·ISSN 2372-0352

AIMS Environmental Science

Volume No. 10

Issue No. 3

September - December 2023

ENRICHED PUBLICATIONS PVT. LTD

S-9, IInd FLOOR, MLU POCKET,
MANISH ABHINAV PLAZA-II, ABOVE FEDERAL BANK,
PLOT NO-5, SECTOR-5, DWARKA, NEW DELHI, INDIA-110075,
PHONE: - + (91)-(11)-47026006

·ISSN 2372-0352

AIMS Environmental Science

Aims and Scope

AIMS Environmental Science is an international Open Access journal devoted to publishing peer-reviewed, high quality, original papers in the field of Environmental science. We publish the following article types: original research articles, reviews, editorials, letters, and conference reports.All published papers' full text from 2016 will be indexed by WoS.

Aim and scope

AIMS Environmental science is an international Open Access journal devoted to publishing peer-reviewed, high quality, original papers in the field of Environmental science. We publish the following article types: original research articles, reviews, editorials, letters, and conference reports.

AIMS Environmental science welcomes, but not limited to, the papers from the following topics:

- Global climate change & adaption
- Air-water-biota-rock interfaces
- Environmental epidemiology & ecology
- Pollution control & mitigation
- Environmental materials
- Natural resource management
- Waste management
- Geo-hazards
- Environmental risk analysis
- Air & water quality

·ISSN 2372-0352

AIMS Environmental Science

·ISSN 2372-0352

·ISSN 2372-0352

·ISSN 2372-0352

·ISSN 2372-0352

·ISSN 2372-0352

·ISSN 2372-0352

·ISSN 2372-0352

Wei Wu	Department of Coastal Sciences, Gulf Coast Research Laboratory, The University of Southern Mississippi, 703 East Beach Drive,Ocean Springs, MS 39564 USA
Wei-Min Wu	Department of Civil & Environmental Engineering 473 Via Ortega, B23, Stanford University, Stanford, CA 94305-4020, USA
Wassana Yantasee	Department of Biomedical Engineering, OHSU School of Medicine, Portland, Oregon 97239 USA
Hong Zhang	School of Engineering and Built Environment, Griffith University, Australia
Jia-Zhong Zhang	Ocean Chemistry Division, Atlantic Oceanographic and Meteorological Laboratory, 4301 Rickenbacker Causeway, Miami, Florida 33149, USA
Jiping Zhu	Exposure and Biomonitoring Division, Health Canada, Ottawa, Ontario, Canada

·ISSN 2372-0352

AIMS Environmental Science

(Volume No. 10 Issue No. 3 September - December 2023)

Contents

·ISSN 2372-0352

Depletion of Indonesian oil palm plantations implied from modeling oil palm mortality and Ganoderma boninense rot under future climate

Robert Russell Monteith Paterson1,2, *

1 CEB–Centre of Biological Engineering, Gualtar Campus, University of Minho, Braga 4710-057, Portugal

2 Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysiaal

A B S T R A C T

Currently there are increasing land-cover change and land-management issues related to oil palm plantations. Palm oil is highly valuable and it is added to numerous commodities internationally with Indonesia being the most voluminous producer. The cultivation of oil palm affects the environment negatively, per se, threatening plantation sustainability from deforestation. Furthermore, the fungus Ganoderma boninense causes basal stem rot of oil palm, a major problem for plantation managers in Indonesia. Suitable future climate for growth of Indonesian oil palm, determined by employing the simulation programme, CLIMEX, has already been employed to create scenarios of oil palm mortality and diseases. This was achieved by combining CLIMEX with narrative models, a technique often employed in climate studies. Determinations of the percentage of oil palm that survive climate change and do not have basal stem rot have also been provided. The current paper considers these scenarios for Indonesia and regions for the first time. The models assume "no change" in the future climate scenarios and that effective mitigation is not taken. The effects on plantation sustainability are considered. Climate change will not affect the incidence of basal stem rot greatly until 2050, but the situation will deteriorate thereafter. Sumatra and Java are especially affected and plantations may be unsustainable quickly, whereas Papua and Sulawesi may be able to sustain the crop longer. However, deforestation in Papua and Sulawesi is highly undesirable. Methods to manage climate change effects on oil palm and reduce basal stem rot are described. Plantation managers can use the earlier assessments herein to indicate if the later ones are accurate and plan to ameliorate the problems. Conclusions indicate that palm oil production may be unsustainable in Indonesia by 2050 from loss of plantations thus requiring action immediately.

Keywords: global heating; Elaeis guineensis; basal stem rot; Sumatra; Kalimantan; CLIMEX

1. Introduction

There are increasing land-cover change and associated land-management issues in relation to oil palm plantations [1]. Demand for palm oil has transformed these landscapes in numerous ways including by highly increased deforestation compared to the previous century. There is increasing information on how 21st-century climate change is likely to decrease the sustainability of plantations with serious implications for food security, biodiversity loss and the economies of palm oil producing, and other countries, that use the oil in numerous commodities [2–6].

Palm oil is very important [7]. The commodity is employed (i) in cooking, (ii) in food and cosmetics,

(iii) as detergent, (iv) in plastics, (v) as chemicals and (vi) as biodiesel [1]. Indonesia's economy is supported by high financial returns from palm oil and it accounts for more than 30% of global demand for vegetable oil. Current global production indicates that 61% comes from Indonesia [8] which is the world's largest manufacturer. Lam et al. [8] indicate that most Indonesian oil palm are located in Sumatra and Kalimantan and that the greatest potential for developing new plantations are in Sulawesi and Papua, although it is essential to preserve the natural forest for environmental reasons. In 2009, 65, 25 and 3% of plantations were located in Sumatra, Kalimantan and Sulawesi respectively with the remainder being loosely distributed over other regions including Papua and Java [9].

Oil palm cultivation increased from 10 to 17 million ha between 2000 and 2012 which was expected to increase [8]. Paterson et al. [10,11] indicated that climate change will affect oil palm production severely, especially after 2050 and production may not increase, at least by as much that is anticipated [8]. Oil palm production may decrease from 10 to 41% if temperature rises from 1 to 4 °C [6]. In addition, basal stem rot of oil palm, a serious disease caused by the fungus Ganoderma boninense, is likely to increase, making the industry much less sustainable in South East Asia [2–5].

Oil palm is associated with deforestation and conversion of peat soils, inevitably involved in climate change and haze creation: The negative effects on biodiversity are concerning [12], even greater deforestation may occur with other oil crops [13]. However, palm oil may have a higher greenhouse gas footprint [8]. Climate change affects detrimentally oil palm agronomy potentially leading to low sustainability [1,2–5], whereas a lowered ability to grow oil palm may reduce climate change because less greenhouse gas will be produced. Palm oil is one of the highest causes of greenhouse gas currently from global land use and cover change [8]. The effect on human health from consuming palm oil and growing oil palm is also of concern, adding to the commodity's general importance [14]. Zoonotic disease in humans may increase from conversion of natural forest to agriculture crops [15] and changes in the ability to grow oil palm will affect deforestation. The effect of climate change on oil palm agronomy requires greater understanding, although there is much information on the effect of palm oil agronomy on climate change. Climate change effects on oil palm is relevant to the sustainability of the industry through risks to plantations, with economic and social implications [1]. All stakeholders will be affected, e.g. oil palm companies, food manufacturers, smallholders, cosmetic manufactures and biodiesel producers and consumers. There are three areas where a decline in oil palm production could occur: (a) concerns about biodiversity and increased greenhouse gases, (b) climate change [10,11] and (c) health concerns of palm oil [14,15].

Southeast Asia is predicted to face the highest loss in crop production with the rise of temperature. Oil palm fruit production has declined due to the uncertainties of climate change and it has estimated to have significant nonlinear impacts on the net revenue of oil palm production. The land for oil palm production can become dry and degraded, and oil palm plantations may become susceptible to diseases and pests due to temperature rise [6].

Indeed, oil palm is threatened by various diseases [7] and many fungal ailments are anticipated to become more prevalent as climate change progresses [6]. Pathogens live in a dynamic environment with the host where the disease may take hold if the plant becomes less resistant. Basal stem rot by Ganoderma boninense has been prevalent for 80 years [7] and is the most serious disease in S E Asia. The level of disease currently can be considered as containable, however, it will increase if palms are grown under suboptimal or unsuitable climates [1,2–5,7].

Ganoderma species concepts are ineffectively described [17] and G. boninense will be able to adapt to climate change more readily than oil palm by the selection of more virulent strains or species [2–5,16,18,19]. G. boninense can reduce yields by 50–80% and the economic loss is $365 million per annum [19]. Basal stem rot can kill 80% of a plantation at 50% of their economic life span [7].

Expansion of industrial oil palm cultivation and basal stem rot disease began early in Sumatra, Indonesia (and peninsular Malaysia) [2] where adaptation of G. boninense to the environments is most likely to occur [8]. This region contains the highest levels of disease, implying an association between time of existence of oil palm plantations and high disease levels. The above data indicate a trend for increased basal stem rot with projected climate change.The climate for growing oil palm is optimal currently and has been for many decades [1,2–5,7]: The increase in disease previously reported will be from increased virulence of the fungus, rather than decreased resistance of oil palm from inclement climate. Some regions may become suitable for oil palm, providing an opportunity to grow disease-free oil palm [10,11]. Two scenarios resulting in more disease with climate change are recognized by the current author: an increase in virulence of G. boninense and a less suitable climate for oil palm, making it less resistant.

The effect of climate on oil palm growth and disease is relevant to spatial planning, oil palm management and sustainable development, as are the interfaces between dynamic models and social ecological planning of oil palm landscapes. Paterson [2] determined the effect of changes in climate for growing oil palm on the infection levels of basal stem rot in Sumatra, Indonesia using similar methods employed in the current report. The developed scenario indicated that basal stem rot would become even more serious after 2050 and that the climate for growing oil palm would deteriorate greatly. Weather is a major factor in crop maladies and when crops suffer cold, heat or dry stress, they may be more susceptible. However, only the changes in highly suitable and suitable climate were considered and the effect on the mortality of oil palm was not assessed. Paterson [3] employed similar methodology of simulation modeling to produce a postulated scheme of how basal stem rot would advance under future climates in Malaysia. The assessments of basal stem rot were qualitative and indicated, nevertheless, that the levels of infection would also increase a great deal after 2050. The

methods developed in [2,3] were further refined in Paterson [4,5]. An assessment of mortality of oil palm with future climates was undertaken for Kalimantan, Indonesia and alternative oil palm producing countries in SE Asia such as Thailand and the Philippines [4]. Similarly, scenarios of mortalities of oil palm were determined for Malaysia, Indonesia and South American countries such as Colombia and Brazil [5]. Furthermore, an assessment of basal stem rot in the countries considered in [4] were also undertaken. Finally, a similar procedure was undertaken to determine future incidences of Phytophthora palmivora disease of oil palm in the SE Asian and South American countries [5].

The objectives of the work described in the current paper were to provide quantitative data for Indonesia regarding oil palm mortality and BSR incidence until 2100 by employing methodsdeveloped in [4,5] especially. The information will be of considerable utility to oil palm plantation managers, environmentalists, economists and politicians. Importantly, various regions/states of the country were also considered. Mitigation issues are also discussed.

2. Materials and method

2.1. General

Paterson et al. [10,11] developed numerical simulation models describing suitable climate scenarios for growing oil palm under climate change, which were employed in the current study to provide novel developed schemes for future infection of oil palm with basal stem rot and oil palm mortalities in Indonesia: The methods for creating narrative scenarios of oil palm diseases and mortalities were established in Paterson [4,5] especially. Briefly, the maps provided were magnified to enhance Indonesia and the amounts of highly suitable, suitable marginal and unsuitable climates were determined by assessing visually the areas of each designated colour on the maps.

Future suitable climate scenarios methods for oil palm growth in Indonesia are described fully in Paterson et al. [10]. In summary, the distribution model for oil palm under current and future climate was developed using CLIMEX for Windows Version 347 (Hearne Scientific Software Pty Ltd, Melbourne 2007) and climate data and climate change scenarios were carried out using CliMond 10´gridded climate data. The potential future climate was characterized using the same five variables based on two Global Climate Models (GCMs), CSIRO-Mk3.053 and MIROC-H (Centre for Climate Research, Japan) with the A1B and A2 SRES scenarios which were available as part of the CliMond dataset. The fitting of CLIMEX Parameters employed the Global Biodiversity Information Facility, a database of natural history collections around the world for various species. Information on the global distribution of oil palm was used in parameter fitting and 124 records were used. South East Asian distribution data were reserved for validation of the model.

The oil palm (Elaeis guineensis Jacq.) distribution was determined by the Global Biodiversity Information Facility (GBIF) (http://www.gbif.org/, accessed 9 November 2015) and additional literature on the species in CAB Direct (http://www.cabdirect.org/web/about.html, accessed October 2015), and formed the basis for the collection of data on the E. guineensis distribution in Paterson al. [11] with 2465 records utilized in fitting the parameters. These records may be described as geographically representative of known distribution of the species. The global study used CSIRO Mk3·0 and MIROC-H GCM global climate models (GCMs) to model potential future distribution of oil palm. CLIMEX in conjunction with the A2 Special Report on Emissions Scenarios (SRES) scenario, a mechanistic niche model using CLIMEX software supports ecological research incorporating the modelling of species' potential distributions under differing climate scenarios and assumes that climate is the paramount determining factor of plant and poikilothermal animal distributions. CLIMEX output categorized areas according to highly suitable climate, suitable climate, marginal climate and unsuitable climate.

2.2. Disease assessment

The basidiomata [2] were counted on oil palm on specific areas of plantations and compared to oil palms without symptoms within the same area. The incidence of basal stem rot were assessed as postulated schemes given that projected climate change is likely to result in more disease of oil palm and highly stressed oil palm. The incidences were determined by (a) considering the extent to which basal stem rot would change at each time period because of altered climate for oil palm growth and (b) attributing a plausible scenario of a 3% increase in basal stem rot every 10 years to take into account the virulence of G. boninense per se as discussed in the Introduction. The infection rate of 3% every 10 years was a compromise figure between increasing virulence initially and then a potential decrease in virulence as the conditions may become unsuitable for the fungus. The decreased resistance of oil palm was taken into account to arrive at the final levels used in the figures. These assessments are discussed in Paterson [4].

The level of existing basal stem rot was determined from the literature [2] and from estimates based on length of time of existence of plantations in the particular region and proximity to regions where data exists for the level of disease [3]. The current level of infection in Sumatra was provided in Paterson [2]. The level in Java was considered to be high from its proximity to Sumatra and the fact the plantations have been in existence for a long time. The level in Kalimantan was determined by its proximity to Sarawak and Sabah where the levels are known [3] and by the fact the plantations are recent compared to Sumatra. They are dispersed at greater distances throughout the region and likely to have less disease. In Sulawesi and Papua the plantations are recent compared to Kalimantan, and dispersed and the scenario employed here was that basal stem rot would be low. The initial incidences of the disease are

The initial incidences of the disease are provided in Figure 1 as CT. The basic methods are published in Paterson [4].

2.3. Oil palm mortality

The mortality of oil palm was determined particularly by the increase in marginal and unsuitable climate for growing oil palm where mortality would be inevitable in the marginal (or "borderline") climate. Some oil palm would be in the process of dying in the case of unsuitable climate.

3. Results and discussion

The current time incidence of infection of basal stem rot in all regions, Sumatra, Java, Kalimantan, Sulawesi and Papua were 19, 39, 30, 19, 10 and 5% respectively (Figure 1a,b). The data indicate that highly suitable climate was maintained or increased for all Indonesia, Kalimantan, Sulawesi and Papua (Figures 2, 5, 6 and 7 respectively) from current time until 2070. Hence, oil palm mortality from inclement climate will remain similar to that of current time and the palms will remain healthy and resistant to basal stem rot, at least from the suitability of climate for growing oil palm point of view. However, the virulence of G. boninense will increase under the described scheme from the value of 3% per 10 years as discussed in the methods.

Figure 2 demonstrates the changes in climate which are suitable for growing oil palm and the mortality of oil palm from adverse climate scenarios for all Indonesian regions. Unsuitable climate decreased until 2050 and then maintained a low level. Marginal climate remained low until 2070 and then increased to a significant amount in 2100. The mortality of oil palm correspondingly increased in 2100 to 10%. The pattern of basal stem rot incidence was similar to that of Kalimantan (Figure 1a): The incidence increased sharply to 70% by 2100. Hence, the 90% of oil palm that are living from by 2100, will have an incidence of 70% basal stem rot under these scenarios.

