G-protein coupled ACh receptors of *C. elegans* (GAR-1, GAR-2 and GAR-3) have sequence homology with five known subtypes of mammalian mAChRs. To identify mAChRs responsible for regulation of behavior response to hyperthermia we investigated effects of loss-of-function mutations in *gar-1*, *gar-2* and *gar-3* genes on the sensitivity of behavior thermotolerance to neostigmine and pilocarpine. Among them only loss-of-function *gar-3* mutation caused insensitivity of *C. elegans* behavior thermotolerance to neostigmine and pilocarpine. Thus it is GAR-3 mAChR that mediates rise in behavior thermotolerance produced by ACh-esterase inhibitor neostigmine or agonists of mammalian mAChRs.

Keywords: *Caenorhabditis elegans*, behavior thermotolerance, acetylcholine, acetylcholine esterase, GAR-3 muscarinic cholinoreceptor

1. Introduction

Temperature is one of the most important variables that determines distribution and abundance of species (Cossins & Bowler, 1987; David, Allemand, Van Herrewege, & Cohet, 1983; Hoffmann, Sørensen, & Loeschke, 2003). Each invertebrate species tends to have own temperature niche with a distinct optimum and a range of permissible temperatures (David et al., 1983). Nevertheless certain threshold temperatures generally limit reproduction and development in most tropical and temperate species when invertebrates are continuously exposed to constant temperatures (Cossins & Bowler, 1987; David et al., 1983; Hoffmann et al., 2003). At extreme high temperature cells and tissues died. Long before this point, however, invertebrates' organisms experiencing hyperthermia endangered by impaired neural performance that prevents coordinated behavior and hampers vital motor patterns (Robertson, 2004a, 2004b). In humans heat stroke is characterized by central nervous system dysfunction that results in delirium, convulsions and coma (Bouchama & Knochel, 2002). It is known that many invertebrates maintain their behaviors at great elevation of environmental temperature above their temperature optimum (Hoffmann et al., 2003; Robertson, 2004a, 2004b). Therefore it is evident that nervous systems of free-living invertebrates are particularly susceptible to detrimental direct effects of heat stress, as their body temperature is determined to a large extent by environmental temperature.

The possible mechanisms of neural circuits dysfunction caused by extreme high temperature are very complex because of high sensitivity to elevated temperature of all processes taking place at all levels of nervous system organization.

In the electrophysiological investigations hyperthermic disturbances of neural circuits are revealed in the disturbances of synaptic transmission (Barclay & Robertson, 2000, 2001; Dawson-Scully & Robertson, 1998; Kelty, Noseworthy, Feder, Robertson, & Ramirez, 2002), and prior adaptation to the thermal extremes by thermal preconditioning can improve synaptic thermoprotection (Barclay & Robertson, 2000, 2001; Dawson-Scully & Robertson, 1998; Kelty et al., 2002). At the present time it is evident that the nervous system is a target for hyperthermic failure of invertebrates behavior (Robertson, 2004b; Kalinnikova, Kolsanova, & Gainutdinov, 2012) Therefore hyperthermic failure of different forms of invertebrate's behavior can be used for investigation of mechanisms, which determine both impair of the whole nervous system functions and protection of these functions against hyperthermia.

Since all types of synaptic transmission are sensitive to hyperthermia it is evident that all of them can be potential targets for heat stress effects. However it is possible that role of separate types of synaptic transmission in the whole nervous system dysfunction produced by heat stress can be different. Cholinergic synaptic transmission plays extremely important role in the large majority of functions of human and animals' organisms including thermoregulation (Conti-Tronconi & Raffery, 1982; Ellis et al., 2006; Erskine et al., 2004; Feiro & Gould, 2005; Gomeza et al., 1999; Lanzafame, Christopoulos, & Mitchelson, 2003). Therefore it is possible that cholinergic system plays important role in the response of whole nervous system to hyperthermia including its dysfunctions and compensatory responses.

The aim of this work was to check the hypothesis assuming that cholinergic system is a target for hyperthermic failure of invertebrates' behavior.

However such investigations using organisms of higher invertebrates as a model have many difficulties: (i) complexity of higher invertebrates' organisms and their nervous system which include neural circuits regulating locomotion, respiration, circulation and other functions of multicellular organism; (ii) hematoneural barrier of higher invertebrates is poor permeable for many chemicals which can be used for neuropharmacological analysis of behavior under optimal or stressful conditions. That's why we have proposed that more simple organism of microscopic soil nematode *Caenorhabditis elegans* can be used as a model to investigate mechanisms of the nervous system thermotolerance. *C. elegans* has several advantages in comparison with higher invertebrates: (i) the absence of circulatory and respiratory systems, and consequently neural circuits which regulate these systems in the organisms of higher invertebrates and can be the targets of hyperthermia effect (Armstrong, Shoemaker, Money, & Robertson, 2006); (ii) the absence of hematoneural barrier, which protect neural circuits of higher invertebrates against chemicals from internal environment and thus complicates neuropharmacological analysis of nervous system's thermotolerance in behavioral tests; (iii) the nervous system of the adult hermaphrodite consists of only 302 neurons subdividing into 118 types and for many years is used as an ideal model to investigate genetic, molecular and cellular mechanisms of nervous system's functions (Bargmann, 1993). Therefore molecular and cellular mechanisms of many forms of *C. elegans* behavior are known (Chase & Koelle, 2007; Hart, 2006), and this knowledge can be used for investigation of *C. elegans* behavior under hyperthermic conditions.

Acetylcholine (ACh) is the major neurotransmitter not only in vertebrates but also in the simple organism of soil nematode *Caenorhabditis elegans* and more than one third of neurons in *C. elegans* nervous system release ACh. In *C. elegans* molecular mechanisms of ACh effects on postsynaptic neurons and muscles are similar with such in mammals and are realized by ACh binding with either nicotinic or muscarinic receptors (Changeux & Edelstein, 1998; Fleming, et al., 1997; Kim, Shin, Park, & Cho, 2008; Lee et al., 2000; Liu, LeBoeuf, & Garcia, 2007; Park, Kim, Shin, Choi, & Cho, 2003; Satelle, 2009; Steger & Avery, 2004). Nicotinic receptors (nAChRs) are ligand-gated ion channels and responsible for the initial fast depolarization in the postsynaptic neurons and muscles (Changeux & Edelstein, 1998; Culetto et al., 2004; Satelle, 2009; Unwin, 2005). Muscarinic receptors (mAChRs) are coupled with variety of G-proteins and thereby extend neurotransmission into multiple intracellular signaling processes (Caulfield & Birdsall, 1998; Langmead, Watson, & Reavill, 2008). In order to reveal the possible role of cholinergic system in the *C. elegans* thermotolerance we have examined effects of ACh-esterase inhibitor neostigmine, agonists of nAChRs and mAChRs and antagonist of mAChRs atropine on thermostability of *C. elegans* swimming induced by mechanical stimulus. The possible role of mAChRs in behavior thermotolerance was examined by comparison of behavior thermotolerance sensitivity to neostigmine and agonist of mAChRs pilocarpine of wild-type worms and worms with loss-of-function mutations of three mAChRs genes (*gar-1*, *gar-2* and *gar-3*). These studies showed that hyperthermia causes in *C. elegans* organism ACh deficiency which is revealed in the lowering of behavior thermotolerance and can be compensated both by

ACh-esterase inhibition and action of mAChRs agonists. In addition it was shown that compensation of ACh deficiency by ACh-esterase inhibition or by mAChRs agonists is a result of activation of GAR-3 mAChRs.

2. Methods

2.1 Worms' Strains And Growth

Caenorhabditis elegans were grown at 22 °C in Petri dishes with standard Nematode Growth Medium (NGM) (3 g/l NaCl, 17 g/l Bactoagar, 2.5 g/l Bactopeptone, 1 ml/l, 5 mg/ml cholesterol, 1 ml/l 1 M $CaCl_2$, 1 ml/l 1 M $MgSO_4$, 25 ml/l potassium phosphate buffer [pH6.0]) seeded with E.coli OP50 (Brenner, 1974). The following strains were used in this study: N2 Bristol, *gar-1 (ok755)*, *gar-2 (ok520)* and *gar-3 (vu78)*. All strains were received from Caenorhabditis Genetics Center. *gar-1 (ok755)* and *gar-2 (ok520)* were generated by *C. elegans* gene Knockout Consortium. N2 Bristol is a wild type strain used in numerous genetic and molecular physiology investigations of *C. elegans*. Mutant strains *gar-1 (ok755)*, *gar-2 (ok520)* and *gar-3 (vu78)* are strains with loss-of-function mutations of one of three genes coding *C. elegans* mAChRs GAR-1, GAR-2 and GAR-3 respectively.

2.2 Behavior Thermotolerance Assays

Experiments on measuring the behavior stability to heat stress action were performed in NG buffer (0.3 % NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM, pH 6.0, potassium phosphate/liter) with young adult hermaphrodites from 3-days old culture of worms. For each experiment worms were washed from agar surface into Petri dish 40 mm in diameter and then transferred with pipette into glass centrifuge tube. In this tube worms were rinsed from growth medium, bacteria and metabolites. For this purpose 10 ml of NG buffer was added into tube. After worms' settling on tube's bottom the supernatant was removed. This procedure was repeated three times. The total rinse time was about 30 minutes. After such procedure worms were transferred into clean Petri dish 40 mm in diameter with NG buffer and then transferred with pipette 10 µl into glass tubes with 1 ml of NG buffer (one worm in each tube). To measure thermotolerance of behavior tubes with worms were placed into water bath with temperature 36 °C immediately or after preadaptation to elevated temperature by short-term (120 minutes) exposure to constant temperature 30 °C tolerated by *C. elegans*. The indexes used to characterize behavioral stability under exposure to a constant high temperature 36 °C were as follows: (i) the average exposure time leading to the appearance of uncoordinated behavior (swimming induced by a mechanical stimulus (shaking of the tube with the nematode)), and (ii) the average exposure time leading to loss of worms' ability to swimming induced by a mechanical stimulus. The signs of uncoordinated behavior were: (i) partial incoordination of body muscle contraction necessary for sinusoidal body movements; (ii) inability to sustained forward swimming during 10 seconds after mechanical stimulus.

2.3 Neuropharmacological Analysis of Behavior Thermotolerance

For neuropharmacological analysis of behavior thermotolerance were used inhibitor of ACh-esterase neostigmine bromide, agonists of nAChRs levamisole hydrochloride and nicotine hemisulfate, non-selective agonist of ACh receptors carbamylchloride chloride (carbachol), agonists of mAChRs pilocarpine nitrate and oxotremorine methiodide (oxotremorine M) and mAChRs antagonist atropine methyl bromide. In all experiments were used freshly prepared solutions of reagents. All substances were dissolved in distilled water and added to tubes with worms just before the exposure to temperature 36 °C or 23 °C in control experiments. Reagents were obtained from Sigma.

It is necessary to note that concentrations of drugs and toxicants used in our neuropharmacological analysis of *C. elegans* thermotolerance in most cases were very high (10^{-3} M or more). Such high concentrations are explained by well known specificity of *C. elegans* as a model organism. *C. elegans* organism has very low sensitivity to most chemicals from environment because its cuticle is extremely impermeable to most organic and inorganic chemicals. Therefore concentrations of drugs and toxicants effective for changes of *C. elegans* behavior are very high (Anderson, Cole, & Williams, 2004; Boyd, Cole, Anderson, & Williams, 2003; Carnell, Illi, Hong, & McIntire, 2005; Davies et al., 2003; Davies, Bettinger, Thiele, Judy, & McIntire, 2004; Johnson & Nelson, 1991; Nurrish, Ségalat, & Kaplan, 1999; Sawin, Ranganathan, & Horvitz, 2000; Schafer & Kenyon, 1995; Singer, Bellingham, & Berger, 1996; Tissenbaum et al., 2000).

All experiments presented in this paper were performed in September, October and November. Each datum on the graphics is the mean thermotolerance for 20 worms incubated individually. In each case were performed 4 or 5 independent experiments with similar results, but in the article are shown results of only one of these experiments.

3. Results

Locomotion is the most important ACh-mediated behavior of *C. elegans*, involving by far the greatest number of cholinergic neurons. The involvement of ACh in *C. elegans* locomotion includes not only neuromuscular transmission, but also neuron-neuron transmission (Culetto et al., 2004; Esmaeili, Ross, Neades, Miller, & Ahringer, 2002; Satelle, 2009). Therefore we propose that cholinergic signaling may be one of the major targets of hyperthermia effect on *C. elegans* swimming, induced by mechanical stimulus. To check this hypothesis we tested the influence of drugs affecting cholinergic signaling on *C. elegans* ability to maintain swimming induced by mechanical stimulus at constant high temperature 36 °C.

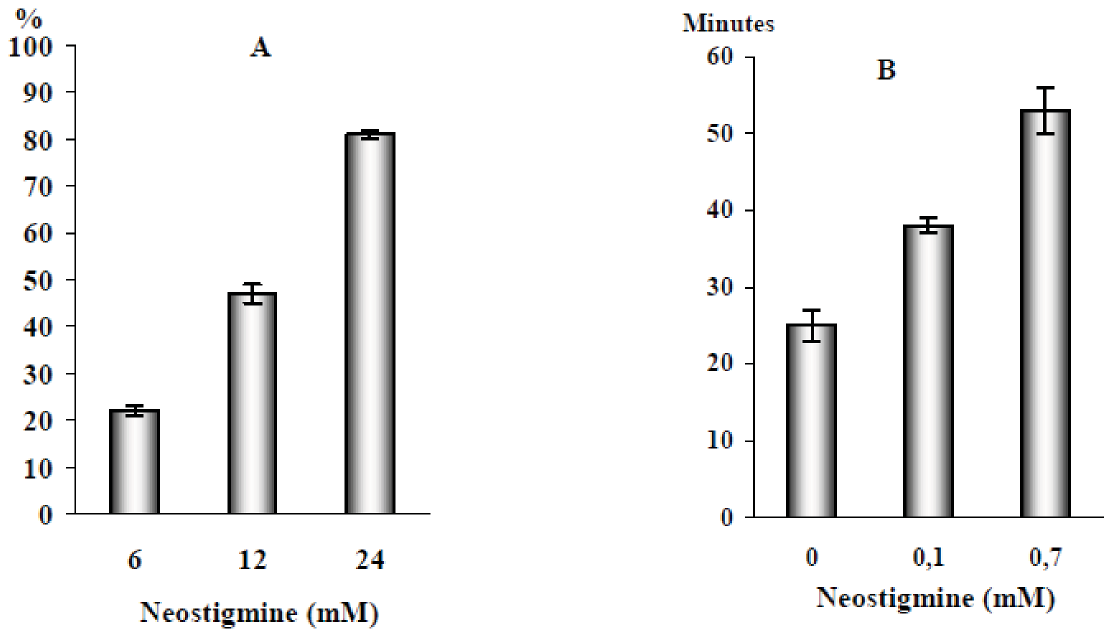

Figure 1. Toxic and "therapeutic" effects of neostigmine on *C. elegans* behavior

A – The ordinate shows the percentage of worms with uncoordinated behavior (swimming, induced by mechanical stimulus) after 90-minute exposition to neostigmine at 22 °C. The abscissa shows neostigmine concentration (mM).

B – The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows neostigmine concentration (mM).

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Twenty nematodes of wild type strain N2 were used in each variant of experiment.

Neostigmine is a reversible inhibitor of ACh-esterase, and its effects on mammals' organisms are mediated by the rise of ACh (Harvey, Champe, Finkel, Cubeddu, & Clark, 2009). It is known that inhibition of ACh-esterase by strong inhibitors such as aldicarb, used as pesticide, is very toxic not only for insects and mammals, but also for *C.elegans* (Govorunova et al., 2010; Nurrish et al., 1999). However neostigmine and other reversible inhibitors of ACh-esterase are used for therapy of many disorders linked to a deficiency of ACh (Bartus, Dean, Beer, & Lippa, 1982; Harvey et al., 2009; Perry et al., 1978; Tabet, 2006). In our experiments aldicarb-like toxic effects of neostigmine, such as worms' paralysis were revealed only at very high concentrations of this drug (6 mM and above) at normal temperature (22 °C) (Figure 1A). However at lower concentrations (0.1-0.7 mM) neostigmine caused rise in behavior thermotolerance in the dose-dependent manner (Figure 1B). This therapeutic effect of neostigmine was revealed in the rise of mean time course of the worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at constant high temperature 36 °C. Protection of *C. elegans* behavior against heat stress by neostigmine was shown both for basal thermotolerance and for thermotolerance induced by short-term adaptation of worms to high temperature, such as 2-hour exposure to temperature 30 °C (Figure 2). The dependence of behavior thermotolerance from ACh rise caused by ACh-esterase inhibition shows, that one of hyperthermia effects responsible for behavior failure is ACh deficiency in the *C. elegans* organism.