Figure 3 demonstrates the changes in climate suitable and the mortality of oil palm from adverse climate scenarios for Sumatra. The climate was highly suitable until 2050 and decreased considerablyby 2070. There was a dramatic increase in marginal climate and a corresponding increase in oil palm mortality from 0 to 25% for 2070 and 2100 under this scheme. Figure 1b indicates the increase in basal stem rot incidence throughout this period. The incidence of basal stem rot in current time was high [7] which increased moderately until 2050 and then rapidly to 2100. Hence, of the 75% of oil palm that survived the adverse climate by 2100, 94% will have basal stem rot under this derived scheme.

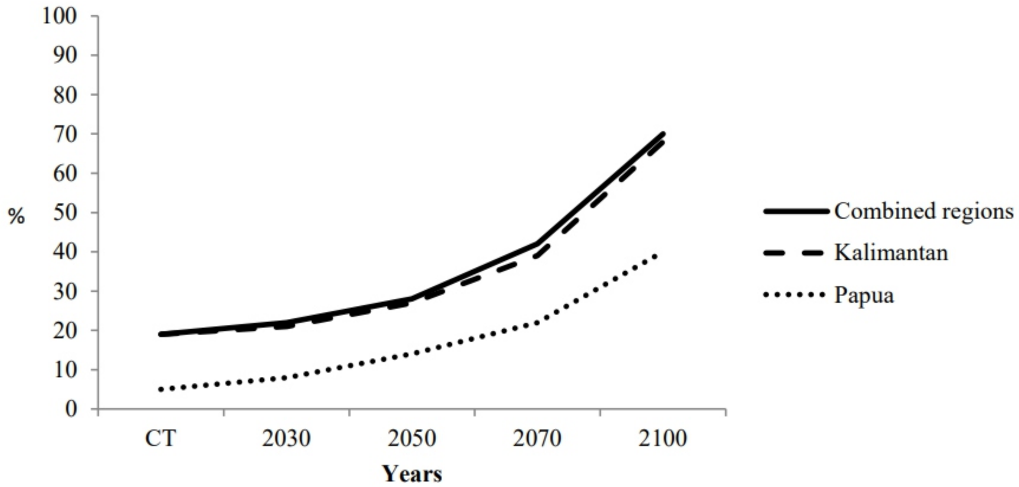

Figure 1a. Percentage of oil palm with basal stem rot in various Indonesian regions. CT = current time.

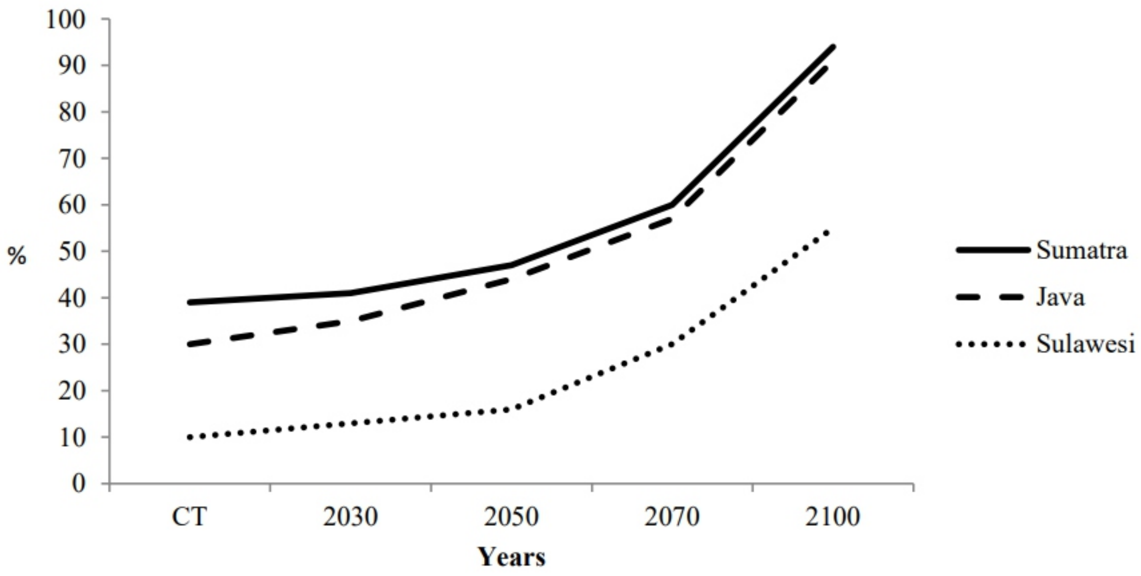

Figure 1b. Percentage of oil palm with basal stem rot in various Indonesian regions. CT = current time. The incidences were determined by (a) considering the extent to which basal stem rot would change at each time period because of altered climate for oil palm growth and (b) attributing a plausible scenario of a 3% increase in basal stem rot every 10 years to take into account the virulence of G. boninense per se.

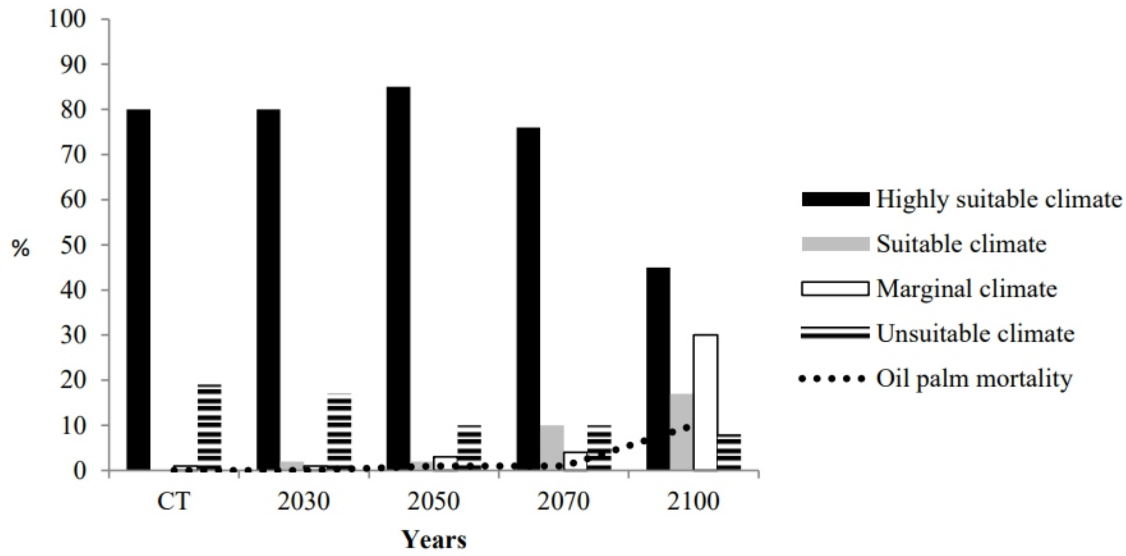

Figure 2. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for all Indonesian regions. CT = current time. The maps provided in Paterson et al. [10,11] were magnified to enhance Indonesia and the amounts of highly suitable and suitable climates were determined by assessing visually the areas of each designated colour on the maps. These were used to provide quantitative narrative models for oil palm mortality. CLIMEX output categorized areas according to highly suitable climate, suitable climate, marginal climate and unsuitable climate.

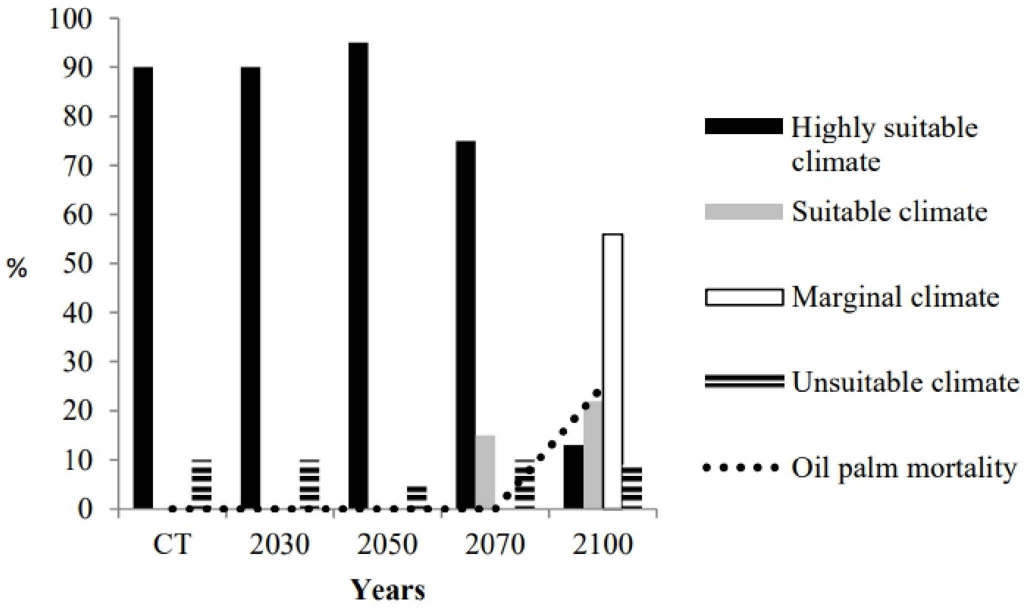

Figure 3. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for Sumatra (refer to Figure 2 legend for a full explanation).

The change in suitable climate for growing oil palm and the increase in oil palm mortality data for Java are provided in Figure 4. There was a linear decrease in highly suitable climate between current time until 2070 with unsuitable and suitable climate increasing until 2050. A large amount (25%) of marginal climate was indicated by 2070 under this scenario with an associated increase in oil palm mortality. The marginal climate increased further to 54% by 2100 with 35% oil palm mortality. A rapid increase in basal stem rot was determined under these scenarios from an initial level of 30%. Basal stem rot incidence increased from 2050 until 2100, when the highest amount of 91% was determined (Figure 1b). Hence 91% of oil palm would have basal stem rot of the 65% surviving palms by 2100.

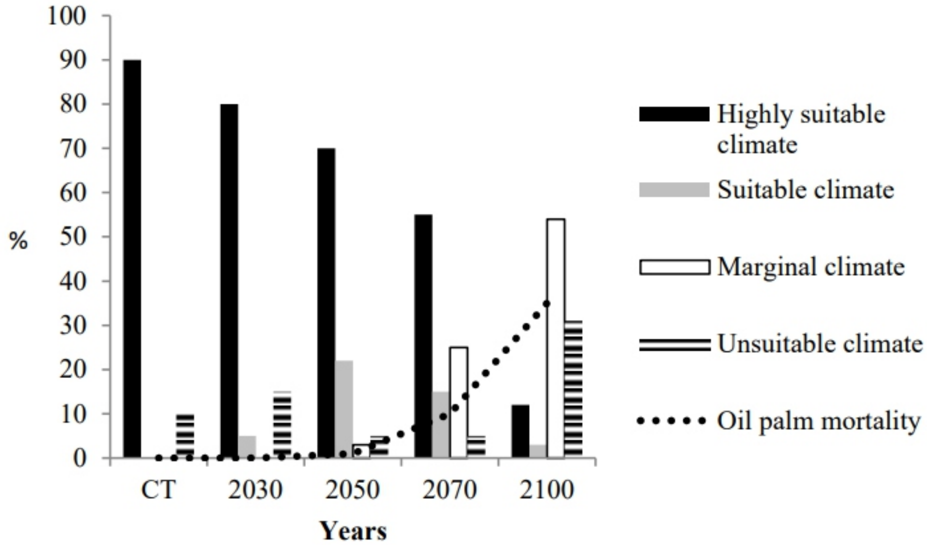

Figure 4. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for Java. CT = current time (refer to Figure 2 legend for a full explanation).

The scenario for Kalimantan is demonstrated in Figure 5. Highly suitable climate was maintained until 2070 and then decreased moderately to 2100. There was a decrease in unsuitable climate by 2070, indicating that the climate will be suitable for growing oil palm compared to other regions. A medium increase in marginal climate was suggested by 2100 which corresponded to a modest increase of 8% in oil palm mortality under this scenario. Overall, the climate appears suitable for growing oil palm even under climate change as outlined herein. The change in basal stem rot incidence was significant after 2050 (Figure 1a) and basal stem rot incidence were at 39 and 68% by 2070 and 2100 respectively under this derived scheme. By 2100, the surviving 92% of oil palm would have a basal stem rot incidence of 68%. The situation in Sulawesi (Figure 6) is different from the previous examples. Highly suitable climate for growing oil palm was low at 75% in current time and increased to 95% by 2050. A concomitant decrease in unsuitable climate indicating that climate change would not necessarily have a negative effect on oil palm growth. The highly suitable climate decreased after 2050 but remained at a high level until 2100. Unsuitable climate increased after 2050. There was only a small level of marginal climate for growing oil palm throughout and mortality from climate of oil palm would be low during these periods. The amount of basal stem rot remained low according to these devised schemes (Figure 1b) with the maximum disease incidence being 55% in 2100. The surviving 99% of oil palms in 2100 will have 55% basal stem rot which is comparatively

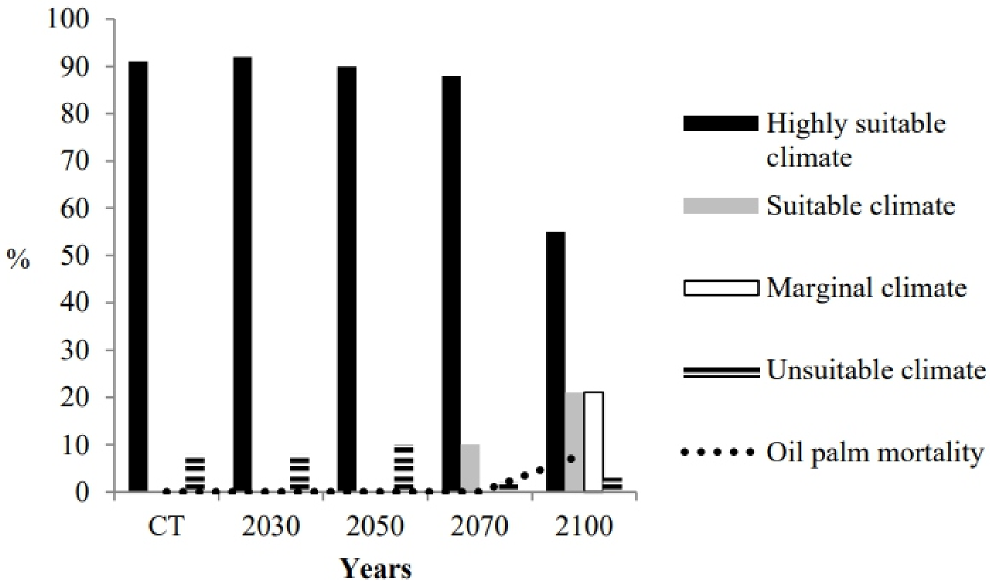

Figure 5. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for Kalimantan (refer to Figure 2 legend for a full explanation).

A low amount of highly suitable climate for growing oil palm was recorded in Papua (Figure 7). A level of approximately 70% was maintained until 2070. A high amount of unsuitable climate was recorded throughout, although this deceased slightly by 2100. Marginal climate increased only by 2100 leading to a scenario with a moderate increase in mortality of the oil palm. There was only a slight increase in disease until 2100 from a low level in current time (Figure 1a). By 2100 there was 40% basal stem rot in the 91% of oil palm surviving from current time, again a comparatively benign situation.