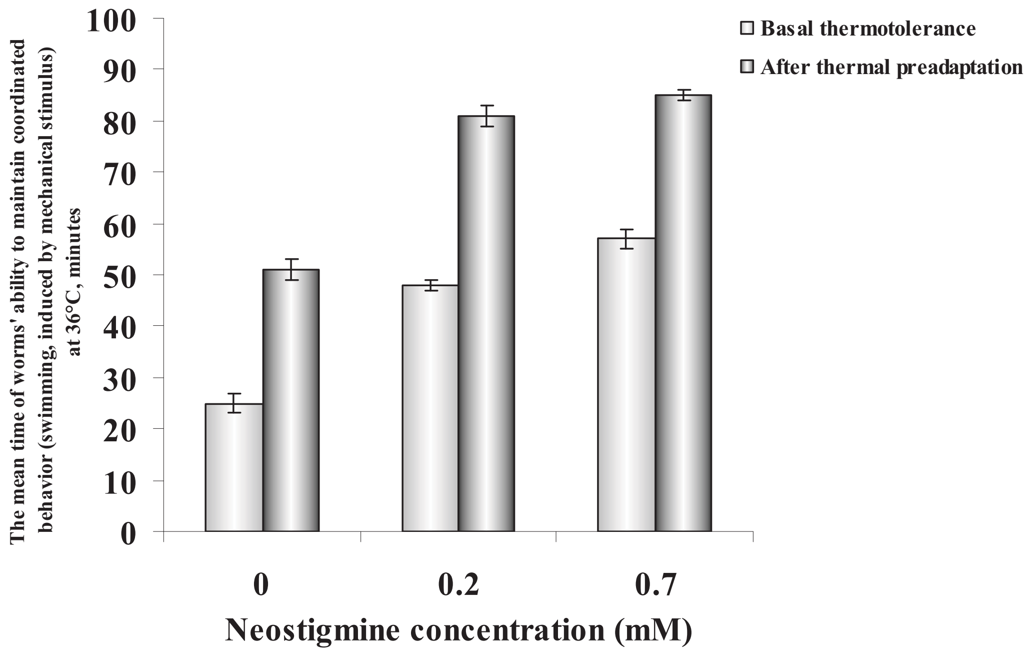

Figure 2. The dependence of *C. elegans* thermotolerance sensitivity to neostigmine from prior adaptation to elevated temperature

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows neostigmine concentration (mM). The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Prior adaptation to high temperature consisted in 2-hours incubation of individual worms in 1 ml of liquid medium at 30 °C. Twenty nematodes of wild type strain N2 were used in each variant of experiment.

Fast excitatory cholinergic signaling is mediated by activation of nicotinic acetylcholine receptors (nAChRs) in neurons and muscles of *C. elegans* (Culetto et al., 2004; Esmaeili et al., 2002; Satelle, 2009). Therefore the rise in *C. elegans* behavior thermotolerance caused by ACh-esterase inhibition (Figure 1B, 2) can be a consequence of nAChRs overactivation. The *C. elegans* cholinergic signaling contains pharmacologically distinct subtypes of nAChRs: levamisole-sensitive nAChRs and levamisole-insensitive nAChRs (Satelle, 2009). That is why we tested effects both of nicotine and levamisole on time course of failure of worms' swimming induced by mechanical stimulus at constant high temperature 36 °C. As shown in Figure 3, both nicotine and levamisole caused dose-dependent decrease of swimming thermotolerance revealed in the lowering of mean time of loss of worms' ability to swim. Nicotine and levamisole reduced *C. elegans* thermotolerance in concentrations 0.03% and 16 μM consequently. These concentrations of nicotine and levamisole did not cause the loss of worms' ability to swimming induced by mechanical stimulus at temperature 22 °C (data not shown). These data indicate that overactivation of nAChRs can not be a mechanism of thermotolerance elevation by neostigmine. Moreover, it sensitizes *C. elegans* behavior to hyperthermia.

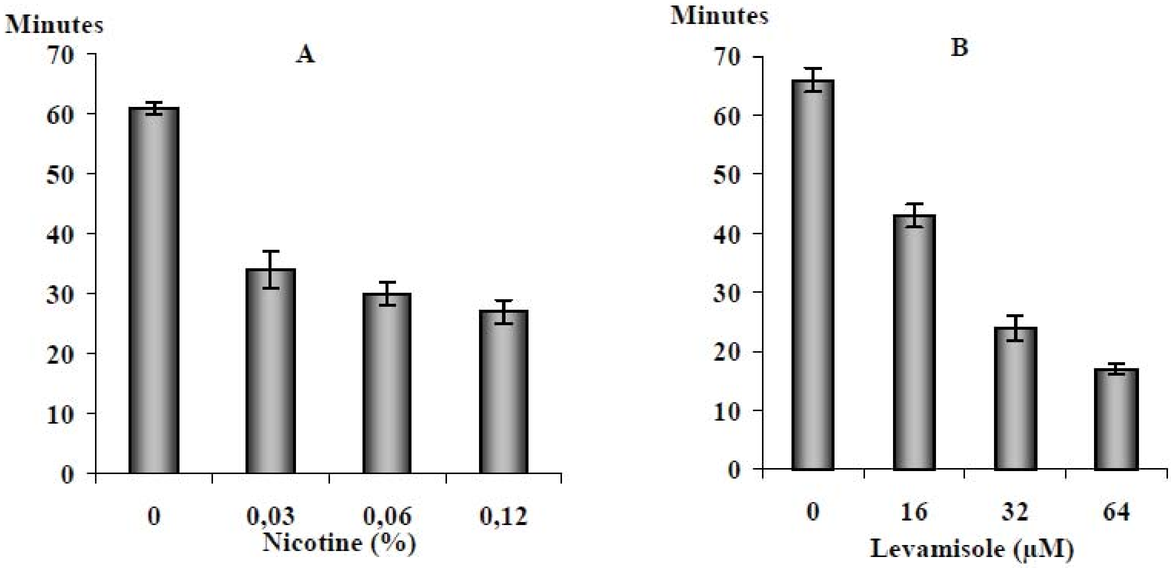

Figure 3. The influence of nicotine and levamisole on thermotolerance of *C. elegans* behavior

The ordinate shows the mean time of worms' ability to maintain swimming, induced by mechanical stimuli at 36 °C. In these experiments was registered complete loss of worms' ability to swimming. The abscissa shows: A – concentration of nicotine (%); B – concentration of levamisole (µM). Twenty nematodes of wild type strain N2 were used in each variant of experiment.

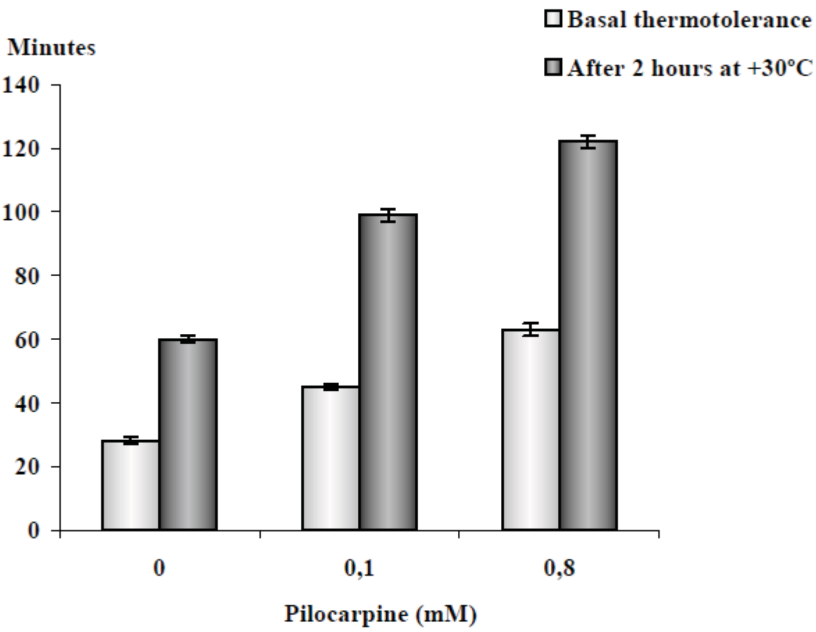

Figure 4. The effect of pilocarpine on *C. elegans* behavior thermotolerance

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows pilocarpine concentration (mM).

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Prior adaptation to high temperature consisted in 2-hours incubation of individual worms in 1 ml of liquid medium at 30 °C. Twenty nematodes of wild type strain N2 were used in each variant of experiment.

Actions of acetylcholine on neurons and muscles of *C. elegans* are the result of activation not only nAChRs, but also metabotropic muscarinic acetylcholine receptors (mAChRs) (Kim et al., 2008; Lee et al., 2000; Liu et al., 2007; Park et al., 2003; Steger & Avery, 2004). In order to determine the possible role of mAChRs in the behavior thermotolerance we tested the influence of agonists of these receptors on the ability of *C. elegans* to maintain swimming, induced by mechanical stimulus at extreme high temperature 36 °C. Pilocarpine and oxotremorine M are agonists of mammalian mAChRs. As shown in Figures 4 and 5, addition of pilocarpine or oxotremorine M to NG buffer elevated behavior thermotolerance revealed in the rise of mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at extreme high temperature 36 °C. Carbachol, non-selective agonist of mammalian ACh receptors, acts on *C. elegans* behavior thermotolerance similarly with pilocarpine and oxotremorine M (Figure 5). Therapeutic effect of carbachol on *C. elegans* behavior in hyperthermic conditions was completely blocked by addition of mAChRs antagonist atropine (Figures 5, 6). Therefore it is evident that carbachol effect on *C. elegans* behavior thermotolerance is mediated by activation of mAChRs. Data in Figures 5 and 6 show that atropine reduced swimming thermotolerance not only in the presence, but also in the absence of carbachol in the medium. This effect of atropine can be a consequence of blocking of mAChRs activation by endogenous ACh. The data in Figure 6 show that atropine strongly attenuated protection of behavior thermotolerance caused by neostigmine. These data support that activation of mAChRs is the general mechanism of the thermotolerance rise caused by inhibition of ACh-esterase.