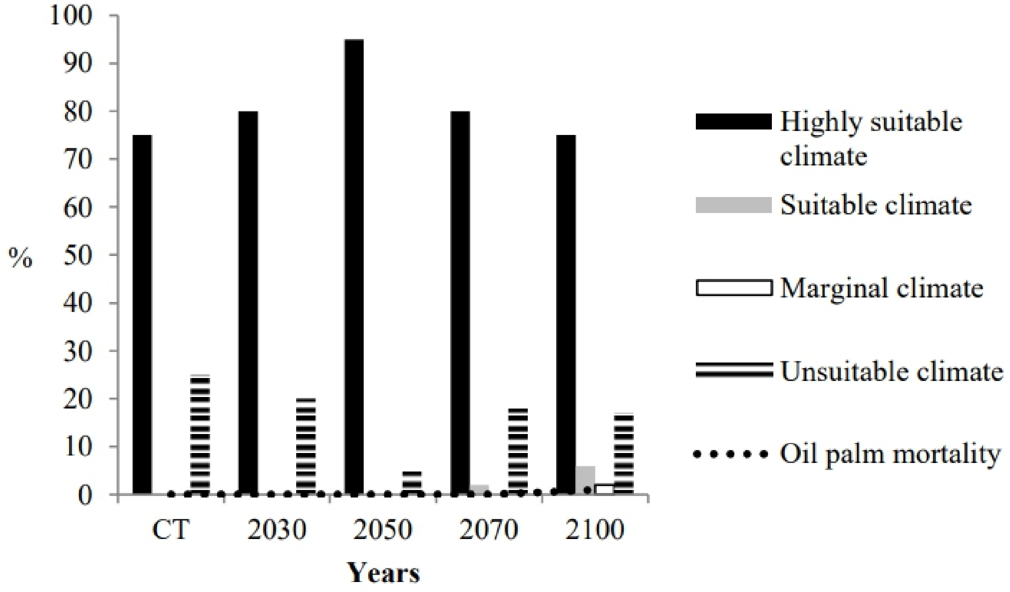

Figure 6. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for Sulawesi (refer to Figure 2 legend for a full explanation).

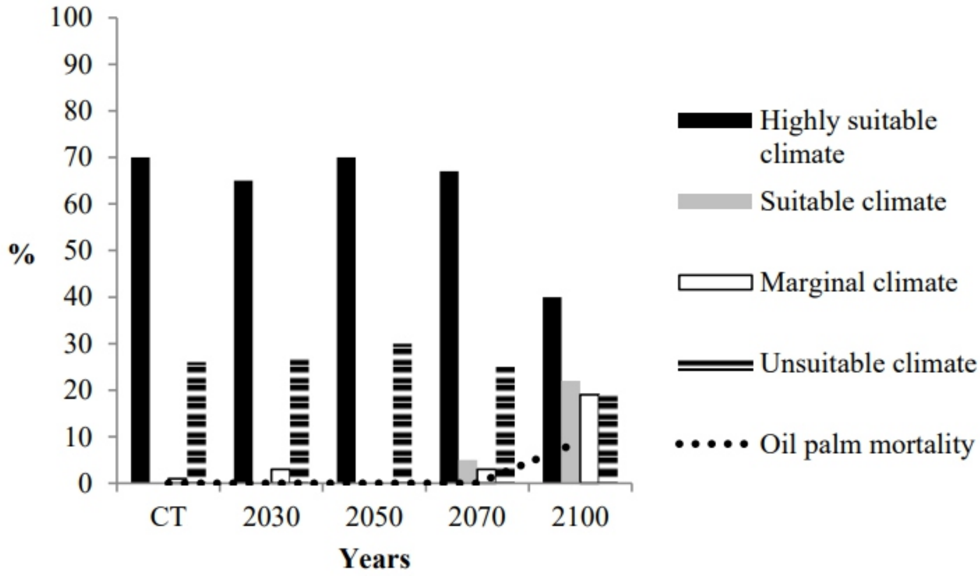

Figure 7. Percentage change in suitable climate for growing oil palm and mortality of oil palm from inclement climate for Papua (refer to Figure 2 legend for a full explanation).

The percentages of oil palm that survived the climate changes and had no basal stem rot until 2100 are presented in Figure 8a,b. The figures for all regions combined were initially 81% which decreased moderately until 2030 and then rapidly to 27% by 2100 (Figure 8a). The data for Kalimantan are very similar to these percentages (Figure 8a). Papua had a high percentage of oil palms that survive climate change and without basal stem rot with a value for 2100 of 64% (Figure 8a). Java was affected dramatically under this scenario with oil palm mortality and basal stem rot levels similar to Sumatra (see below) (Figure 8b). From an initial figure of 70%, the percentages fell slowly until 2050 and rapidly thereafter to 6% by 2100. Sumatra had a lower initial level of 61% and then followed a similar pattern to Java (Figure 8b). Sulawesi had a high value in current time of 90% which fell moderately until 2050 and then rapidly until 2100 to 45% (Figure 8b). These data indicate severe problems of sustainability for the oil palm industry.

Cold stress played an important role in the changes in climatic suitability for oil palm in Indonesia [10]: A reduction in cold stress was observed by 2100 in Sumatra and Java. An increase in heat stress was prominent in Java, Sumatra and Kalimantan which would lead to increased oil palm mortality in these regions. However, the increased heat stress may be too great for further basal stem rot infection and so an increase in disease may not be exponential which is a reason that the increase in disease of was maintained at 3% throughout the test period in the present paper, rather than employing, for example, an exponential increase. Interestingly, dry stress was confined to the most southerly parts of Indonesia which were not considered in the current paper as they have few significant plantations. The map definition in [11] was insufficient to make similar assessments.

The Figures 2–7 above do not consider mortality of oil palm from other reasons such as increased pollution, inadequate fertilization, other diseases and/or inefficient management more generally. Hence, there will likely be greater disease and mortality than suggested by the current data. The information represents large decreases in the sustainability of the oil palm industry in most cases from depletion of oil palm in plantations. In summary, the industry may become unsustainable after 2050 under these developed schemes, although Papua and Sulawesi represent more sustainable conditions [1]. However, deforestation must not occur if these regions were developed because this is highly detrimental to the environment. The area available for oil palm in Sulawesi is small in comparison to other areas and would not compensate adequately for losses in the major production regions.

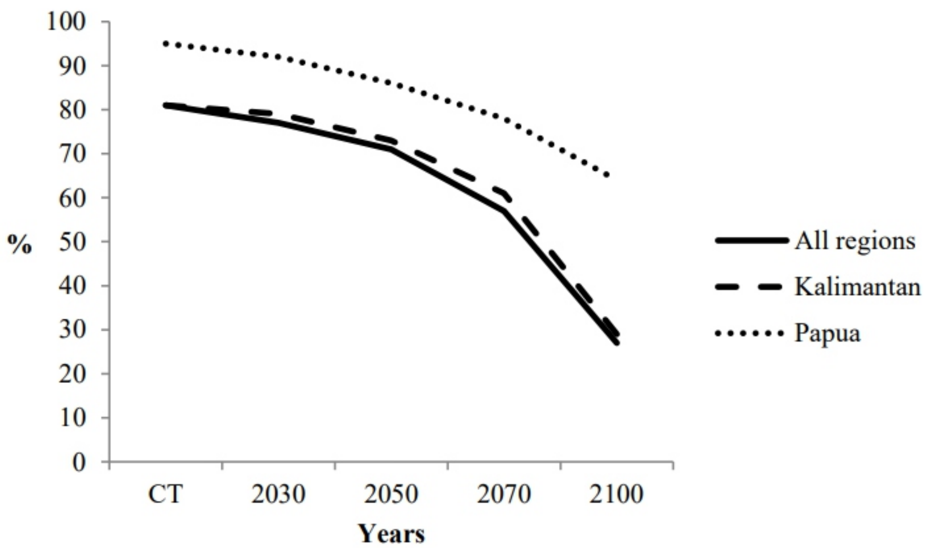

Figure 8a. Percentage of oil palm that survived inclement climate and have no basal stem rot for various regions. CT = current time.

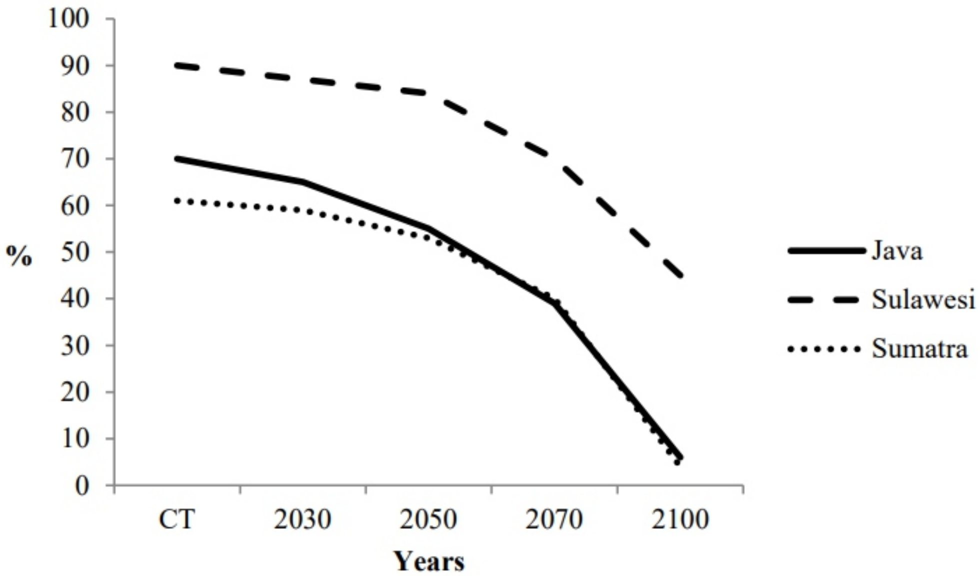

Figure 8b. Percentage of oil palm that survived inclement climate and have no basal stem rot for various regions. CT = current time.

The results herein for Sumatra can be compared to the equivalent postulated schemes for Sumatra in [2], where the disease reached 100% by 2100. In the present work, 75% oil palm will survive the inclement climate of which 94% will have basal stem rot by 2100. Both results indicate a very serious situation for the Sumatran oil palm industry. However, Papua may be able to compensate to some extent, although it is extremely important not to deforest this region. Paterson [3] described the problem of G. boninense infection becoming moderately worse by 2070 and much worse by 2100 in Malaysia, using similar methods of big data and simulation technology to those described in the present paper. The data in Paterson [4] suggest that Kalimantan and the Philippines may be more suitable for growing oil palm than Thailand or Myanmar, with Papua New Guinea being intermediate by using similar methods to those in the present paper. The mortalities of oil palm in South America was assessed to be much higher in South America compared to Indonesia and Malaysia in Paterson [5] using similar scenarios.

The negative relationship between climate change and oil palm production indicates a reduction related to rises of temperature and sea level [6]. The findings demand implementation of mitigation and adaptation strategies to reduce the negative impacts of climate change on the oil palm sector. Mitigation strategies include (a) conservation of carbon stocks, forest biomass and soil carbon; (b) reduction of carbon losses from biota, better soil and ecosystem management; (c) prevention of deforestation and (d) enhancement of carbon sequestration in soils and biota through increased forestation [1,6]. Adaptation strategies include improved land tenure, appropriate choices of technology, better food security, and an ecosystem-based approach to development, by improving adaptive capacity and responding to changes in water demands.

Developing climate resilient oil palm varieties is crucial for the changing climate conditions [1]. Soil and water conservation are critical to increase water infiltration and reduce run-off that can help overcome climate change. The application of organic matter improves the water-holding capacity and reduces inorganic fertilizer application. Mulching, weed and cover crop can prevent soil erosion, maintain soil moisture, improve water infiltration, reabsorb CO2 emission from land and reduce water evaporation. It is necessary to involve multiple stakeholders, such as (a) private sector decision makers, including industry, individual producers and consumers, and (b) public decision makers who are important to facilitate mitigation and adaptation strategies in the oil palm sector. Thus, the sharing of costs and benefits amongst governments, industries and farmers are key concerns in understanding mitigation and adaptation in the oil palm sector [6].

The present paper demonstrates dynamic models which can be employed for social-ecological planning of oil palm landscapes. Plantation managers, inter alia, can use the assessments herein to indicate if their control measures ameliorate climate change effects on oil palm. The scenarios are based on a "no change" situation and do not include any steps that might be taken to ameliorate the effect of climate change on oil palm. Neither do they consider whether climate change will be more benign or severe than is considered likely at present.

4. Conclusion

Urgent action is required to control climate change and maintain the palm oil industry. Paterson and Lima [1] suggested practices and methods for oil palm development in the future. These include using leguminous crops, empty oil palm bunches, earthworms, arbuscular mycorrhizal fungi and biochar to protect against climate change and disease, indicating how biological heterogeneity can be used to reconcile production and conservation. The Indonesian government must consider collaborating closely with other nations to implement the recommendations of the Paris climate agreement and other more recent COP conferences, as the most likely way to reduce climate change to the benefit of the oil palm industry (C:/Users/Russell/Downloads/cp2019_L10E.pdf).

Acknowledgments

The author greatly appreciates the IOI Professorial Chair he received at Universiti Putra Malaysia for 2018–2019. This research received no external funding.

Conflict of interest

The author declares no conflict of interest

References

1. Paterson RRM, Lima N (2018) Climate change affecting oil palm agronomy, and oil palm cultivation increasing climate change, require amelioration. Ecol Evol 8: 452–461.

2. Paterson RRM (2019) Ganoderma boninense disease of oil palm is expected to significantly reduce production after 2050 in Sumatra if projected climate change occurs. Microorganisms 7:24.

3. Paterson RRM (2019) Ganoderma boninense disease deduced from simulation modelling with large data sets of future Malaysian oil palm climate. Phytoparasitica 47: 255–262.

4. Paterson RRM (2020). Oil palm survival under climate change in Kalimantan and alternative SE Asian palm oil countries with future basal stem rot assessments. Forest Pathol 50: e12604.

5. Paterson RRM (2020) Future scenarios for oil palm mortality and infection by Phytophthora palmivora in Colombia, Ecuador and Brazil, extrapolated to Malaysia and Indonesia. Phytoparasitica 48: 513–523.

6. Sarkar SK, Begum RA, Pereira JJ (2020) Impacts of climate change on oil palm production in Malaysia. Environ Sci Pollut R 27: 9760–9770.

7. Corley RHV, Tinker PB (2015) The Oil Palm, Wiley Blackwell.

8. Lam YW, Kulak M, Sim S, et al. (2019) Greenhouse gas footprints of palm oil production in Indonesia over space and time. Sci Total Environ 688: 827–837.

9. Rianto B (2010) Overview of palm oil industry in Indonesia. Pricewaterhouse Coopers Indonesia. Available from: https://www.pwc.com/id/en/publications/assets/palm-oil-plantation.pdf

10. Paterson RRM, Kumar L, Taylor S, et al. (2015) Future climate effects on suitability for growth of oil palms in Malaysia and Indonesia. Sci Report 5.
11. Paterson RRM, Kumar L, Shabani F, et al. (2017) World climate suitability projections to 2050 and 2100 for growing oil palm. J Agric Sci 155: 689–702.
12. Dislich C, Keyel AC, Salecker J, et al. (2017) Wiegand, K. A review of the ecosystem functions in oil palm plantations, using forests as a reference system. Biol Rev 49: 1539–1569.
13. Meijaard E, Garcia-Ulloa J, Sheil D, et al. (2018) Oil palm and biodiversity: A situation analysis by the IUCN Oil Palm Task Force. 2018.
14. Kadandale S, Marten R, Smith R (2019) The palm oil industry and noncommunicable diseases. Bull World Healt Organ 97: 118–128.
15. Gibb R, Redding DW, Chin KQ, et al. (2020). Zoonotic host diversity increases in human dominated ecosystems. Nature 584: 398–402.
16. Paterson RRM, Sariah M, Lima N (2013) How will climate change affect oil palm fungal diseases? Crop Prot 46: 113–120.
17. Zhou LW, Cao Y, Wu SH, et al. (2015) Global diversity of the Ganoderma lucidum complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. Phytochem 114: 7–15.
18. Merciere M, Boulord R, Carasco-Lacombe C, et al. (2017) About Ganoderma boninense in oil palm plantations of Sumatra and peninsular Malaysia : Ancient population expansion, extensive gene flow and large scale dispersion ability. Fungal Biol 121: 529–540.
19. Paterson R R M, Lima N. Ecology and biotechnology of thermophilic fungi on crops under global warming[M]//Fungi in Extreme Environments: Ecological Role and Biotechnological Significance. Springer, Cham. 2019: 81–96.