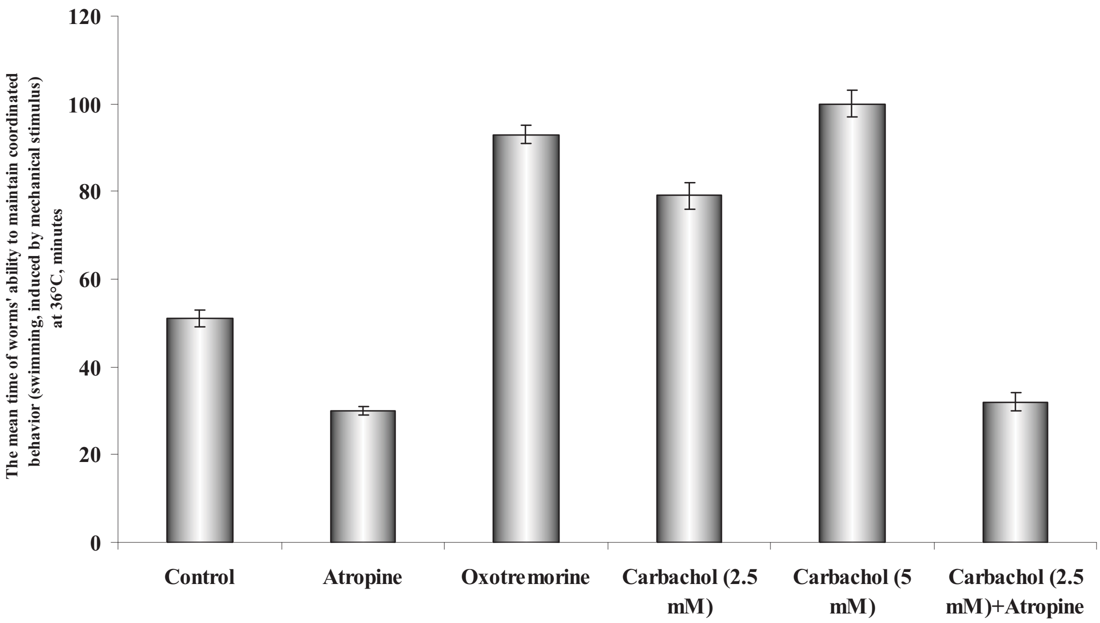

Figure 5. The effects of atropine and mAChRs agonists on *C. elegans* behavior thermotolerance

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows conditions of experiments. Drugs concentrations were as follows: atropine – 1.5 mM, oxotremorine – 5.0 mM, carbachol – 2.5 or 5 mM.

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Twenty nematodes of wild type strain N2 were used in each variant of experiment.

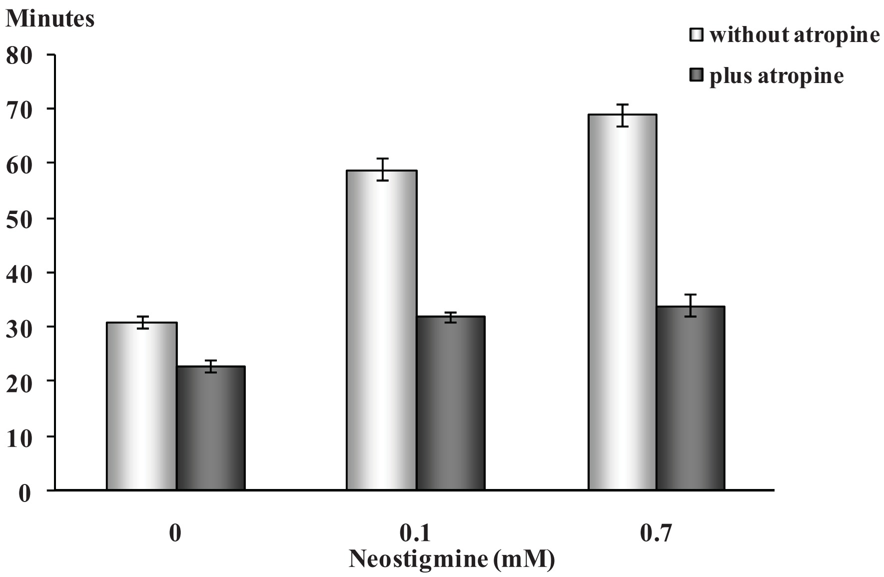

Figure 6. The effects of neostigmine and atropine on *C. elegans* behavior thermotolerance

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows concentrations of neostigmine (mM). Atropine concentration was 1.5 mM.

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Twenty nematodes of wild type strain N2 were used in each variant of experiment.

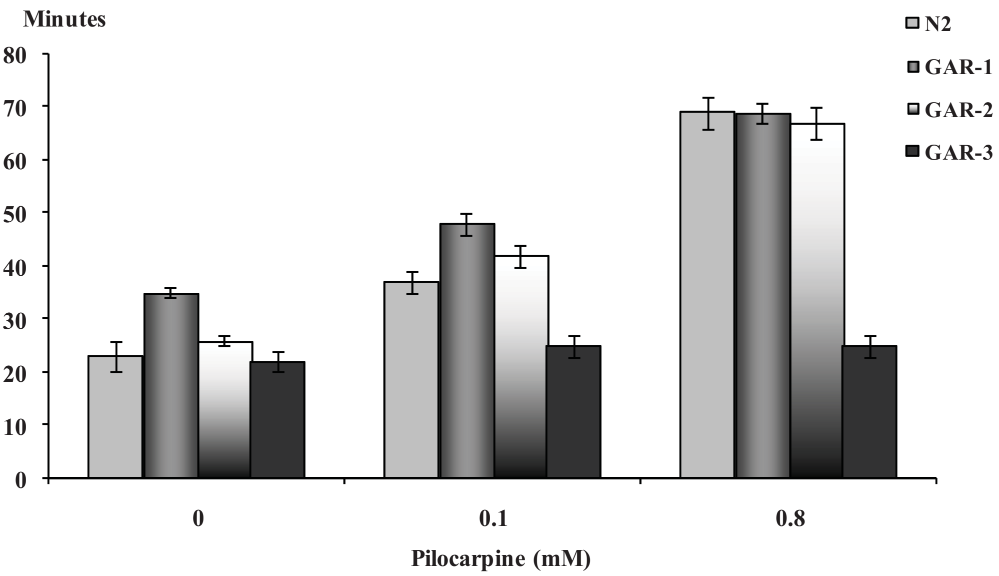

Figure 7. Thermotolerance sensitivity to pilocarpine of C. elegans wild type strain N2 and three mutant strains: *gar-1(ok755)*, *gar-2(ok520)* and *gar-3(vu78)*

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows pilocarpine concentration (mM).

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Twenty nematodes were used in each variant of experiment.

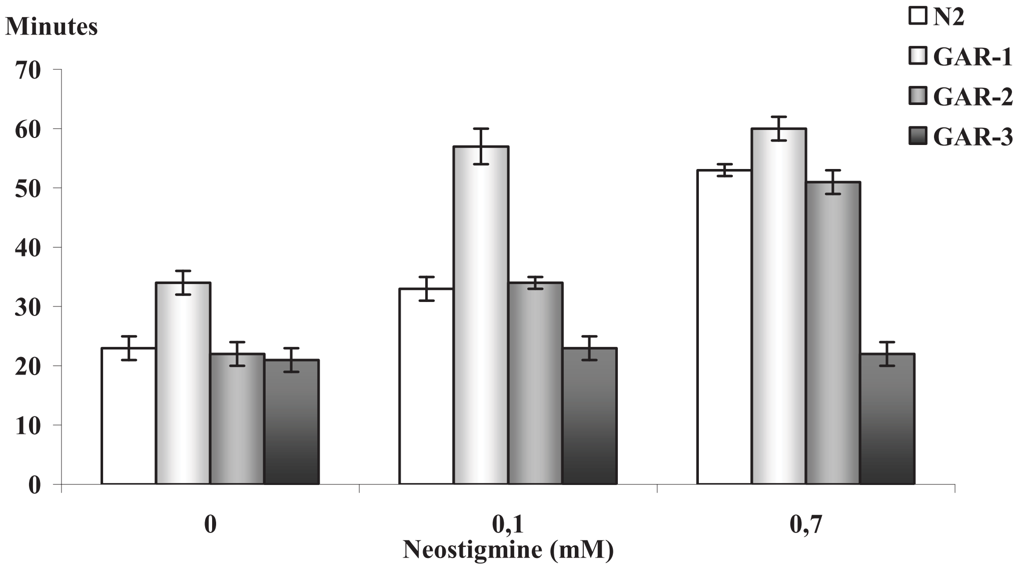

Figure 8. Thermotolerance sensitivity to neostigmine of *C. elegans* wild type strain N2 and three mutant strains: *gar-1(ok755)*, *gar-2(ok520)* and *gar-3(vu78)*

The ordinate shows the mean time of worms' ability to maintain coordinated behavior (swimming, induced by mechanical stimulus) at 36 °C. The abscissa shows neostigmine concentration (mM).

The signs of uncoordinated behavior were as follows: (i) inability to sustained forward swimming during 10 seconds after mechanical stimulus; (ii) partial incoordination of body muscle contraction necessary for sinusoidal body movements. Twenty nematodes were used in each variant of experiment.