·ISSN 2372-0352

Development of sample preparation method for organochlorine pesticides analysis in soil samples

Thanawat Jumepaeng1,* and Nirand Pongpun2
1 Faculty of Sports Science and Health, Thailand National Sports University Udon Thani Campus, Udon Thani, Thailand
2 Faculty of Science, Srinakharinwirot University, Bangkok, Thailand

A B S T R A C T

OCPs are the persistent organic pollutants which are highly toxic to the environment and can accumulate in humans by foods such as rice, vegetable, and fruits. The aim of this research was to develop a rapid and easy sample preparation method for determination 4 OCPs, aldrin, dieldrin, β endosulfane, and p-p' DDT in soil samples by gas chromatography with electron capture detection. The analysis was performed by using HP-5 capillary column with the isothermal condition at 250 oCand carrier gas flow rate of 1 mL/min. The results show that the 4 OCPs and internal standard, pentachloronitrobenzene, were analyzed within 6 min. The optimized condition of the sample preparation method was using ultrasonic assisted solvent extraction with 20 mL of hexane and ethyl acetate (9:1 v/v) as an extractant for 15x2 min. The results obtained from the proposed sample preparation method have demonstrated that the limit of detection was 0.628–3.68 µg kg−1. Percent recoveries were in the range of 81.42 to 110.7% and RSD were 1.68–9.43%. Correlation coefficients of calibration graphs were more than 0.99. The developed sample preparation method exhibited simplicity rapid and low chemical reagent consumption with satisfactory results.

Keywords: none clean-up method; ultrasonic extraction; Thai soil sample; electron capture detector

1. Introduction

Organochlorine pesticides (OCPs) were widely used after World War II due to cheap, high stability, and bioaccumulation property. However, these persistent chemicals can be transferred and accumulate in the environment [1]. They are the large group of persistent organic pollutants (POPs) with high lipophilicity, bioaccumulation activities, long half-life in the food chain [2]. OCPs have been banned for agricultural and other uses in Europe, North America South America and many countries of Asia including Thailand because of their carcinogenic, mutagenic, and toxicity properties. However, OCPs still can be found in environmental samples such as water, agriculture products, animal, and soils caused by their long half-life and non-polar properties [1]. It is widely known that OCPs still contaminated in soils because they do not decompose due to their physical properties and it is widely recognized that most non-polar organic compounds, such as the OCPs retained in soils, are bound to solid organic matter with covalent bonds, and hydrophobic interactions [3]. Mostly, the interaction between soil matrices and OCPs is stronger than in several agriculture products [4].

Therefore, the development of sample preparation method for analysing and monitoring OCPs in soil samples is still important. Many studies have shown that the extraction and clean-up of OCPs from soils are problematic steps in sample preparation methods and OCPs analysis [5]. Numerous papershave been published about OCPs extraction from soils, namely applying the soxhlet extraction [6] solid phase microextraction [7] and tradition shake flask extraction [8] followed in some cases by a clean-up step with solid-phase extraction. Tor and co-worker present the ultrasonic technique combined the solvent extraction of OCPs from environment matrices and clean-up by solid phase extraction method [9] but this methodology still require expensive equipment, high amount of toxicity solvent, complexation procedure, and long-time extraction. Thus the aim of this work was to develop a cheap, safety, simple, and rapid sample preparation method with satisfying precision and accuracy for determination of OCPs in soils.

2. Material and methods

The four standard compounds of OCPs namely, aldrin, dieldrin, β-endosulfane, and p-p'-DDT were purchased from Chem service (USA) and pentachloronitrobenzene as an internal standard (IS) were purchased from Sigma aldrich (USA). The stock solutions of these compounds were prepared by dissolving in ethyl acetate (RCI Labscan, Thailand) and kept storing in the dark place at 4 oC. The extraction solvents namely, hexane, ethyl acetate, dichloromethane were purchased from Acros Organics (USA) and sodium chloride was purchased from Merck (Germany). Two types of clean-up materials including Alumina and C-18 cartridges were purchased from Merck (Germany) and Chromabound (USA), respectively. The deionized water was obtained by using a Milli-Q water purification system.

2.1. Reference soil samples

The reference soil samples were collected from the Department of Agriculture, Thailand. The soils were collected in plastic bags (1 kg) from 10 to 20 cm in the ground. The reference sample was heated for the elimination of OCPs in soils by drying oven at 100 oC for 24 h and ground sample to powder. Soil samples were spiked with a mixture of the standard solution prior to analysis by GC-ECD under each optimization parameter.

2.2. Sample preparation procedure

2.2.1. Optimization of type of extraction solvent and extraction time

Soil powder (10.00 grams) that spiked with 10.00 µg kg−1of 4 OCPs were placed into 100 mL of the beaker. Then, 20 mL of two different types of extraction solvents (mixture solvent between hexane:ethyl acetate 9:1 v/v and dichloromethane) were added and the sample was extracted by ultrasonic technique for five different time periods (5, 10, 15, 20, and 25 min). The sample matrix was then filtrated with Whatman no.42 and extraction again. After the twice extraction, the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of each extraction solvents and then immediately injected into the GC-ECD to prevent the analytes from losing in the injection step.

2.2.2. Optimization of clean up methods.

The spiked soil powder was placed into 100 mL of the beakers. Then, 20 mL of mixed solvents were added and the soil sample was extracted by ultrasonic technique for 15 min. The supernatant was then filtrated and extracted again. After the twice extraction, The clean-up was carried out for three different methods (Alumina, C-18, and without clean-up). The extracts were clean-up by using Alumina and C-18 solid phase extraction with hexane:ethyl acetate 9:1 v/v as an eluent solution. All of the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of mixed solvent and then immediately injected into the GC-ECD

2.3. Real sample analysis

Soil samples such as S101, S102, S103, and S104 were obtained from several regions of Department of Agriculture, Thailand. Soils were ground to powder and added 20 mL of mixture extraction solvent and extracted by ultrasonic technique. The sample matrix was then filtrated with Whatman no.42 and extraction again. After the twice extraction, the extracts were evaporated under nitrogen stream condition until dryness and redissolved with 0.50 mL of mixed solvent between hexane and ethyl acetate (9:1 v/v) and then immediately injected into the GC-ECD.

Table 1. The Gas chromatography (GC-ECD) conditions.

Parameters	Optimized conditions
Column temperature	250 °C
Detector temperature	320 °C
Injection system	split mode: split ratio 64.4:1
Injection temperature	260 °C
Injection volume	1.0 µL
Carrier gas	Helium
make up gas	Nitrogen
Rate of carrier gas	1 mL/min

2.4. GC-ECD analysis of soil samples

The extracts of OCPs in soil samples were analyzed by gas chromatography with electron capture detector (GC-ECD) (Hewlett-Packard). The capillary column was HP-5 (5%-phenyl 95%-dimethylpolysiloxane 30.0 m x 320 µm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL min−1. The isothermal program was set at 250 oC (6 min). Other operating conditions and the retention time of 4 OCPs are shown in Table 1 and the chromatograms of 4 OCPs and IS in reference soil samples are shown in Figure 1.

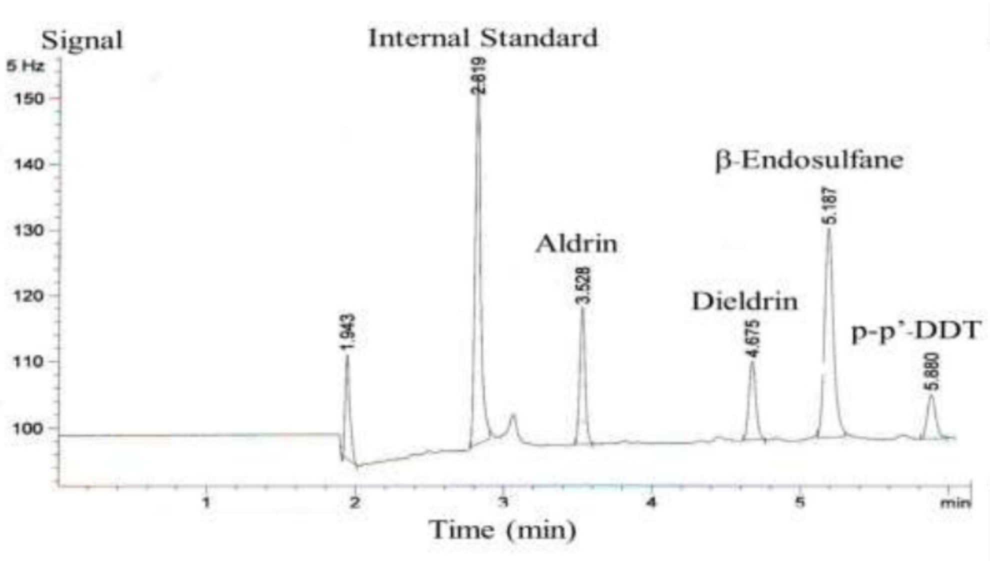

Figure 1. The chromatogram of 4 OCPs and internal standard

3. Results and discussion

3.1. Optimization of sample preparation method

The effects of some sample preparation parameters were studied including extraction solvent, extraction time, and type of clean–up methods, affecting to the extraction efficiency of the sample preparation method.

3.1.1 Optimization of type of extraction solvent

The characteristic requirements of the extraction solvent in sample preparation are composed of suitable volatility, low viscosity, and polarity nearly to OCPs. The extraction solvents which have been used for the ultrasonic assisted solvent extraction that gave good results in earlier work, namely, mixture solvent between hexane and ethyl acetate (9:1 v/v) and dichloromethane were investigated in detail as shown in Figure 2A. It was indicated that the highest signal of these OCPs was obtained by using mixture solvent between hexane and ethyl acetate (9:1 v/v) as the extraction solvent. In fact, the trend of extraction efficiency between mixture solvent and dichloromethane were closely but p-p'-DDT was not found when used dichloromethane as extraction solvent. Thus, mixture solvent between hexane and ethyl acetate (9:1 v/v) was choosing as the extraction solvent in this study.

3.1.2. Optimization of extraction time

Extraction time is the time to extract 4 OCPs from the soil samples by extraction solvent. The effect of the extraction time on the signal of OCPs in soils was performed from 5 to 25 min shows that in Figure 2B. It was found that the signal trend of 4 OCPs increased with an increase of the extraction time up to 15 min after that they slightly decrease. It might relate to the back extraction of the analytes caused by a long time of the extraction. So, the extraction time of 15 min was an appropriate case in this study.

3.1.3. Optimization of clean-up methods

Various clean-up methods have been used by different research groups for extract OCPs from soil samples. In this work, ultrasonic assisted solvent extraction with column chromatography, C-18 cartridge, and the extraction without clean-up method was studied. Figure 2C indicates that the ultrasonic assisted solvent extraction without the clean-up method provided a slight increase in the extraction efficiencies of 4 OCPs as compared with the other two clean-up procedures for soil samples. It might have been the OCPs extracts were loss in the clean-up methods and affecting to the signal of 4 OCPs. From the signal trends with all of three methods, without the clean-up method is the best choice for the sample preparation of 4 OCPs compounds from soil samples.

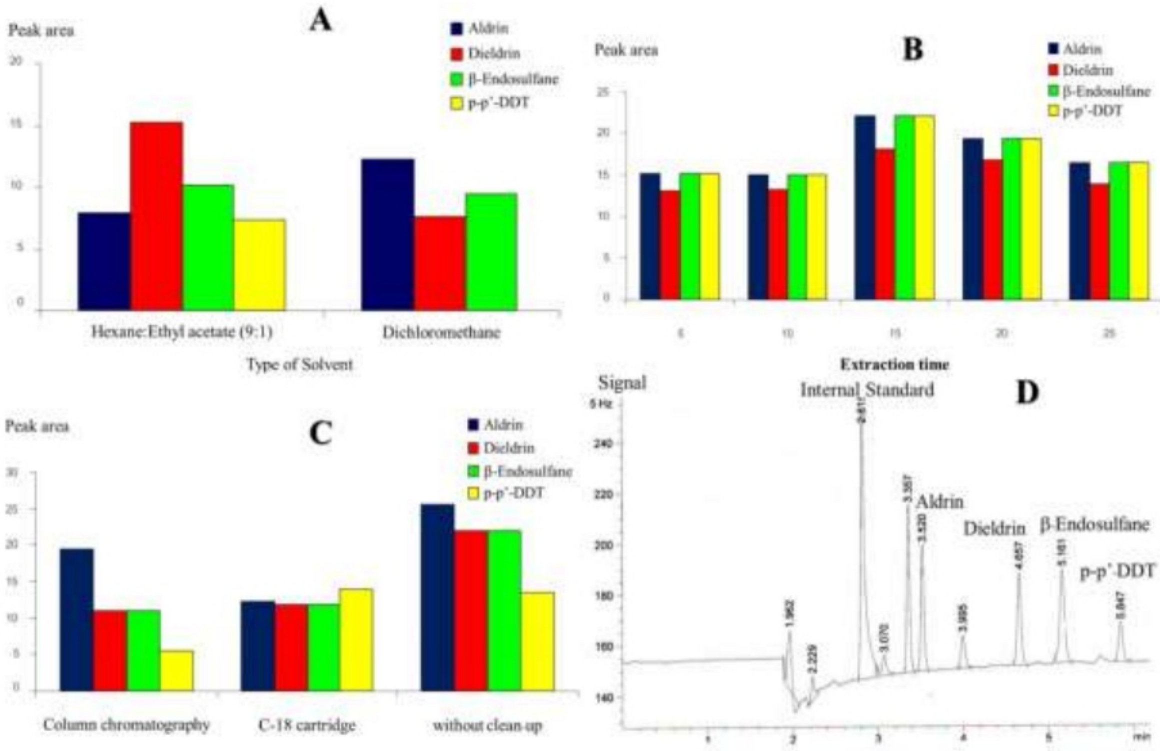

Figure 2. The effect of A) extraction solvent B) Extraction time C) clean-up methods on 4 OCPs analysis and D) the chromatogram of 4 OCPs in real soil samples.

3.2. Features of the analytical method

To validate the optimum conditions of propose sample preparation method, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (%RSD) and accuracy (% recovery) were investigated. The results of the validated methods for sample preparation and GC-ECD are shown in Table 2. Linearity for the five-point calibration curves were satisfied with linear coefficient values greater than 0.99 at the concentration range of 5–200 μg kg−1for 4 OCPs. The obtained LOD values were in the range from 0.628−3.68 μg kg−1 while LOQ were in the range between 2.093−12.27 μg kg−1. Both LOD and LOQ demonstrate that the proposed sample preparation method is sensitive enough to determine these OCPs in soil samples. The method precision was expressed as %RSD in the range of 1.68−9.43 %. The recovery (%) studied was determined by spiking of the mixed solution of OCPs standard into the soil sample which was prepared to be a blank without any residual OCPs. Then the spiked sample was analysed under the optimum conditions of the proposed sample preparation-GC-ECD method. The analytical recoveries of these OCPs represent a satisfactory value in the range from 81.42–110.7%. It is shown that the developed method gives an acceptable precision and accuracy.

3.3. Analysis of OCPs in soil samples

The developed sample preparation method was then applied for the determination of 4 OCPs in soil samples. The chromatogram of 4 OCPs are shown in Figure 2D and Table 3, respectively. It was found that there were high contents of Dieldrin in S101−S103 and β-Endosulfane in S101 and S104. Additionally, Aldrin and p-p'-DDT was not detectable in all samples. From the results, it is indicated that the developed sample preparation method with GC-ECD is a rapid, simple and suitable method for the determination of these OCPs from the soil samples.

Table 2. Linear range of calibration curve, LOD, LOQ, %RSD and % recovery of the proposed method.

OCPs	Linear range (μg kg^{-1})	R^2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	%RSD (n=6)	%Recovery (n=3)
Aldrin	5–150	0.991	1.186	3.954	1.68	110.7±11.1
Dieldrin	5–150	0.997	0.628	2.093	9.08	105.2±6.57
β-Endosulfane	5–150	0.991	2.259	7.530	9.43	106.4±11.6
p-p'-DDT	10–200	0.998	3.681	12.268	4.46	81.42±10.77

Table 3. The contents of some OCPs found in soil samples determined by the proposed method and GC-ECD (mean ±SD, n=5, ND = not detected).