The *C. elegans* genome encodes three mAChRs, GAR-1, GAR-2 and GAR-3 (Kim et al., 2008; Lee et al., 2000; Liu et al., 2007; Park et al., 2003; Steger & Avery, 2004). All of them show high similarity in amino acids sequences to the known mammalian mAChRs M_1, M_2, M_3, M_4 and M_5, which are coupled to different G-proteins (Caulfield & Birdsall, 1998; Langmead et al., 2008; Lanzafame et al., 2003). To identify mAChRs responsible for the rise in behavior thermotolerance we analyzed available deletions mutants of all of three genes of these receptors by estimation of thermotolerance sensitivity to agonist of mAChRs pilocarpine and inhibitor of ACh-esterase neostigmine. The behavior thermotolerance in the absence of pilocarpine or neostigmine was not significantly changed in *gar-2* or *gar-3* loss-of-function mutants, while *gar-1* loss-of-function mutant was more resistant to constant high temperature 36 °C in comparison with that of wild type strain N2 (Figures 7, 8). In contrast, sensitivity of the behavior thermotolerance to pilocarpine was not significantly changed in *gar-1* or *gar-2* mutants, but loss-of-function mutation of *gar-3* gene almost completely ceased protection of worms' behavior against heat stress caused by pilocarpine (Figure 7). Similar data were obtained in experiments, in which we compared the sensitivity of behavior thermotolerance to neostigmine of N2 strain and *gar-1*, *gar-2* and *gar-3* mutants. While neostigmine induced rise in behavior thermotolerance of *gar-1* and *gar-2* mutants with dose-dependent response similar to this of N2 worms, the sensitivity of behavior thermotolerance in *gar-3* mutants to neostigmine was very slight (Figure 8). These data indicate that *gar-3* function is required for effects of both agonist of mAChRs pilocarpine and elevated endogenous ACh concentration on behavior thermotolerance of *C. elegans*.

4. Discussion

We have showed that reversible inhibitor of ACh-esterase neostigmine (Harvey et al., 2009) protected *C. elegans* behavior against hyperthermia (Figures 1, 2). Inhibition of the ACh-esterase activity prevents ACh hydrolysis and, as a result, elevates ACh concentration and prolongs its action on the postsynaptic receptors. Effects of ACh-esterase inhibitors on the functions of the whole human or animal organism can be either toxic (Govorunova et al., 2010; Nurrish et al., 1999) or therapeutic (Bartus et al., 1982; Harvey et al., 2009; Perry et al., 1978; Tabet, 2006). In the first case ACh inhibitors disturb cholinergic transmission by enormous rise of ACh concentration resulting in overactivation of postsynaptic AChRs. Such enormous rise of ACh concentrations leads to the paralysis or even death of organism (Govorunova et al., 2010; Nurrish et al., 1999). On the other hand, inhibition of ACh-esterase can be therapeutic for the organism if the complementary rise of ACh concentration is required for the compensation of ACh deficiency which has been established a core pathophysiological feature in Alzheimer's disease, Parkinson's disease, vascular dementia and multiple sclerosis's dementia (Bartus et al., 1982; Harvey et al., 2009; Perry et al., 1978; Tabet, 2006). Therefore inhibitors of ACh-esterase are used in agriculture as pesticides and in medicine as drugs for neurologic disorders. In studies of cholinergic signaling in *C. elegans* for inhibition of ACh-esterase usually is used aldicarb. Aldicarb is known as pesticide caused enormous rise of ACh in the neuron-muscle ACh transmission revealed in worm's paralysis (Govorunova et al., 2010; Nurrish et al., 1999). In contrast, reversible inhibitor of ACh-esterase neostigmine is usually used in medicine and neurophysiological studies of mammals (Harvey et al., 2009; Naguib & Yaksh, 1997). In our experiments with *C. elegans* aldicarb-like toxic effect of neostigmine was shown for concentrations 60-fold higher than those effective for its therapeutic effect revealed in the rise of behavior thermotolerance (Figure 1). Since therapeutic effects of ACh-esterase inhibitors are shown only in the ACh deficiency states of cholinergic system (Bartus et al., 1982; Harvey et al., 2009), protection of *C. elegans* behavior against heat stress by neostigmine (Figures 1, 2) indicates that one of consequences of hyperthermia action is the deficit of ACh in *C. elegans* organism. Three possible reasons of ACh deficit caused by hyperthermia are: (i) inhibition of ACh secretion by cholinergic neurons; (ii) increase of ACh hydrolysis by ACh-esterase caused by extreme high temperature; (iii) requirement of supplementary ACh for compensation of other disturbances caused by hyperthermia in nervous system. Independently of the reason of ACh deficiency in *C. elegans* organism in hyperthermic conditions it is evident that this deficiency is one of reasons of behavior disturbances caused by heat stress, since these disturbances are attenuated by neostigmine (Figures 1, 2).

It is known that resistance of poikilotherms' behavior to thermal extremes can be increased by prior adaptation to elevated temperature. Long-term exposure to constant elevated temperature within the normal viable temperature range of an organism is usually termed "acclimation" (Cossins & Bowler, 1987). Since ACh deficiency is one of reasons of behavior disturbances caused by hyperthermia the possible mechanism of that prior adaptation to high environmental temperature can be prevention or lowering of this deficiency. In this case the sensitivity of *C. elegans* behavior thermotolerance to ACh-esterase inhibitor must be diminished or completely lost after prior adaptation to constant high temperature tolerated by worm's organism. However, effects of prior adaptation to elevated temperature 30 °C and neostigmine on behavior thermotolerance are additive or slightly synergic (Figure 3). Therefore it is evident that prior adaptation to high environmental temperature, used in this work, did not prevent ACh deficiency induced by hyperthermia although it is possible that it extends time-course of its appearance.

ACh effects on excitable cells are mediated by its binding with two types of receptors, namely nAChRs and mAChRs (Culetto et al., 2004; Esmaeili et al., 2002; Kim et al., 2008; Lee et al., 2000; Liu et al., 2007; Park et al., 2003; Steger & Avery, 2004; Satelle, 2009). Therefore, two possible mechanisms of behavior protection against hyperthermia by ACh rise due to inhibition of ACh-esterase are overactivation of nAChRs or overactivation of mAChRs. Cholinergic system of *C. elegans* consists of two subtypes of nAChRs, namely levamisole-sensitive and levamisole-insensitive (Satelle, 2009). Both levamisole and nicotine did not protect *C. elegans* behavior against hyperthermia but evoked the opposite effect - sensitization of *C. elegans* behavior to high extreme temperature (Figure 3). Therefore overactivation of L-subtype or N-subtype nicotinic receptors can not be a mechanism of the protection of *C. elegans* behavior against heat stress via rise of ACh content in its organism. Moreover it is possible that overactivation of nAChRs not only by nicotine or levamisole but also by enormous rise of ACh can diminish thermotolerance of *C. elegans* behavior.

In contrast to effects of agonists of nAChRs, agonists of mammalian mAChRs pilocarpine and oxotremorine M mimic protective effect of neostigmine on *C. elegans* behavior in hyperthermia conditions while antagonist of mammalian mAChRs atropine had opposite effect and greatly attenuated protective effect of neostigmine (Figures 4-6). These results suggest that ACh protects *C. elegans* behavior against hyperthermia by activation of mAChRs.

Muscarinic acetylcholine receptors are known to regulate numerous fundamental physiological processes including muscarinic actions of acetylcholine on peripheral effector tissues and a multitude of central sensory, vegetative and motor functions (Ellis et al., 2006; Erskine et al., 2004; Feiro & Gould, 2005; Lanzafame et al., 2003). Many studies have shown that central muscarinic receptors play an important role in the regulation of body temperature in mammals (Gomeza et al., 1999) and therefore are necessary for survival of these animals and humans at hot environment. However the possible role of mAChRs in the thermotolerance of poikilotherms is still unknown.

The *C. elegans* genome encodes three mAChRs, namely GAR-1, GAR-2 and GAR-3 (Kim et al., 2008; Lee et al., 2000; Liu et al., 2007; Park, Kim et al., 2003; Steger & Avery, 2004). All of them show high similarity in amino acid sequences to the known mammalian mAChRs M_1, M_2, M_3, M_4 and M_5, which are coupled to different G-proteins (Caulfield & Birdsall, 1998; Langmead et al., 2008; Lanzafame et al., 2003).