Sample code	OCPs (μg kg^{-1})			
	Aldrin	Dieldrin	β-Endosulfane	p-p'-DDT
S101	ND	17.09±0.69	15.96±0.52	ND
S102	ND	280.50±6.8	ND	ND
S103	ND	5.68±0.78	ND	ND
S104	ND	ND	265.25±7.92	ND

4. Conclusion

In this study, an optimized ultrasonic extraction without the clean-up procedure followed by analysis with GC-ECD has been indicated a good alternative method for the analysis of toxicity organochlorine pesticides in soil. This method exhibited none complexion procedure and lower chemical reagent consumption than tradition extraction method with satisfactory precision and accuracy results. Moreover, the ultrasonic extraction without clean-up method have shown the rapid sample preparation method because the OCPs extracted from soil samples by ultrasonic extraction and filtrated were clean enough for injecting to GC-ECD analysis.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Chung S, Chen B (2011) Determination of organochlorine pesticide residues in fatty foods: A critical review on the analytical methods and their testing capabilities. J Chromatogr A 1218: 5555–5567.

2. Fernandes V, Pestana D, Monteiro R, et al. (2012) Optimization and validation of organochlorine compounds in adipose tissue by SPE-gas chromatography. Biomed Chromatogr 26: 1494–1501.

3. Zhang N, Yang Y, Tao S, et al. (2011) Sequestration of organochlorine pesticides in soils of distinct organic carbon content. Environ Pollut 159: 700–705.

4. Rissato S, Galhiane M, Apon B, et al. (2005) Multiresidue Analysis of pesticides in soil supercritical fluid extraction/gas chromatography with electron-capture detection and confirmation by gas chromatography−mass spectrometry. J Agric Food Chem 53: 62–69.

5. Stoytcheva M (2011) Pesticides—strategies for pesticides analysis. London Intechopen.

6. Fatoki O, Awofolu R (2003) Methods for selective determination of persistent organochlorine pesticide residues in water and sediments by capillary gas chromatography and electron-capture detection. J Chromatogr A 983: 225–236.

7. Chunzhou D, Zhaorui Z, Ming Y (2005) Determination of organochlorine pesticides and their

derivations in water after HS-SPME using polymethyl-phenylvinylsiloxane-coated fiber by GCECD. Water Res 39: 4204–4210.
8. Dabrowska H, Dabrowski L, Biziuk M, et al. (2003) Solid-phase extraction clean-up of soil and sediment extracts for the determination of various types of pollutants in a single run. J Chromatogr A 1003: 29–42.
9. Tor A, Mehmet A, Senar O (2006) Ultrasonic solvent extraction of organochlorine pesticides from soil. Anal Chim Acta 559: 173–180.

·ISSN 2372-0352

Assessment of methods practiced in the disposal of solid waste in Eastleigh Nairobi County, Kenya

Joseph Muiruri1,*, Raphael Wahome2 and Kiemo Karatu3
1 Wangari Maathai Institute for Peace & Environmental Studies (WMI), College of Agriculture and Veterinary Sciences, University of Nairobi, P. O. Box 29053-00625 Nairobi, Kenya
2 Department of Animal Production, University of Nairobi, Kenya
3 Department of Sociology, University of Nairobi, Kenya

ABSTRACT

Solid waste management is a documented threat to health and the environment to many countries in sub-Saharan Africa. Rapid industrial development and urbanization have seen a rise in urban population which translates to massive production of solid waste. Though most urban and city planners have adopted new technologies such as landfills and incineration these alone cannot work without training residents on best practices that will guide them on how to manage their waste. Both health and environmental implications are associated with solid waste management and amounting in urgency especially in developing countries. The study aimed to assess the methods used by residents of Eastleigh South Ward in Nairobi County to dispose of their solid waste at the household level. Various waste disposal methods were documented during field observation and interviews while secondary data was obtained from records and reports on the management of waste in Nairobi County. About 48% of the resident indicated they discard waste along the road in heap/drainage, further 35% indicated putting waste in dust bins which eventually ends up in undesignated areas. The study also documents challenges related to waste management including infrequent /irregular waste collection, illegal dumping, low levels of information on poor waste disposal, and lack of concern among residents. Other challenges included irresponsible waste management approaches by the Nairobi County Government as well reluctance to pay private garbage collectors and high cost of disposal services charged by private garbage collectors. Promoting awareness through public education on the management of solid waste will enhance proper solid waste management practices. The study further recommends allocation of more resources to allow for effective management of solid waste.

Keywords: solid waste; disposal; environment; public awareness; practices

1. Introduction

Mahar et al. [1] defines solid waste as biodegradable and non-biodegradable material along with other refuse occasioned by human and animal activities which are of little value and mostly done away with. Empirical evidence from the literature on urban development show that rapid population growth has led to a massive generation of solid waste resulting from poor waste disposal systems in cities and urban areas.

According to Nabegu [2], urban waste management sector is facing numerous challengesglobally especially due to the large amounts of municipal and industrial wastes produced daily. Areport by UNEP [3] acknowledges that it is estimated that every year about 3.4 to 4 billion tons of solid waste and up to 300 million tons of hazardous waste are produced globally. Thus, the huge increase in the volume of solid waste will witness unprecedented scale of environmental risks such as diseases, ecosystem degradation contamination of soil and water, global warming and climate extremes. According to Ayodeji [4], the risks posed by poor management and disposal methods are more obvious in the developing countries who are the greatest consumers of industrial materials as well as outdated and obsolete technological products.

In the last two decades environmental degradation has continued to attract the attention within the global community a situation that has made more people to become increasingly conscious of variety of environmental issues such as global warming, air, water and land pollution. Most literature on environmental sustainability shows that almost all types of environmental pollution result from human induced activities. Fernando [5] believes that it is important for residents to understand the need for sustainable disposal mechanisms as a solution to the dangers posed by environmental pollution.The main purposes of Solid Waste Management strategies are to address theenvironmental, health, aesthetic, economic and land-use concerns attributed to improper wastedisposal for nations, municipalities, corporations, and individuals around the world [6,7]. Asmawatiet al. [8] argues that there is no material in this world that is not useful in one-way or the other orwhich is created out of nothing but it is through ignorance that man considers certain things as wasteand others as useful.

Wilson et al. and Munyaga, N [9,10] opined that management of solid waste systems have resulted in major challenges for emerging cities more so in underdeveloped countries. Further, Safiuddin et al. [11] observed waste management problem continue to deepen owing to heightened human activities. Wilson et al. [12] pointed out that the main challenge with disposal of waste stems from poorly grounded strategies that occasion littering, illegal waste disposal including burning. According to Kassim S.M Ali [13] waste occasioned by human activities should be discarded to reduce risk to the environment and health of humans and animals. Further, inadequate collection of poorly disposed of solid waste results in an increase of pathogens that cause air and water-borne diseases since they play hosts to other disease vectors such as mosquitoes, flies and rodents. Kassenga et al., Omofonmwan er al. and Leah Oyake-Ombis [14–16] argued that dumpsites being the initial waste collection sources, become reservoirs of most polluting agents making them environmental polluting zones for soil, air, ground and surface water. In the last two decades, Kenyahas recorded tremendous industrial development in line with the vision 2030, a situation that has seen an influx of population to the urban centers which are likely to increase solid waste generation to higher levels, hence the need for urgent waste disposal mechanisms. Management of solid waste in urban areas of Kenya is a real challenge while the existing disposal systems are haphazard and inefficient. Otieno and Gakungu [17,18] opined that a good proportion of all solid waste generated in urban areas ranging between 30%–40% remain uncollected, while solid waste generation rate exceeds one kilogram per capita per day, [19]. Moreover, urban management bodies have failed to implement solid waste management systems that are sustainable resulting in enhanced illegal dumping in open fields that pose unfavorable effects on the environment hence negatively impacting public health systems.

Data obtained from KNBS [20] estimates that of the 47.5 million people, 34.8% translating to 10 million of the total population in Kenya are inhabitants of urban centres with the five major urban centres of Nairobi, Mombasa, Kisumu, Nakuru and Eldoret accounting for a third of the total urban population. Nairobi County's population is estimated to be about 4. 3 million people who generate about 2400 tons of solid waste daily, a worrying trend and King'oo [21] observed that if proper measures for the management of waste are not put into place, this scenario will continue to foster further socio-economic, environmental and institutional challenges for Nairobi county.

Nairobi city like some other cities in Africa lacks effective systems of solid waste management resulting in negative short and long-term impacts on human health and the environment in general [22].

According to Musyoka [23], if these challenges are not addressed by 2030 the county could generate 35% more waste than it does today. Muniafu et al. [24], observed that various legislations in Kenya mandates local authorities with the task of managing solid waste. However, the implementation of these policies and legal provisions have not been systematic and lack coordination. According to Nairobi Solid Waste Management Plan [25], of all waste generated in Nairobi 68% is domestic, making it prudent to focus on types of solid wastes generated as well as the methods used in disposing of waste by the residents.

The research was carried out in Eastleigh suburb one of the most populated zones in Nairobi County, mainly due to high influx of Somali population as well as the emergence of numerous wholesale and retail businesses. The population is a mix of middle and low-income neighbourhoods. 1st Avenue the main street in Eastleigh has buildings with multiple uses such as business malls, with the basements used as storage facilities, while the upper floors have been converted into residential areas. Further, open spaces are used as open-air garages, parking lots while the alleys and pedestrian walks are used by small scale traders and hawkers selling all sorts of goods such as clothes, shoes, electronics as well as vegetables and fruits. The activities carried out have led to generation of high volumes of solid waste which is poorly stored and disposed posing a serious public health and environmental hazards. Residents' behavioural practices towards generation, handling and disposal of solid waste is wanting because they use unorthodox methods disposing of waste anywhere they find convenient irrespective of whether it is a designated site or not. A big percentage of waste is discarded along the road, in open spaces and drainage channels. The study sought to answer the research question; What are the methods used by the residents in solid waste management in Eastleigh Nairobi County? .The study was premised on the researcher's hypothesis that methods of solid waste adopted by residents of Eastleign in Nairobi County are poor.

2. Materials and method

2.1. Study site

This research was carried out in Eastleigh South Ward within Kamukunji Sub-County in Nairobi County, Kenya. The area of study is situated in the eastern part of Nairobi city at geographical coordinates of; 1.2734°S, 36.8481°E. The study area is cosmopolitan and is one of the busy commercial hubs of Nairobi County. Many of the businesses are owned by the Somali community.

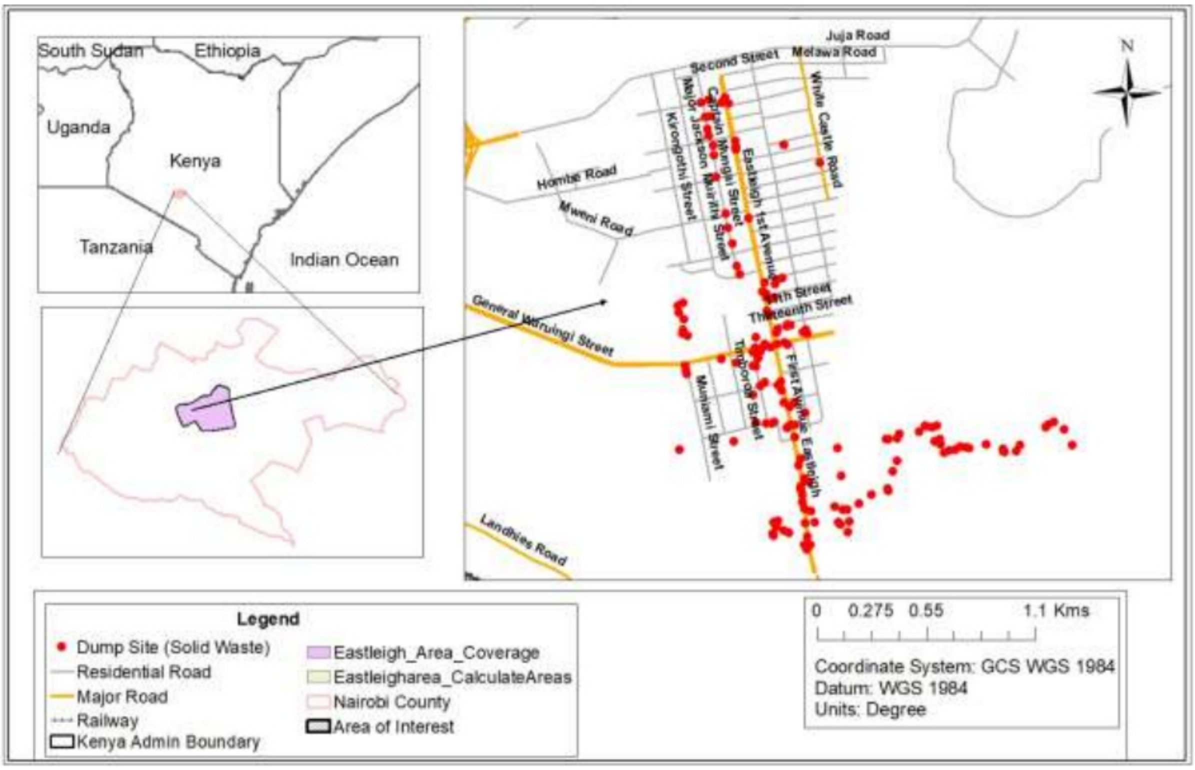

Figure 1. Map of area of study.

2.2. Sample size and sampling

Eastleigh South Ward is within Kamukunji Sub-County, Nairobi County which covers an area of 12 square kilometres carrying a population of 263462 persons. Eastleigh South Ward the main focus of the study covers approximately 4.02431 square kilometres carrying a population of 89968 persons and an estimated 29022 households. The area has an average household size of 3.1 persons spread over 10.552 square kilometres. For the study a sample size of 188 households was derived which was rounded up to the nearest hundred to a sample size of 200 households using the formula below;

n =(z2x p x q x N)

e2(N – 1) + (z2x p x q), where:

n = Sample size (being determined)

N = Population size (29022)

p = Sample proportion (assumed to be 0.02, if not given)

q = 1 – p

e = 0.02 (since the acceptable error should be 2%)

z = Standard deviation at a given CI (z = 1.96 at 95% CI)

2.3. Data collection

Primary data was collected through random sampling targeting households within the area of Study from April through to May 2019. Quantitative data was obtained through use of numeric data (outline) that is measurable while the qualitative method involved both descriptive and conceptual data (outline) to provide in-depth information. Data collection involved use of questionnaires, interviews, field observation and document analysis. The study used a face to face interview with respondents and key informants from randomly selected households, shop attendants, street vendors and hawkers. The questionnaires employed involved both structured and semi-structured questions to gather more data from the respondents on methods used to manage generated waste. Sampled households were picked depending on accessibility, availability and willingness of the respondents to participate in the survey while secondary data was obtained through a review of census and survey reports as well as previous research studies on the management of solid waste. Further, field observations documented important information on modes of waste disposal through the use of Figure 2, 5 and 7 and physical observation. Mixed methods approach enabled the researcher to cross check the data to enable valid and credible results [26].

2.4. Data analysis

Both quantitative and qualitative data obtained from questionnaires and interviews were coded and analyzed using Statistical Package for Social Sciences (SPSS) version 21.0. Descriptive statistics were obtained and presented in frequencies and percentages for the following variables; methods, waste composition, level of solid waste, waste storage and demographic characteristics. The study assessed the relationship between household demographic characteristics and management of solid waste practices using cross-tabulation and chi-square analysis at a significance level of less than 5%.

3. Results and discussion

3.1. Sample distribution

According to population studies, demographic characteristics consist of age, family size, material status, education level and income, which according to Castagna et al. and Chu et al [27,28]are critical when dealing with the evaluation of the management of solid waste practices within populations. The demographic characteristics of the respondents are presented in Table 1, where 57.6% male and 42.4% female with a mean age of 25–34 years were interviewed. Of these about 55.1%were married. On education, the majority had attained secondary level (55.9%) while (35.6%) had middle-level college education. Slightly less than half of the individuals (43.2%) had a monthly income of between Ksh. 10000–20000.

Table 1. Responses to demographic characteristics.

Demographic characteristics			Male	Female	Total
Age group	18–24 Years	N	17	15	32
		%	14.40%	12.70%	27.10%
	25–34 Years	N	26	23	49
		%	22.00%	19.50%	41.50%
	35–44 Years	N	15	5	20
		%	12.70%	4.20%	16.90%
	45–54 Years	N	9	6	15
		%	7.60%	5.10%	12.70%
	Over 55 Years	N	1	1	2
		%	0.80%	0.80%	1.70%
	Total	N	68	50	118
		%	57.60%	42.40%	100.00%

Marital status	Married	N	36	29	65
		%	30.50%	24.60%	55.10%
	Single	N	29	16	45
		%	24.60%	13.60%	38.10%
	Separated	N	1	3	4
		%	0.80%	2.50%	3.40%
	Widowed	N	2	2	4
		%	1.70%	1.70%	3.40%
	Total	N	68	50	118
		%	57.60%	42.40%	100.00%
Education level	None	N	1	0	1
		%	0.80%	0.00%	0.80%
	Primary	N	5	4	9
		%	4.20%	3.40%	7.60%
	Secondary	N	38	28	66
		%	32.20%	23.70%	55.90%
	College	N	24	18	42
		%	20.30%	15.30%	35.60%
	Total	N	68	50	118
		%	57.60%	42.40%	100.00%
Monthly income	<Ksh. 10000	N	14	5	19
		%	11.90%	4.20%	16.10%
	Ksh. 10000–20000	N	28	23	51
		%	23.70%	19.50%	43.20%
	Ksh. 20000–50000	N	21	21	42
		%	17.80%	17.80%	35.60%
	Ksh. 50000–100000	N	5	1	6
		%	4.20%	0.80%	5.10%
	Total	N	68	50	118
		%	57.60%	42.40%	100.00%

3.2. Responses to the composition of solid waste

Much of the waste generated within the area is organic (51.5%) and the other half is inorganic (48.5%) (Table 2).The study identified packaging materials are increasingly becoming a critical component of solid waste as earlier observed by Firdaus et al. [29] who contends that as the gross national product and urban population growth rises, paper and packaging waste will also increase. Ampofo et al. [30] contends that food-stuffs waste is greatly produced at the stage of wrapping, preparation and use. A variety of containers used for wrapping most items for sale is dumped in the environment, this has led to the development of many illegal dumpsites which creates unhealthy environment and blockage of sewerage systems resulting in flooding especially during the rainy season.

As a result, a substantial amount of the waste within the designated collection sites remained uncollected which accumulate into filthy huge moulds that negatively impact on the quality of air in the surrounding

Figure 2. A heap of solid waste along 1st Avenue, Eastleigh.

Table2. Responses on the composition of solid waste.

Type of waste	Number of persons in a household			
	<3	4–6	7–9	Total
Organic waste	(56) 47.8%	(63) 53.2%	(65) 55.0%	(61) 51.5%
Inorganic waste	(62) 52.2%	(55) 46.8%	(53) 45.0%	(57) 48.5%
Total	(118) 100.0%	(118) 100.00%	(118) 100.00%	(118) 100.00%

3.3. Levels of solid waste generated

The study sought to rate the level of waste generated from respondents. Figure 3 indicates how respondents rated the levels of waste in their residential area.40.70% reported that the levels were neutral with 33.10% indicating levels were high while only 12.70% said the levels were very high.

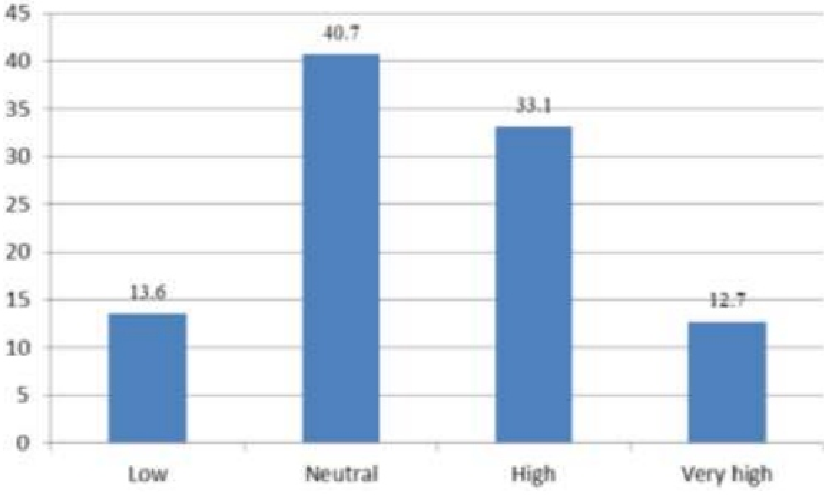

Figure 3. Responses on the level of waste generated.

With the rapid increase of population from natural causes and rural-urban migration, waste generation has increased tremendously much of which is poorly handled ending up in undesignated dump sites. In a study by Dhamija [31], India in 2001, the population in the urban areas had increased to 93.0% from 52.7% in 1901. This led to a significant increase in solid waste in urban areas resulting in a hazardous dimension. The waste estimate generation increased from 4500 Metric Tonnes/day (MT/day), 6,500 MT/day to 12000 MT/day in the year 1981, 1991, and 2001 respectively. This is expected to rise to 17000–25000 MT/day by 2021with assumption of 6–8% growth rate. In Kenya, there is a similar situation of population increase where from 1969 to 2019 the population has grown from 10.9M to 47.6M (Figure 4) with about 26.3% population increase from year 2009–2019 [20].

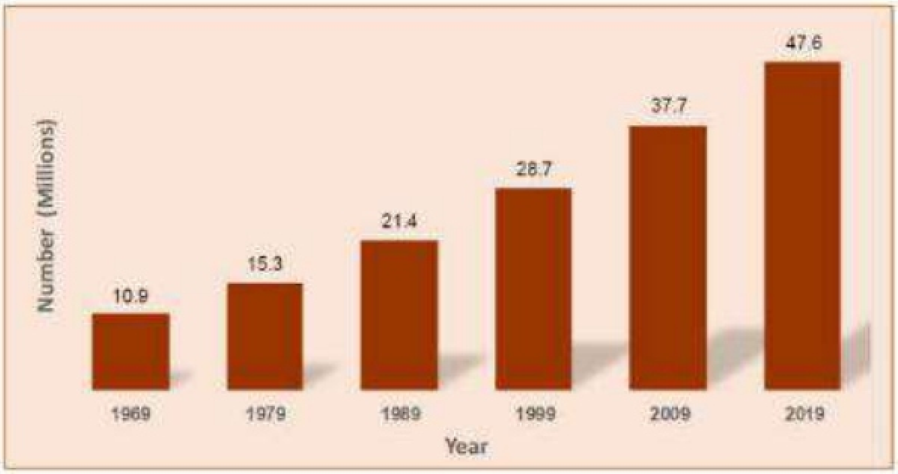

Figure 4. Population trend, 1969 to 2019 [20].

During the field visit (Figure 5) observations showed waste was usually disposed unsorted in heaps in undesignated areas. According to Firdaus et al. [29], waste generated is drastically increased when relevant agencies charged with collecting and disposing are unable to deal with the large quantities produced on daily basis. This results in the accumulation and rotting of uncollected garbage at collection sites.

Figure 5. Solid waste dumped on the road (Eastleigh 2nd Avenue).

3.4. Storage and collection

The study investigated types of containers used for storage and collection of waste in Eastleigh suburb. Figure 6 highlights the responses to the study question. Responses revealed that containers made of plastic (bags and buckets) were the most preferred at 52% and 28% respectively; evidently these are the most commonly used. The study observed that 15% of the respondents discarded their waste in undesignated areas such as roadsides and drainage tunnels with a paltry 5% storing their waste in covered bins. However, the study observed that though some respondents stored their waste in plastic buckets, plastic bags and metallic bins, most of this waste was disposed at the undesignated dumping sites as shown in the Figure below. The haphazard dumping of solid waste in open spaces are a great risk to the environment as it leads to air, water and land pollution.

3.5. Solid waste disposal management

Regarding solid waste disposal management, 31.8% indicated that residents within the area managed collection and disposal by enlisting private garbage collectors. However, 68.2% indicated they are not involved in any form of management of solid waste services, resulting in most of the generated waste being poorly disposed. The respondents indicated that while some residents paid for the services by private service providers, others opted to take their waste at designated garbage collection points where it is collected at specific times by the County Government garbage trucks. The study was able to identify other waste disposal options available to the residents. A good number (48.0%) opts to discard waste along the road, in the drainage channels and other undesignated points with 25.1% indicating they stored waste in dustbins which they eventually took to designated collection points. A small number of residents (4.7%) prefer storing waste in dustbins and later empty it in the county skips usually found in designated sites within the area, with only (8.8%) preferring burning, nature of waste notwithstanding. Out of the total number of interviewed only 3.5% indicated that they recycled some of the waste (Table 3). The study also established waste collection intervals ranged between once a week at 61.0%, twice a week at 28.8% while 3.4% indicated that waste was irregularly collected. However, 5.1% of the respondents indicated waste is never collected at all (Table 3).

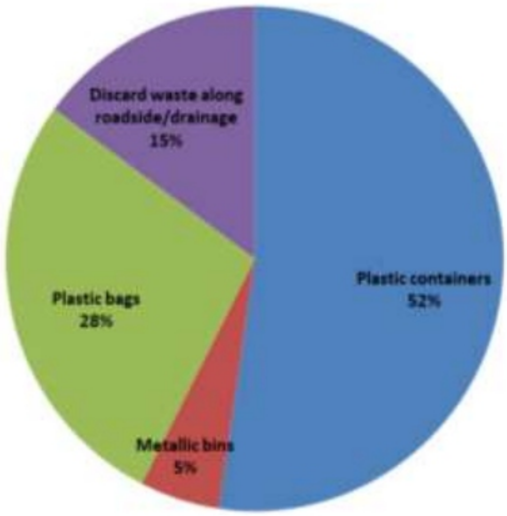

Figure 6. Responses on methods used in waste storage.

Table 3. Responses to methods of waste disposal and collection frequency.

Methods of disposal	N	%	Frequency of collection	N	%
Discarding along the road in a heap/drainage	82	48.0	Once a week	72	61.0
Putting in a dust bin	60	35.1	Two times a week	34	28.8
Burning	15	8.8	Everyday	2	1.7
Disposing at designated collection points	8	4.7	Irregular	4	3.4
Recycling	6	3.5	Zero collection	6	5.1
Total	171	100.0	Total	118	100

Evidence from field observation reveals that most respondents at 61.0 % discard their wastes in drainage channels and by the roadside. This is occasioned by a haphazard manner of waste collection by private waste collectors and Nairobi County government. Waste stored in dustbins ends up being discarded on the roadside or in drainage channels since some residents default on payment to service providers while others cannot afford it. When the discarded waste piles up some residents result in burning affecting the air quality within the vicinity an argument supported Giusti [32] who contends that continuous burning of solid waste in the open causes health problems to those exposed to inhalation of the ambient gases released into the air and in turn leads to respiratory and eye problems.

Figure 7. Burnt solid waste off 2nd Avenue, Eastleigh.

According to Ampofo S et al. and Leah Ombis [16,30] several issues identified that rendered solid waste unmanageable, include; resident's reluctance to pay for private service providers, inefficiency over waste collection and disposal services by the relevant county authority, inadequate public awareness and unreliable means of transporting waste to Dandora landfill. For Giusti [32], to overcome these challenges, Nairobi county government should adopt sustainable management of solid waste including but not limited to waste minimization/prevention, waste re-use, recycling and composting. Ampofo et al [30] further argues that waste that is not recyclable, reused or composited other methods of solid waste is key to achieving sustainable, clean and safe environment.

3.6. Reasons for poor solid waste disposal

Un-Habitat [34] report on sustainable cities, has identified poor management of solid waste as a major challenge towards the promotion of a sustainable environment and livelihoods. Table 4highlights responses on the challenges affecting the management of solid waste in the studied area with 37.2% indicating that they were not bothered by the state of poorly disposed waste, 16.8% identified lateness of service providers in waste collection, 12.4% cited lack of information on appropriate waste management methods while 5.8% indicated reluctance in paying service providers fee with another 9.5% identifying inefficiency by the Nairobi county government as the main challenge. 5.1% indicated high charges of waste collection fee and poor cooperation among residents and service providers as the reason for poor management in waste disposal.

Table 4. Responses on reasons for poor management of solid waste in Eastleigh.

Reasons for Poor Management of solid waste	N	Percent
Lack of concern	51	37.2%
Lateness in the collection of waste	23	16.8%
Reluctance to pay private waste collectors	8	5.80%
High charges of waste collection	7	5.10%
Poor cooperation among residents and private waste collectors	7	5.10%
Lack of appropriate information on the management of waste	17	12.40%
Failure by Nairobi County Government to take their responsibility seriously	13	9.50%
Poor transportation	1	0.70%
Poor storage	2	1.50%
Informal settlements	6	4.40%
Poorly managed collection points	1	0.70%
Increase in population	1	0.70%
Total	137	100.0%

Findings conclude that lack of concern by residents on good management practices of solid waste has led to negative impacts on solid waste disposal damaging the environment. Ampofo et al.opines that some residents shun appropriate management methods of waste to avoid meeting the costs of service providers. Further Findings indicate that some residents decry the services rendered by private collectors as unsatisfactory owing to delays and irregular collection hence the unwillingness to pay, leaving them with no choice but to use unorthodox means of disposing waste [30], citing UN-Habitat Report [34], argues that the generation of waste is inevitable due to the rapid increase in urban population, hence the need for concerted efforts by national governments and urban authorities in the development of sustainable cities. According to Tiwari [35], though most urban authorities developed environmental policies and legislation, implementation remains a pipe dream. To address these challenges Boadi et al., Parrot et al. and Henry et al. [36–38] highlights the need to adopt sustainable management strategies to enable address negative consequences related to the unregulated management of solid waste practices.

4. Conclusion

After careful analysis of the data gathered it is imperative to conclude that uncollected solid waste has become the most visible environmental problem particularly among the low and middle income neighborhoods within Nairobi's Eastleigh South Ward. This scenario is supported by Leah O. Ombis [16], that Nairobi city county government waste management system is fraught with many problems and has no clear systems and process to deal with an estimated 2400 tons of waste generated daily. The study was able to identify the challenges that Eastleigh residents experienced in regard to solid waste disposal and collection systems. Various challenges were identified which include; failure by the county government authority to prioritize implementing various guidelines and legislation on waste management, inefficiency in waste collection and poor infrastructure. In addition, activities of multiple actors involved are poorly coordinated, while in some sections of the area of study, waste collection systems are non-existent and even where services are found, they are riddled with many challenges that include residents' inability to pay.

5. Recommendations

Nairobi County Government should enforce existing management of solid waste policies and legislations as spelled out in Environment Management and Coordination Act 2019, the Kenyan Constitution (2010) [39] and in the Nairobi county solid waste management Act 2015. Nairobi County government should engage other stakeholders in sensitizing residents on sustainable management systems on the solid waste that include separation, reuse and recycling. Nairobi county government should institutionalize management of waste processes by investing in efficiency and infrastructural capacity by providing skips, trucks, bins, as well as guidelines on modalities to service providers on proper waste Management. The findings of the study can be replicated in other urban centres of developing Countries. This would help mitigate environmental issues associated with poor waste management systems.

Acknowledgments

I'm grateful to my supervisors Prof. Wahome and Dr. Kiemo for their devotion and guidance, Wangari Maathai Institute staff for their unwavering support. I would also like to thank all my respondents in Eastleigh estate for their contributions. All the research was self-funded.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Mahar A, Malik R, Qadir A (2007) Review and analysis of current management of solid waste situation in urban areas of Pakistan. In Proceedings of International Conference. SustainableSolid Waste Management. 5–7 Sept. 2007. Chennai, India, 34–41, gongolwa PG (2007) Evaluation.

2. Nabegu A (2010) An analysis of municipal solid waste in Kano Metropolis, Nigeria. J Human Ecol 31: 11–119.

3. United Nations Environmental Programme (UNEP) (2011) Towards a green economy. Pathway to Sustainable Development and Poverty Eradication. Nairobi: United Nations.

4. Ayodeji I (2010) Exploring secondary school students' understanding and practices of waste management in Ogun State, Nigeria. Intl J Env Sci Educ 5: 201–215.

5. Fernando R (2019) Solid waste management of local governments in the Western Province of Sri Lanka: An implementation analysis. Waste Manage 84: 194–203.

6. Henry R, Yongsheng Z, Jun D (2006) Municipal management of solid waste challenges in developing countries–Kenyan case study. Waste Manage 26: 92–100.