Results of pharmacological analysis of *gar-1*, *gar-2* and *gar-3* ectopically expressed in cell cultures (Kim et al., 2008; Lee et al., 2000; Park et al., 2003) and data presented in this paper show that activation of GAR-3 mAChRs is a general mechanism of the increase of *C. elegans* behavior thermotolerance by agonists of mammalian mAChRs or by rise of ACh content as a result of ACh-esterase inhibition: (i) loss-of-function *gar-3* mutants are insensitive to pilocarpine and neostigmine effects on behavior thermotolerance while the sensitivity of loss-of-function *gar-1* and *gar-2* mutants to this substances at hyperthermia conditions is comparable with such of wild type worms (Figures 7, 8); (ii) the sensitivity of cultured cells to agonists of mammalian mAChRs can be induced by ectopic expression of *gar-3* but not *gar-1* or *gar-2* (Kim et al., 2008; Lee et al., 2000; Park et al., 2003). Therefore the sensitivity of behavior thermotolerance to such agonists of mammalian mAChRs, as pilocarpine, oxotremorine M and carbachol (Figures 4, 5) can be a consequence of activation of GAR-3 mAChRs but not of GAR-1 or GAR-2 mAChRs; (iii) only *gar-3* expression in cultured cells produced significant sensitivity to non-selective antagonist of mammalian mAChRs atropine while cells with *gar-1* expression had only slight sensitivity to atropine and cells with *gar-2* expression were completely insensitive to atropine (Lee et al., 2000). Therefore the most possible explanation of decrease of *C. elegans* thermotolerance by atropine (Figures 5, 6) is the block of GAR-3 muscarinic receptors activation by ACh.

gar-3 is expressed in many neurons and peripheral tissues of *C. elegans* including body wall muscles (Liu et al., 2007; Steger & Avery, 2004). It is known that GAR-3 muscarinic receptor regulates feeding and male mating behaviors of *C. elegans* (Liu et al., 2007; Steger & Avery, 2004). However possible role of GAR-3 in *C. elegans* locomotion is not revealed. Moreover phenotypes of *gar-3* loss-of-function mutants are almost wild-type with exception of faster pharyngeal pumping (Steger & Avery, 2004), suggesting that GAR-3 does not regulate *C. elegans* locomotion under optimal temperature conditions. However, as it is shown in this article, GAR-3 can play important role in the maintenance of *C. elegans* locomotion at extreme high temperature.

The major question has arisen from our data is why loss-of-function *gar-3* mutants in conditions without overactivation of mAChRs either by exogenous agonists or by the rise of endogenous ACh content due to ACh-esterase inhibition have behavior thermotolerance similar with such of wild type worms (Figure 7, 8)? The study of mAChRs knockout animals is an important tool to understanding the role of these receptors in the functions of rodents' and *C. elegans* nervous system. However the physiological relevance of muscarinic signaling is sometimes difficult to pinpoint in these studies. Knockout mice lacking any one of the five mammalian muscarinic receptors subtypes are viable and generally healthy (Gomeza et al., 1999; Hamilton et al., 1997; Zhang et al., 2002). For example M_2 muscarinic receptor knockout mice have normal movement and temperature control, while pharmacological analysis revealed the major role of M_2 mAChR in the thermoregulation and locomotion (Gomeza et al., 1999). The similar data were obtained in experiments with loss-of-function mAChRs mutants of *C. elegans*, since *C. elegans* lacking GAR-2 or GAR-3 mAChRs have generally normal behavior, and differences of all forms of behavior between worms lacking GAR-1 mAChRs and wild type worms are not detectable at optimal temperature (Liu et al., 2007; Steger & Avery, 2004).

It is known that the effect of knockout mutation on animal organism includes both defects of organism functions caused by direct effects of mutations and opposite compensatory effects which allow to remain behavior or other functions of organism in the absence of gene functions necessary for wild type animal (Valet, Tavernier, Castan-Laurell, Sébastien, & Langin, 2002).

Therefore almost normal or fully normal behavior of mutant mice and *C. elegans* lacking one of mAChRs subtypes must be the result of compensatory response of organism on mutation effect. On the other hand the major role of mAChRs in many behaviors in mammals and *C. elegans* is identified by pharmacological analysis of these behaviors using agonists and antagonists of these receptors (Gomeza et al., 1999; Hamilton et al., 1997; Liu et al., 2007; Steger & Avery, 2004; Zhang et al., 2002). Direct effect of single mutation lacking one of mAChRs subtypes was revealed in mice and *C. elegans* in the insensitivity of behavior to agonists and antagonists' action, and this insensitivity allows identifying the possible key role of mAChRs subtypes in the multiple forms of behavior. For example, in mice disruption of M_2 mAChR gene caused great insensitivity of thermoregulation and M-current activity in sympathetic neurons to agonists of mAChRs (Gomeza et al., 1999), and in *C. elegans* loss-of-function *gar-3* mutants are insensitive to effects of mAChRs agonists on feeding and mating behaviors (Liu et al., 2007; Steger & Avery, 2004). Therefore insensitivity of behavior thermotolerance to agonists of mAChRs caused by loss-of-function *gar-3* mutation reveals the key role of GAR-3 mAChR in the regulation of *C. elegans* thermotolerance while the absence of this mutation effect on thermotolerance in the medium without agonists of mAChRs or ACh-esterase inhibitor neostigmine indicates that lacking of *gar-3* gene can be compensated by *C. elegans* organism.

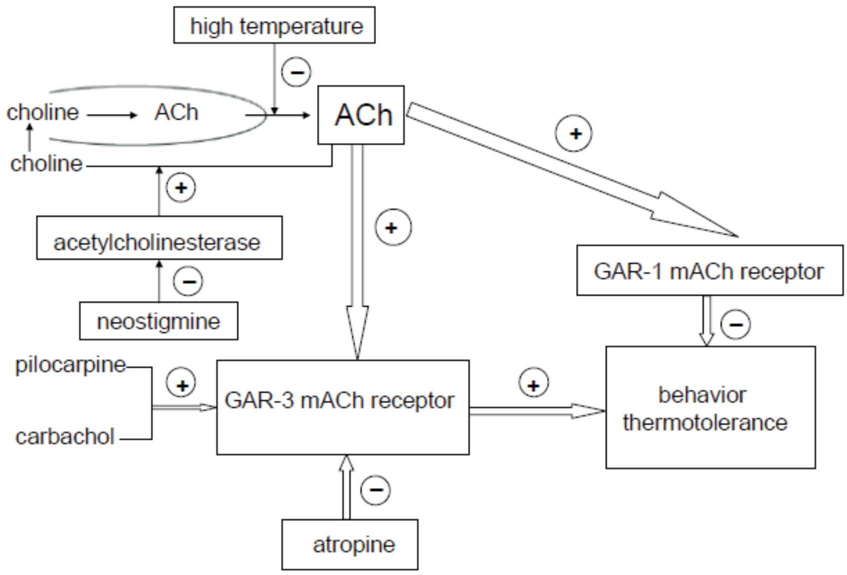

Figure 9. The regulation of *C. elegans* thermotolerance by activation of mAChRs

It is interesting that binding of ACh by GAR-1 mAChRs had opposite to activation of GAR-3 mAChRs effect on behavior thermotolerance since loss-of-function *gar-1* mutation caused rise in *C. elegans* thermotolerance (Figure 7, 8 and scheme in Figure 9). Opposite effects of ACh binding by two subtypes of mAChRs were shown for functions of mammalian and *C. elegans* nervous systems not only at hyperthermia but also at optimal temperature conditions. These effects are explained by differences of expression of genes of mAChRs subtypes in different parts of nervous system and by differences of G-proteins coupled with certain subtypes of mAChRs. For example, in mammals M_1, M_3 and M_5 subtypes of mAChRs are selectively linked to $G_{q/11}$ proteins and activate phospholipase C whereas M_2 and M_4 subtypes are preferentially coupled to $G_{j/0}$ proteins and inhibit adenylate cyclase (Caulfield & Birdsall, 1998; Langmead et al., 2008). Therefore ACh binding by M_2 subtypes receptors in many cases inhibits neurotransmitters release from neurons (Zhang, Chen, & Pan, 2007) while activation of M_1 receptors in contrast stimulates neurotransmitters release (Bauer, Woolley, Teschemacher, & Seward, 2007). In *C. elegans* GAR-2 mAChRs similarly with mammalian M_2 mAChRs mediate negative feedback in cholinergic motor neurons by inhibiting ACh release whereas ectopic expression of *gar-3* in these neurons caused acceleration of ACh secretion (Dittman & Kaplan, 2008).

While the rise in behavior thermotolerance caused by binding of ACh with GAR-3 mAChRs can be explained by stimulation of neurotransmitter release, the opposite effect of ACh binding with GAR-1 mAChRs can not be explained since its physiological relevance is unknown and rise in behavior thermotolerance is the first identified phenotypic feature caused by *gar-1* loss-of-function mutation.

Locomotion is the most important ACh-mediated behavior involving by far the greatest number of cholinergic neurons. The involving of ACh in locomotion includes both neuron-neuron transmission and neuromuscular transmission since ACh is excitatory neurotransmitter depolarizing locomotory muscles. Therefore two possible mechanisms of regulation of locomotion thermotolerance by mAChRs signaling are its effects on the thermostability of neuron-neuron transmission or regulation of thermostability of neuromuscular transmission by ACh binding with GAR-3 or GAR-1 mAChRs. While *gar-3* expression was shown not only in many neurons but also in the body wall muscles (Steger & Avery, 2004), investigation of neuromuscular transmission did not reveal the influence of GAR-3 mAChRs signaling on the sensitivity of locomotory muscles to excitation by nAChRs signaling. However these investigations showed that GAR-3 signaling stimulates neurotransmitter release from cholinergic motor neurons (Dittman & Kaplan, 2008). Therefore it is evident that overactivation of GAR-3 mAChRs can stabilize neuromuscular transmission against heat stress only on presynaptic but not on postsynaptic level if hyperthermia inhibits ACh release from motor neurons. On the other hand, it is possible that neuron-neuron synaptic transmission in the neural network regulating locomotion is more sensitive to hyperthermia than neuromuscular transmission and in this case agonists of mAChRs can elevate thermotolerance of *C. elegans* locomotion by stimulation of neurotransmitters release from interneurons.