7. Nemerow N, Agardy F, Salvato J (2009) Environmental engineering: environmental health and

safety for municipal infrastructure, land use and planning, and industry. John Wiley & Sons.
8. Asmawati D, Nor Ba'yah A, Fatimah Y (2010) A Study on the Knowledge, Attitudes, Awareness Status and Behaviour Concerning Solid Waste Management School of Psychology and Human Development, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Malaysia.
9. Wilson D, Velis C, Cheeseman C (2006) Role of informal sector recycling in waste management in developing countries. Habitat Int 30: 797–808.
10. Munyaga N (2016) Challenges of solid waste disposal in Gachororo, Kiambu County. University of Nairobi Press.
11. Safiuddin M, Jumaat M, Salam M, et al. (2010) Utilization of solid wastes in construction materials. Int J Phy Sci 5: 1952–1963.
12. Wilson D, Rodic L, Scheinberg A, et al. (2012) Comparative analysis of solid waste management in 20 cities. Waste manage res 30: 237–254.
13. Kassim S, Ali M (2006) Solid waste collection by the private sector: Households' perspective in Dar-es-Salaam city, Tanzania. Habitat Int 30: 769–780.
14. Kassenga G, Mbuligwe S (2009) Impacts of a Solid Waste Disposal Site on Soil, Surface Water and Groundwater Quality in Dar es Salaam City, Tanzania. J Sust Dev Africa 10: 73–94.
15. Omofonmwan S, Eseigbe J (2009) Effects of solid waste on the quality of underground water in Benin Metropolis, Nigeria. J Human Ecol 26: 99–105.
16. Leah O (2018) How Nairobi can fix its serious waste problem. Quartz Africa.
17. Otieno J (2010) Management of solid waste in Uganda: Issues Challenges and Opportunities. POVIDE programme Workshop. The Netherlands.
18. Gakungu N, Gitau A, Njoroge B, et al. (2012) Management of solid waste in Kenya: A case study of public technical training institutions. ICASTOR J Engineer 5: 127–138.
19. Tilahun E, Bayrakdar A, Sahinkaya E, et al. (2017) Performance of polydimethylsiloxane membrane contactor process for selective hydrogen sulfide removal from biogas. Waste manage61:
20. KNBS (2019) Kenya Population and Housing Census Volume I: Population by County and Sub County.
21. Stephen N (2019) Assessment of Household Domestic Waste Management Practices, Kiambu County- Kenya. South-Eastern Kenyan University (SEKU) Repository.
22. Njoroge B, Kimani M, Ndunge D (2014) Review of Municipal Management of solid waste: A Case Study of Nairobi, Kenya. Int J Engineer Sci 4: 2319–6483.
23. Musyoka M (2019) Assessment of Community Perception, Policies and Land use factors in relation to Climate Change Processes in Nairobi city (Doctoral dissertation, JKUAT-IEET).
24. Muniafu M, Otiato E (2010) Solid Waste Management in Nairobi, Kenya. A case for emerging economies. J Lang Technol & Entre Africa 2: 342–350.
25. National Environment Management Authority (2014) National Management of solid waste Strategy, Nairobi. Kenya.
26. Bryman A (2004) Social research methods (2 ed.). New York: Oxford University.
27. Castagna A, Casagranda M, Zeni A, et al. (2013) 3R'S from Citizens Point of View and Their Proposal from a Case-Study. UPB Sci Bull 75: 253–264.
28. Chu Z, Wang W, Wang B, et al. (2016) Research on factors influencing municipal household solid waste separate collection: Bayesian belief networks. Sustainability 8: 152.
29. Firdaus G, Ahmad A (2010) Management of urban solid waste pollution in developing countries. Int J Env Res 4: 795–806.
30. Ampofo S, Soyelle J, Abanyie S (2016) The Negative Impacts of Poor Municipal Management of solid waste on Livelihoods in Walewale Township, West Mamprusi District, Ghana: A Social Survey and Assessment. International Institute for Science, Technology and Education.

31. Dhamija U (2006) Sustainable solid waste management: Issues, policies, and structures.Academic Foundation.

32. Giusti L (2009) A review of waste management practices and their impact on human health. Waste manage 29: 2227–2239.

33. Saveyn H, Eder P, Ramsay M, et al. (2016) Towards a better exploitation of the technical potential of waste-to-energy. Seville: European Commission.

34. Un-Habitat (2010) Management of solid waste in the world's cities. UN-HABITAT.

35. Tiwari A (2019) Integrated Management of solid waste Turns Garbage into Gold: A Case Study of abalpur City. Waste Manage Res Effi 385–392.

36. Boadi K, Markku K (2005) Environment and health impacts of household solid waste handling and disposal practices in the third world cities: the case of Accra metropolitan area, Ghana. J Env Health. 68: 34–36.

37. Parrot L, Sotamenou J, Dia B (2009) Municipal management of solid waste in Africa: Strategies and livelihoods in Yaoundé, Cameroon. Waste Manage 29: 986–995. Republic of Kenya(2012a), County Government Act, Nairobi.

38. Henry R, Yongsheng Z, Jun D (2006) Municipal management of solid waste challenges in developing countries–Kenyan case study. Waste manage 26: 92–100.

39. Constitution of Kenya (2010) Government Press.

·ISSN 2372-0352

Evaluation of the freshwater copepod Acanthocyclops americanus (Marsh, 1983) (Cyclopidae) response to Cd, Cr, Cu, Hg, Mn, Ni and Pb

Alma Sobrino-Figueroa1,*, Sergio H. Álvarez Hernandez2 and Carlos Álvarez Silva C1

1 Alejandro Villalobos Laboratory, Department of Hydrobiology, Metropolitan Autonomous University. Mexico City, Mexico.

2 Applied Phycology Laboratory, Department of Hydrobiology, Metropolitan Autonomous University. Mexico City, Mexico

ABSTRACT

The toxic effect of cadmium, chromium, copper, mercury, manganese, nickel and lead on adult Acanthocyclops americanuscopepods was evaluated to determine the sensitivity of this species to these metals. Toxicity tests were carried out to determine the LC50, after which bioassays were carried out with environmentally relevant sublethal concentrations in order to measure oxidative damage to cell membranes as well as neurotoxic effects. Cadmium was the most toxic metal and manganese was the least harmful. Copper had the greatest oxidative effect (lipid peroxidation) and nickel had the least effect. A 3% to 79% drop was observed in acetylcholinesterase (AchE) activity, with copper causingthe greatest inhibitory effect. A. americanus sensitivity to cadmium, manganese and lead was similar to that recorded for Daphnia magna neonates, but the copepods were less sensitive to chromium, copper, mercury and nickel. The response of A. americanus to exposure to metals makes it possible to propose it as a test organism to evaluate the presence and effect of these elements, particularlycadmium, manganese and lead, in toxicity studies, and possibly for monitoring purposes.

Keywords: Acanthocyclops americanus; metal toxicity; lipoperoxidation; neurotoxic effects;freshwater copepods

1. Introduction

The lacustrine catchment area of the Valley of Mexico presents high indices of disruption caused by various issues among which are the expansion of the urban area, the excessive exploitation of the water table, the desiccation of springs, the modification of riverbeds and the influx of untreated wastewater from industrial, domestic and agricultural activities [1,2]. All this has brought about drastic changes in the environmental conditions of rivers, creeks, ponds, dams and lakes, changes that affectthe organisms that live there, decreasing their distribution and causing some species to be threatened and others to have disappeared [1,3].

High concentrations of contaminants, primarily metals and pesticides, have been found in the water systems of this region [4–9]. Metals are considered to be contaminants that cause serious problems to the environment since they cannot be removed efficiently through natural processes. Their removal requires physicochemical processes that are expensive, while they are highly persistent,accumulate and are biomagnified (e.g., As and Hg) in food chains. They are also toxic to aquatic organisms depending on their concentration [5,10,11].

High levels of Cd, Cr, Cu, Hg, Mn, Ni and Pb are found in the water of aquatic systems in the Valley of Mexico, including the catchment areas of the Lerma River where Cd, Cr, Cu, Hg, Ni and Pb are found in the water of aquatic systems in the Valley of Mexico, including the catchment areas of the Lerma River where Cd, Cr, Cu, Hg, Ni and Pb concentrations vary from11.3 to 980 g L−1(Table 1) [4,5,7,12].In the case of Xochimilco, the concentration of metals detected in the canals varies from0.071 to 9946.6 g L−1 [6,9,13]. Average levels of Cr (1.93 mg L−1), Ni (4.11 mg L−1) and Pb (0.418 mg L−1) detected in thenavigationcanals [9,13] are above those indicated by the NOM-001-SEMARNAT for the Protection of Aquatic Life (Cr = 0.5–1.0 mg L−1, Ni = 2.0–4.0 mg L−1and Pb = 0.2–0.4 mg L−1) [14]

Table 1. Metal concentrations found in waters of aquatic systems in the Valley of Mexico.

Metals	Lerma River(μg/L)	Xochimilco (μg/L)	Sublethal bioassays Concentrations (μg /L)
Cd	11.3 ±7.8	0.5 to 24.5	4.1
Cr	17.2 ±8.0	54.1 to 331.5	331
Cu	119 ±76	351.3 to 2198	90
Hg	118.5±103.5	0.071 to 7.61	26
Mn	980 ±440	1857 to 9946.6	35170.0
Ni	90 ±12	89.4 to 8136.8	1660.0
Pb	116 ±77	5.6 to 431.2	230
Reference	4, 5, 7, 12	6, 9, 13	This study

Metals may cause adverse effects in aquatic organisms depending on the concentration and the exposure time. In elevated concentrations, they cause the death of sensitive organisms. In sublethal exposures, they bring about changes in enzymatic activities at the biochemical level [15]. They cause changes in physiological rates (feeding rate, excretion rate and growth rate), and upheavals in the behaviour, reproduction and survival of neonates and juveniles [11,15]. They also lead to the generation of Reactive Oxygen Species (ROS) that cause oxidative stress in aquatic organisms such as fish, molluscs and crustaceans [16,17]. ROS act on cellular macromolecules such as lipids, proteins and DNA. Enzymatic activity and the structural lipids of cell membranes are the main targets of metals [18]. Prolonged oxidative stress leads to aging, diseases and even cellular death due to necrosis and/or apoptosis [19].

Moreover, acetylcholinesterases (AChE) are enzymes in the esterases group whose function is the hydrolysis of the acetylcholine neurotransmitter (Ach). These enzymes are essential in controlling the transmission of nerve impulses that run from nerve fibers to muscle cells, autonomous ganglia and the central nervous system. Prior studies have demonstrated that metals may be neurotoxic to aquatic species because they affect AChE activity, causing changes in mobility and behaviour that compromise the survival of organisms [20,21].

Acanthocyclops americanusis a copepod species of the order Cyclopoida. These organisms are 0.44 to 1.12 mm in length and are part of the zooplankton community in freshwater aquatic systems [22,23]. They are important from the ecological point of view because they are food for fish, crustaceans and other predatory specimens like axolotls [23].

Apart from Mexico, A. americanus is found in Spain, the United States and France. In Mexico, the presence of populations of this species has been reported for the lacustrine catchment area of the Valley of Mexico [23], while Enríquez and assistants (2011) recently found them in the lake at Huetzalin, Xochimilco [22].

Studies on the effect of contaminants on native organisms are very few and its harmful effects are unknown in many of the species present in subtropical and tropical climates [24]. Since no studies have been carried out on A. americanussensitivity to diverse xenobiotics, an evaluation of the toxicity, oxidative damage and neurotoxic effect of cadmium, chrome, copper, mercury, manganese, nickel and lead on adultA. americanuscopepods was carried out in this study to determine the response of the species to each metal, and whether A. americanus is more sensitive to these elementscompared with other copepod species and neonates of Daphnia magna, a species that is used in Mexico as a test organism to evaluate the toxicity of effluents that are discharged into natural systems [25].

2. Materials and method

2.1. Test organisms

Acanthocyclops americanusadults (985 ± 30 m) were obtained from water samples collected from semi-permanent pools located at the CIBAC (Cuemanco Biological and Fish Farm Research Center). The organisms were grown in the laboratory, in two-litre glass jars (pyrex) with reconstituted water [26], for four months, under the following conditions: temperature 22 ± 2 °C, pH 7.1, total hardness 165 ± 5 mg L−1and dissolved oxygen > 7 mg L−1. The copepods were fed every third day a mixture of microalgae (Monraphidium sp. 1 x 106cel/mL), pulverised fish feed (Tetramin 0.1 g/L) and rotifers (Brachionus sp. 160 ± 20 organisms/mL) [22].

2.2. Chemicals

The metals were prepared as standard solutions [27,28] with deionized water and the following salts: CdCl2, (Baker, 99% purity), K2Cr2O7 (Merk, 99.5%), CuSO4 (Baker, 99.9%), HgCl2 (Merk,99.98%), MnCl24´H2O (Baker, 99.99%), NiCl2 (Merk, 99.5%) and Pb(NO3)2 (Baker, 99%). The standard solutions and the tests were prepared the day the bioassays began.

2.3. Toxicity tests

The adult copepods (917 ± 74 m) obtained from the laboratory cultures were exposed to five nominal concentrations of each metal (for the tests with Cd, Cr, Cu and Hg: 0.1, 0.5, 1.0, 5.0 and 10 mg L−1, and for the bioassays with Mn, Ni and Pb: 1, 10, 25, 50 and 100 mg L−1). One control was left with no toxic substance. Twenty organisms were placed in crystal glasses (pyrex) with 50 mL of each test solution (in triplicate). The conditions during the bioassays were as follows: temperature 22 ± 2 °C, pH 7.1, total hardness 160 ± 5 mg L−1, photoperiod 12 hrs light/12 hr darkness and dissolved oxygen > 7 mg L−1. The copepods were not fed during the test trials. Each bioassay was repeated a minimum of three times.The number of immobile organisms in each test was recorded after 24 and 48 hours of exposure [28,29]. The LC50 (Lethal Concentration 50) was determined with the data obtained following the Probit method via the EPA Probit software. The calculation to compare the LC50 and its confidence intervals was carried out using the statistics described in APHA (1994) [28] to evaluate the statistical significance of the differences recorded among the different treatments:

$$f_{1,2} = antilog\sqrt{(log f_1)^2 + (log\ f_2)^2}$$

$$f_{1,2} = 1.96\ SD/LC50_{1,2}$$

where:

SD = Standard deviation
LC50 = Lethal concentration 50

f = Confidence limit factor
f1 = Upper endpoint of the 95% confidence interval
f2 = Lower endpoint of the 95% confidence interval

2.4. Sublethal bioassays

A sublethal assay was then carried out for 8 days, with a change in the test solutions every 48 hours. The organisms were exposed to the LC1 of each metal (table 1). Fifty copepods were exposed to the metals (in triplicate). At days 2, 4 and 8 of exposure, a random sample of 15 organisms was taken from each replica and put together to create a composite sample (n = 45) (in triplicate). Thesesamples were used to determine the degree of lipid peroxidation and acetylcholinesterase (AchE) activity in copepod tissues to evaluate the oxidative and neurotoxic effect of these metals.

2.5. Determination of biochemical parameters

2.5.1. Lipid peroxidation analysis

The degree of lipid peroxidation was determined in a thiobarbituric reactive species (TBARS) assay. This method is based on the reaction of thiobarbituric acid (TBA) and the subproducts formed by the effect of free radicals (ROS) on cell membranes [30].

The composite samples were homogenised in a 3 mL phosphate buffer (0.02 M, pH 7.2) using a stainless steel homogeniser in an ice bath at 4 °C.

One mL of each homogenate was incubated at 90 ºC in a mixture of thiobarbituric acid (0.5%), trichloroacetic acid (15%) and hydrochloric acid (0.25 M) for 20 minutes. Three replicates per treatment were prepared. The samples were then centrifuged and analysed in a spectrophotometer (Genesis) at 535 nm. The concentration of TBARS was calculated with an extinction coefficient of 1.56 x 105 M/cm, and the results were expressed as nmol TBARS/mg protein.

2.5.2. AChE activity analysis

AChE enzyme activity was measured following the Ellman et al. (1961) method, modified for microplates. One mL of each homogenate was centrifuged at 5000 g and 4 °C for 10 minutes, and the supernatant was collected to determine AChE activity.