It is known that both neuron-neuron and neuron-muscle synaptic transmissions can be a target for reversible disturbances of invertebrates' behavior caused by hyperthermia (Barclay & Robertson, 2001; Janssen, 1992; Kelty et al., 2002; Robertson, 2004b; Tryba & Ramirez, 2003).

The specificity of our experiments consists in measuring of thermotolerance of swimming induced by strong mechanical stimulus in the absence of sensory inputs into nervous system from food and other worms. Two types of behavior disturbances caused by hyperthermia in these conditions were defects of swimming (the lack of coordination of locomotory muscles necessary for sinusoidal movements) and reversible inability of worms to swimming induced by mechanical stimulus. At constant extreme high temperature the first type of above mentioned swimming defects always precedes the loss of ability to swimming. Three parameters determining swimming thermostability in these conditions are: (i) thermotolerance of mechanosensory system function; (ii) thermotolerance of neuronal network formed by the interneurons AVA, AVB and PVC providing input to the A- and B-type motor neurons (responsible for forward and backward movement) and the inhibitory D-type motor neurons involved in the coordinated movement (Leung et al., 2008); (iii) thermotolerance of neuromuscular synaptic transmission.

It is evident that reversible loss of swimming ability can be explained by cessation of neuromuscular synaptic transmission by hyperthermia. However more weak defects of swimming induced by mechanical stimulus without inability to swimming can be explained only by defects of functions of neural networks regulating *C. elegans* swimming or by defects of mechanosensation. Therefore it is evident that prevention of such weak defects of swimming at extreme high temperature by agonists of mAChRs and ACh-esterase inhibitor is a result of protection of neuron-neuron synaptic transmission against disturbances caused by hyperthermia. This conclusion is in accordance with conception assuming that central processes in the nervous system are the most sensitive targets of negative effects of hyperthermia on invertebrates' behavior (Barclay & Robertson, 2001; Dawson-Scully & Robertson, 1998; Kalinnikova et al., 2012; Kelty et al., 2002; Robertson, 2004b).

References

Anderson, G. L., Cole, R. D., & Williams, P. L. (2004). Assessing behavioral toxicity with *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry, 23*, 1235-1240. http://dx.doi.org/10.1897/03-264

Armstrong, G. A. B., Shoemaker, K. L., Money, T. G. A., & Robertson, R. M. (2006). Octopamine mediates thermal preconditioning of the locust ventilatory central pattern generator via a cAMP/protein kinase A signaling pathway. *The Journal of Neuroscience, 26*, 12118-12126. http://dx.doi.org/10.1523/JNEUROSCI.3347-06.2006

Barclay, J. W., & Robertson R. M. (2000). Heat shock-induced thermoprotection of hindleg motor control in the locust. *The Journal of Experimental Biology, 203*, 941-950.

Barclay, J. W., & Robertson R. M. (2001). Enhancement of short-term synaptic plasticity by prior environmental stress. *Journal of Neurophysiology, 85*, 1332-1335.

Bargmann, C. I. (1993). Genetic and cellular analysis of behavior in *C.elegans*. *Annual Review of Neuroscience, 16*, 47-71. http://dx.doi.org/10.1146/annurev.ne.16.030193.000403

Bartus, R. T., Dean, R. L., III., Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science, 217*, 408-417. http://dx.doi.org/10.1126/science.7046051

Bauer, C. S., Woolley, R. J., Teschemacher, A. G., & Seward, E. P. (2007). Potentiation of exocytosis by phospholipase C-coupled G-protein-coupled receptors requires the priming protein Munc13-1. *The Journal of Neuroscience, 27*, 212-219. http://dx.doi.org/10.1523/JNEUROSCI.4201-06.2007

Bouchama, A., & Knochel, J. P. (2002). Heat stroke. *The New England Journal of Medicine, 346*, 1978-1988. http://dx.doi.org/10.1056/NEJMra011089

Boyd, W. A., Cole, R. D., Anderson, G. L., & Williams, P. L. (2003). The effects of metals and food availability on the behavior of *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry, 22*, 3049-3055. http://dx.doi.org/10.1897/02-565

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics, 77*, 71-94.

Carnell, L., Illi, J., Hong, S. W., & McIntire S. L. (2005). The G-protein-coupled serotonin receptor SER-1 regulates egg laying and male mating behaviors in *Caenorhabditis elegans*. *The Journal of Neuroscience, 25*, 10671-10681. http://dx.doi.org/10.1523/JNEUROSCI.3399-05.2005

Caulfield, M. P., & Birdsall, N. J. (1998). International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacological Reviews, 50*, 279-290.

Changeux, J. P., & Edelstein, S. J. (1998). Allosteric receptors after 30 years. *Neuron, 21*, 959-980. http://dx.doi.org/10.1016/S0896-6273(00)80616-9

Chase D. L., Koelle M. R. Biogenic amine neurotransmitters in *C. elegans* (February 20, 2007). In *WormBook* (Ed.), The *C. elegans* Research Community, WormBook. Retrieved from http://www.wormbook.org.

Conti-Tronconi, B. M., & Raffery, M. A. (1982). The nicotinic cholinergic receptor: correlation of molecular structure with functional properties. *Annual Review of Biochemistry, 51*, 491-530. http://dx.doi.org/10.1146/annurev.bi.51.070182.002423

Cossins, A. R., & Bowler, K. (1987). *Temperature biology of animals*. New York: Chapmann and Hall. http://dx.doi.org/10.1007/978-94-009-3127-5

Culetto, E., Baylis, H. A., Richmond, J. E., Jones A. K., Fleming, J. T., Squires, M. D., … Sattelle, D. B. (2004). The *Caenorhabditis elegans unc-63* gene encodes a levamisole-sensitive nicotinic acetylcholine receptor α subunit. *The Journal of Biological Chemistry, 279*, 42476-42483. http://dx.doi.org/10.1074/jbc.M404370200

David, J. R., Allemand, R., Van Herrewege, J., & Cohet, Y. (1983). Ecophysiology: abiotic factors. In M. Ashburner, H. L. Carson, & J. N. Thompson (Eds.), *The genetics and biology of Drosophila*. (pp. 105-107). London: Academic Press.

Davies, A. G., Pierce-Shimomura, J. T., Kim, H., VanHoven, M. K., Thiele, T. R., Bonci, A., … McIntire, S. (2003). A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell, 115*, 655-666. http://dx.doi.org/10.1016/S0092-8674(03)00979-6

Davies, A. G., Bettinger, J. C., Thiele, T. R., Judy, M. E., & McIntire, S. (2004). Natural variation in the *npr-1* gene modifies ethanol responses of wild strains of *C. elegans*. *Neuron, 42*, 731-743. http://dx.doi.org/10.1016/j.neuron.2004.05.004

Dawson-Scully, K., & Robertson R. M. (1998). Heat shock protects synaptic transmission in the flight motor circuitry of locusts. *NeuroReport, 9*, 2589-2593. http://dx.doi.org/10.1097/00001756-199808030-00030

Dittman, J. S., & Kaplan, J. M. (2008). Behavioral impact of neurotransmitter-activated-G-protein-coupled receptors: muscarinic and $GABA_B$ receptors regulate *Caenorhabditis elegans* locomotion. *The Journal of Neuroscience, 28*, 7104-7112. http://dx.doi.org/10.1523/JNEUROSCI.0378-08.2008

Ellis, J. R., Ellis, K. A., Bartholomeusz, C. F., Harrison, B. J., Wesnes, K. A., Erskine, F. F., … Nathan, P. J. (2006). Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *The International Journal of Neuropsychopharmacology, 9*, 175-189. http://dx.doi.org/10.1017/S1461145705005407

Erskine, F. F., Ellis, J. R., Stuber, F., Hogan, K., Miller, V., Moore, E., … Nathan, P. J. (2004). Evidence for synergic modulation of early information processing by nicotinic and muscarinic receptors in humans. *Human Psychopharmacology, 19*, 503-509. http://dx.doi.org/10.1002/hup.613

Esmaeili, B., Ross, J. M., Neades, C., Miller, D. M., III, & Ahringer, J. (2002). The *C.elegans* even-skipped homologue, *vab-7*, specifies DB motoneurons identity and axon trajectory. *Development, 129*, 853-862.

Feiro, O., & Gould, T. J. (2005). The interactive effects of nicotinic and muscarinic cholinergic receptor inhibition on fear conditioning in young and aged C57BL/6 mice. *Pharmacology Biochemistry and Behavior, 80*, 251-262. http://dx.doi.org/10.1016/j.pbb.2004.11.005

Fleming, J. T., Squire, M. D., Barnes, T. M., Tornoe, C., Matsuda, K., Ahnn, J., … Lewis, J. A. (1997). *Caenorhabditis elegans* levamisole resistance genes *lev-1*, *unc-29* and *unc-38* encode functional nicotinic receptor subunits. *Journal of Neuroscience, 17*, 5843-5857.

Gomeza, J., Shannon, H., Kostenis, E., Felder, C., Zhang, L., Brodkin, J., … Wess, J. (1999). Pronounced pharmacologic deficit in M2 muscarinic acetylcholine receptor knockout mice. *Proceedings of the National Academy of Sciences of the USA, 96*, 1692-1697. http://dx.doi.org/10.1073/pnas.96.4.1692

Govorunova, E. G., Moussaif, M., Kullyev, A., Nguyen, K. C. Q., McDonald, T. V., Hall, D. H., & Sze, J. Y. (2010). A homolog of FHM2 is involved in modulation of excitatory neurotransmission by serotonin in *C. elegans*. *PLoS ONE, 5(4)*, e10368. http://dx.doi.org/10.1371/journal.pone.0010368

Hamilton, S. E., Loose, M. D., Qi, M., Levey, B., Hille, B., McKnight, G. S., … Nathanson, N. M. (1997). Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proceedings of the National Academy of Sciences, 94*, 13311-13316. http://dx.doi.org/10.1073/pnas.94.24.13311

Hart A. C. Behavior. (July 3, 2006), *WormBook* (Ed.), The *C. elegans* Research Community, WormBook. http://dx.doi.org/10.1895/wormbook.1.87.1

Harvey, R. A., Champe, P. C., Finkel, R., Cubeddu, L. X., & Clark, M. A. (2009). Neostigmine. *Pharmacology* (4th ed.). Lippincott's Illustrated *Reviews* (p. 51). USA: Lippincott Williams & Wilkins.

Hoffmann, A. A., Sørensen, J. G., & Loeschke, V. (2003). Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology, 28*, 175-216. http://dx.doi.org/10.1016/S0306-4565(02)00057-8

Janssen, R. (1992). Thermal influences on nervous system function. *Neuroscience&Biobehavioral Review*s, *16*, 399-413. http://dx.doi.org/10.1016/S0149-7634(05)80209-X

Johnson, T. E., & Nelson, G. A. (1991). *Caenorhabditis elegans*: A model system for space biology studies. *Experimental Gerontology, 26*, 299-309. http://dx.doi.org/10.1016/0531-5565(91)90024-G

Kalinnikova, T. B., Kolsanova, R. R., & Gainutdinov, M. Kh. (2012). *Caenorhabditis elegans* as a convenient model organism for understanding heat stress effects upon intact nervous system. In S. Josipovich & E.Ludwig (Eds.), *Heat Stress: Causes, Treatment and Prevention* (pp. 113-140). NY: Nova Science Publishers.

Kelty, J. D., Noseworthy, P. A., Feder, M. E., Robertson, R. M., & Ramirez, J. M. (2002). Thermal pre-conditioning and HSP72 preserve synaptic conditioning during thermal stress. *The Journal of Neuroscience, 22*, RC193:1-RC193:6.

Kim, S., Shin, Y., Park, Y-S., & Cho, N. J. (2008). Regulation of ERK1/2 by the *C.elegans* muscarinic acetylcholine receptors GAR-3 in Chinese hamster ovary cells. *Molecules and Cells, 25*, 504-509.

Langmead, C. J., Watson, J., & Reavill, C. (2008). Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacology & Therapeutics, 117*, 232-243. http://dx.doi.org/10.1016/j.pharmthera.2007.09.009

Lanzafame, A. A., Christopoulos, A., & Mitchelson, F. (2003). Cellular signaling mechanism for muscarinic acetylcholine receptors. *Receptors and Channels, 9*, 241-260. http://dx.doi.org/10.1080/10606820308263

Lee, Y. S., Park, Y. S., Nam, S., Suh, S. J., Lee, J., Kaang, B. K., & Cho, N. J. (2000). Characterization of GAR-2, a novel G protein-linked acetylcholine receptor from *Caenorhabditis elegans*. *Journal of Neurochemistry, 75*, 1800-1809. http://dx.doi.org/10.1046/j.1471-4159.2000.0751800.x

Leung, M. C. K., Williams, P. L., Benedetto, A., Au, C., Helmcke, K. J., Aschner, M., & Meyer, J. N. (2008). *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. *Toxicological Sciences, 106*, 5-28. http://dx.doi.org/10.1093/toxsci/kfn121

Liu, Y., LeBoeuf, B., & Garcia, L. R. (2007). $G\alpha_q$-coupled muscarinic acetylcholine receptors enhance nicotinic acetylcholine receptor signaling in *Caenorhabditis elegans* mating behavior. *The Journal of Neuroscience, 27*, 1411-1421. http://dx.doi.org/10.1523/JNEUROSCI.4320-06.2007

Naguib, M., & Yaksh, T. L. (1997). Characterization of muscarinic receptor subtypes that mediate antinociception in the rat spinal cord. *Anesthesia & Analgesia, 85*, 847-853. http://dx.doi.org/10.1097/00000539-199710000-00025

Nurrish, S., Ségalat, L., & Kaplan, J. M. (1999). Serotonin inhibition of synaptic transmission: $G\alpha_0$ decreases the abundance of UNC-13 at release site. *Neuron, 24*, 231-242. http://dx.doi.org/10.1016/S0896-6273(00)80835-1

Park, Y-S., Kim, S., Shin, Y., Choi, B., & Cho, N. J. (2003). Alternative splicing of the muscarinic acetylcholine receptor GAR-3 in *Caenorhabditis elegans*. *Biochemical and Biophysical Research Communications, 308*, 961-965. http://dx.doi.org/10.1016/S0006-291X(03)01508-0

Perry, E. K., Tomilinson, B. E., Blessed, G., Bergmann, K., Gibson, P. H., & Perry, R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal, 6150*, 1457-1459. http://dx.doi.org/10.1136/bmj.2.6150.1457

Robertson, R. M. (2004a). Modulation of neural circuit operation by prior environmental stress. *Integrative and Comparative Biology, 44*, 21-27. http://dx.doi.org/10.1093/icb/44.1.21

Robertson, R. M. (2004b). Thermal stress and neural function: adaptive mechanisms in insect model systems. *Journal of Thermal Biology, 29*, 351-358. http://dx.doi.org/10.1016/j.jtherbio.2004.08.073

Satelle, D. B. (2009). Invertebrate nicotinic acetylcholine receptors - targets for chemicals and drugs important in agriculture, veterinary medicine and human health. *Journal of Pesticides Science, 34*, 233-240.

Sawin, E. R., Ranganathan, R., & Horvitz, H. R. (2000). *C.elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron, 26*, 619-631. http://dx.doi.org/10.1016/S0896-6273(00)81199-X

Schafer, W. R., & Kenyon, S. (1995). A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. *Nature, 375*, 73-78. http://dx.doi.org/10.1038/375073a0

Singer, J. H., Bellingham, M. C., & Berger, A. J. (1996). Presynaptic inhibition of glutamatergic synaptic transmission to rat motoneurons by serotonin. *Journal of Neurophysiology, 76*, 799-807.

Steger, K., & Avery, L. (2004). The GAR-3 muscarinic receptor cooperates with calcium signals to regulate muscle contraction in the *Caenorhabditis elegans* pharynx. *Genetics, 167*, 633-643. http://dx.doi.org/10.1534/genetics.103.020230

Tabet, N. (2006). Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine closing! *Age and Ageing, 35*, 336-338. http://dx.doi.org/10.1093/ageing/afl027

Tissenbaum, H. A., Hawdon, J., Perregaux, M., Hotez, P., Guarente, L., & Ruvkin, G. (2000). A common muscarinic pathway for diapause recovery in the distantly related nematode species *Caenorhabditis elegans* and Ancylostoma caninum. *Proceedings of the National Academy of Sciences of the USA, 97*, 460-465. http://dx.doi.org/10.1073/pnas.97.1.460

Tryba, A. K., & Ramirez, J. M. (2003). Response of the respiratory network of mice to hyperthermia. *Journal of Neurophysiology, 89*, 2975-2983. http://dx.doi.org/10.1152/jn.00743.2002

Unwin, N. (2005). Refined structure of the nicotinic acetylcholine receptor at A4 resolution. *Journal of Molecular biology, 346*, 967-989. http://dx.doi.org/10.1016/j.jmb.2004.12.031

Valet, P., Tavernier, G., Castan-Laurell, I., Sébastien, S. B., & Langin, D. (2002). Understanding adipose tissue development from transgenic animal models. *Journal of Lipid Research, 43*, 835-860.

Zhang, W., Basile, A. S., Gomeza, J., Volpicelli, L. A., Levey, A. I., & Wess, J. (2002). Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *The Journal of Neuroscience, 22*, 1709-1717.

Zhang, H. M., Chen, S. R., & Pan, H. L. (2007). Regulation of glutamate release from primary afferents and interneurons in the spinal cord by muscarinic receptor subtypes. *Journal of Neurophysiology, 97*, 102-109. http://dx.doi.org/10.1152/jn.00586.2006

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