A volume of 0.05 mL of supernatant and 0.25 mL of reaction mixture (DTNB 10 mM, phosphate buffer pH 7.2 and acetylcholine 0.075 M) was placed in a multi-well plate (96 wells).

Activities were measured at 405 nm in an ELISA reader (Multiskan Spectrum). The reaction was monitored for 20 minutes. AchE activity was expressed as the amount of enzyme that catalysed the hydrolysis of 1 nmol of acetylcholine per minute per milligram of protein (nmol/min/mg protein) [31,32].

The concentration of protein in the samples was evaluated following the Bradford method, with100 L of each homogenate and 1 mL of the Bradford reactive. The samples were read in aspectrophotometer (Genesis) at 535 nm. A calibration curve was used with bovine serum albumin as a standard [33].

2.6. Statistical analyses

The Tbars level and AchE activity data obtained in the bioassays with the metals were analysed with a Kolmogorov-Smirnov test to determine normality. Later, the data were analysed with a two-way ANOVA test considering the type of metal and the exposure time as factors. A multiple comparison was then carried out with a Bonferroni test to determine the statistical importance of the differences observed between the control and the different treatments with metals and exposure times. A significance level of <0.05 was considered for the analyses. All statistical analyses were run with the NCSS v 97 software for Windows [34].

3. Results

3.1. Toxicity tests

The data obtained from the lethality bioassays indicated that the most toxic metal for A. americanus adults was cadmium and the least harmful was manganese (Figure 1). Cadmium was 77 times more toxic than chromium, 49 times more toxic than copper, 17 times more toxic than mercury, 1725 times more toxic than manganese, 513 times more toxic than nickel, and 1546 times more toxic than lead.

LC50 values (48 hours) indicated that the toxicity of the metals was, from the most toxic to the least: Cd > Hg > Cu > Cr > Ni > Pb > Mn.

The analysis of the LC50 dataand its confidence intervals indicated no significant differences in Mn and Pb toxicity ($P < 0.05$). However, significant differences were observed in the responses toCd, Cr, Cu, Hg and Ni ($P < 0.05$) (Figure 1).

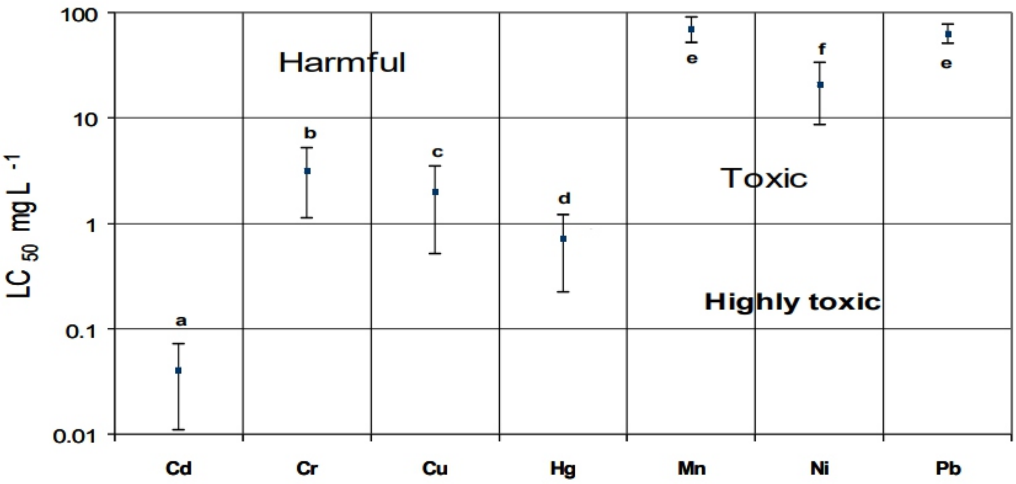

Figure 1. Median lethal concentration (LC50, 48 h) values and confidence limits ($P < 0.05$) for Acanthocyclops americanus copepods exposed to Cd, Cr, Cu, Hg, Mn Ni and Pb. (Different letters indicate significant differences among different metal treatments ($P < 0.05$).)

3.2. Sublethal bioassays

3.2.1. Lipid peroxidation analysis

The mortality rate in the sublethal bioassaysvaried from 0 to 4%, values that lie within acceptable limits for toxicity tests [26–28].

It is important to mention that the Cd, Cr, Cu, Hg, Ni and Pb concentrations used in the sublethal bioassays are environmentally relevant. This is because they fall within the concentration intervals that are reported for water in the aquatic systems of the Valley of Mexico (Table 1).

The average data on the degree of lipid peroxidation recorded in the metal assays ranged from 2.07 ± 0.34 nM Tbars $mg^{-1}$to 22.23 ± 5.47 nM Tbars mg^{-1} (Figure 2). The metal that caused the greatest oxidative effect on the copepods was Cu and the metal with the least effect was Ni. In the case of the tests carried out with Cu, Cr, Hg, Mn and Pb, a direct relationship was observed between the concentration of Tbars and the exposure time, as the degree of lipid peroxidation increased with exposure time (P < 0.05) (Figure 2). In most cases, significant differences were observed in relation to the control group, however, no significant differences were observed at days 2, 4 and 8 of exposure in the bioassays with Ni (P < 0.05). Similarly, no significant differences were recorded in relation to the control group after 2 days of exposure in the tests with Cd and Mn (P < 0.05) (Figure 2).

3.2.2. AChE activity analysis

Regarding AchE activity, significant differences were observed in relation to the control group in thetests with Cd, Cr, Cu, Hg and Pb, for all evaluation times (P < 0.05) (Figure 3). The AchE activity values recorded in these tests were lower than those recorded for the control group, indicating thatthese metals may inhibit AchE activity. This varied from 15% to 79%. The metal that caused the greatest inhibitory effect was Cu (Figure 3). In the case of the tests with the copepods exposed to Mn, AchE activity was observed to be similar to that recorded for the control group 2 days after beginning the bioassay. However, a 36% and 34% decrease in the activity of this enzyme was observed after 4 and 8 days of exposure to this metal, respectively. Ni caused no inhibitory effect on AchE activity (Figure 3).

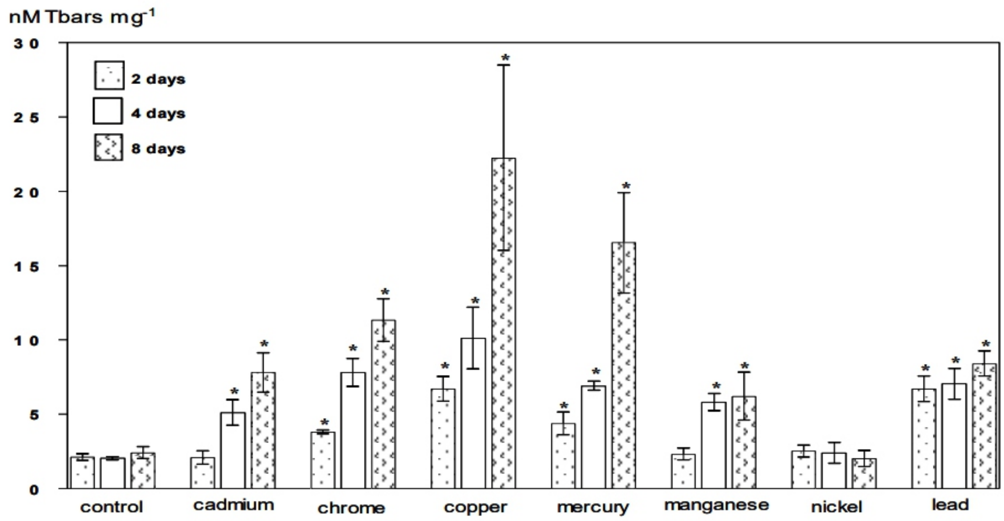

Figure 2. Levels of lipid peroxidation measured as Tbars (nM mg−1) (mean ± standard deviation) in tissues of the copepod Acanthocyclops americanus exposed to Cd, Cr, Cu, Hg, Mn, Ni and Pb. (*Significant differences (P < 0.05) among the exposed organisms and the control group (Bonferroni test).)

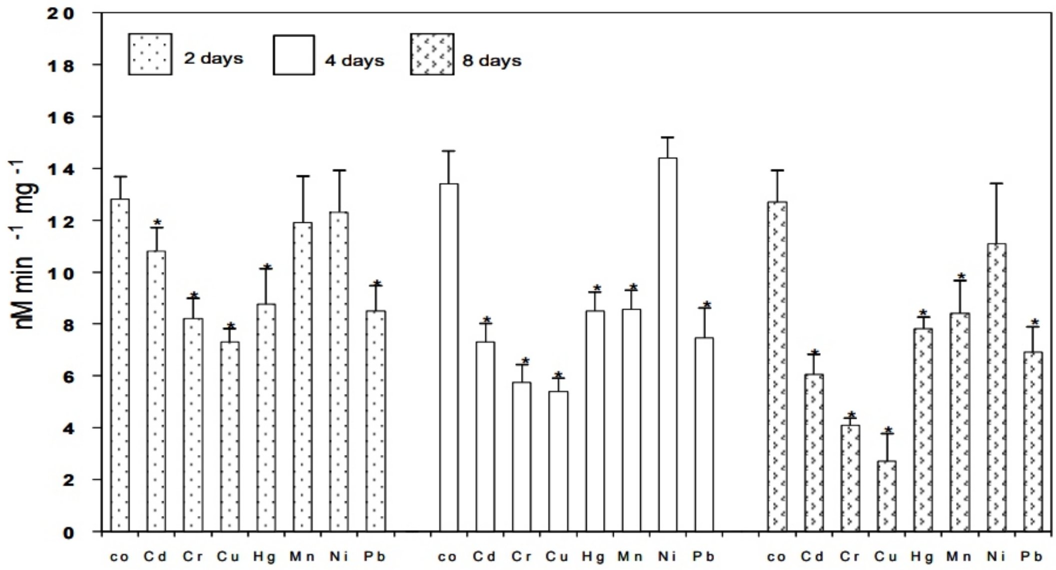

Figure 3. Changes in AchE enzyme activity (nM min−1 mg−1) (mean ± standard deviation) in Acanthocyclops americanus exposed to Cd, Cr, Cu, Hg, Mn, Ni and Pb.(*Significant differences (P < 0.05) among the exposed organisms and the control group (Bonferroni test).

4. Discussion

Most of the studies that have evaluated the toxic effect of different contaminants on copepods have involved species that live in marine systems. The species that are most frequently used in toxicological evaluations are Acartia tonsa and Tigriopus japonicus [35–37]. Very few studies have taken place with freshwater species, however, this research proves that freshwater copepods are quitesensitive to metals [38–40].

When we compared the response ofA. Americanus adults to the different metals, as has been done for other species of freshwater copepods like Cyclops abyssorum and Eudiaptomus padanus that live in oligotrophic environments [41], we observed that they are more sensitive to Cd, Cr, Cu Hg (Table 2) and less sensitive to Ni and Pb (Table 2). Also, A. americanus is less sensitive to Cr, Cuand Ni compared with Mesocyclops pehpeiensis, whereas Tripocyclops prasinus mexicanus and Cyclops sp. are less sensitive to Cd, Cr and Cu than A. americanus [42–44](Table 2). In addition, the LC50 values obtained in the tests with Cd and Cr indicated that A. americanus is more sensitive to these metals than species of marine copepods like Acartia tonsa,Tisbe holothuriae, Tigriopus japonicas, Tigriopus fulvus, Tisbe battagliai and Tigriopus brevicornis [45–51]

Moreover, our results indicate that A. americanus is sensitive to Cd, Mn and Pb, as are Daphnia magna neonates. When comparing the sensitivity of A. americanus adults with that of D. magnaneonates [45,52,53], a species that is used worldwide to evaluate toxicity in samples of water, elutriation and leaching, it is evident that A. americanus adults are less sensitive to Cr, Cu, Hg and Ni (Table 2). The LC50 recorded for A. americanus exposed to Cd was 0.041 ± 0.03 mg L−1, a value that is close to that which is tolerated by D. magna neonates (0.054 mg L−1(0.039 to 0.069 mg L−1) [52,53].

Likewise, A. americanus presented a similar sensitivity to that of D. magna neonates to manganese and lead [45] (Table 2).

Species of the genus Acanthocyclops have been proposed as water quality indicators in cases of contamination by metals. The species A. balcanicus and A. venustusthat inhabit the Aries River in Romania have been proposed as indicator organisms for water with high concentrations of aluminum, copper and zinc associated with particulate and suspended solids. However, these metals, in dissolved form, are very toxic to these species [54]. Acanthocyclops trajani, a species that inhabits the Nile River, has been proposed as a species that is sensitive to water contaminated with metals [55,56]. Krupa (2007) proposedA. rubustus, along with other species of Cyclopoida, indicator of mesotrophy [57]. In addition, this researcher found a highincidence of deformed A. rubustusmales in sites where elevated concentrations of metals were recorded in the Shardarinskoe Lake, Kazakhstan. Finally, Gagneten and Paggi (2009) recorded Cyclopoida, including A. robustus, as more sensitive to metals than rotifers in the Salado River, Argentina [58].

Table 2. Median Lethal 50 values determined for other copepods species in similar test conditions

SPECIES	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Reference
	LC_{50} 48 hours ppm							
Cyclops abyssorum	3.8 ±2.5	10±2.1	2.5±0.5	2.2±1.1	nd	15±10.5	5.5±2.2	41
Eudiaptomus padanus	0.55±0.22	10.1±1.8	0.5±0.15	0.85±0.17	.	3.6 ±1.1	4.0±2.4	41
Mesocyclops pehpeiensis	-	0.51±0.15	0.075±0.046		-	1.191±0.47	-	42
Cyclops sp.	-	10.47	-	0.60	-	-	-	44
Tripocyclops prasinus mexicanus	2.23±1.7	-	2.11±0.79	0.199±0.156	-	-	-	43
Daphnia magna	0.054±0.015	0.77±0.25	0.086±0.03	0.0186±0.0098	61.2±9.8	5.1±4.5	57.1±15.5	45, 52
Marine copepods								
Acartia tonsa	0.337±0.12	11.9±2.7	0.064±0.01	0.019±0.008	-	5.0±1.9	-	45
Tisbe holothuriae	0.97	8.14	0.08	-	-	-	-	46
Tigriopus japonicus	25.2±7.39		3.9±1.97	-	-	-	-	47
Tigriopus japonicus	12.1±3.1	-	-	-	-	17.7±5.1	-	48
Tigriopus fulvus	12.36±4.3	128.16±14.9	-	0.52±0.15	-	-	-	49
Tisbe battagliai	0.340	-	0.088	-	-	-	-	50
Tigriopus brevicornis	0.048±0.021	-	0.150±0.085	-	-	-	-	51
Acanthocyclops americanus	0.041±0.03	3.16±2.05	2.004±1.5	0.712±0.49	70.74±19.05	21.04±12.4	63.40±13.005	This study

The toxicity of metals recorded for A. americanus,according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), was as follows: Cd and Hg were highly toxic, Cr and Cu were toxic, and Mn, Ni and Pb were harmful to this species (i. highly toxic: EC50 ≤ 1 mg L−1; ii. toxic: EC50:1 mg L−1 ≤ 10 mg L−1; iii. harmful to aquatic organisms: EC50 10 mg L−1 ≤ 100 mg L−1; iv. non-toxic: EC50> 100 mg L−1) [59].

The use of biochemical indices to identify sublethal effects produced by xenobiotics has currently increased. These indices are called biomarkers [60,61]. The degree of lipid peroxidation and AchE activity have been proposed as biomarkers that are effective in identifying damage that may be either reversible or permanent, depending on the concentration and the exposure time to metals and other xenobiotics [61,62]. Publications on biomarker evaluations in copepods are scarce,and most have been carried out on marine species.

The results obtained in this study indicate that Cd, Cr, Cu, Hg, Mn and Pb significantly increased lipid peroxidation in copepod tissues after 8 days of exposure. This shows that these metals caused oxidative stress, as the degree of lipid peroxidation indicated that the generation of ROS overcame the antioxidant defenses, causing cellular membranes to be altered [63].

The lipid peroxidation levels recorded in this study demonstrated that the most deleterious metal was Cu (22.23 ± 5.56 nM Tbars mg−1; control = 2.4 ± 0.65 nM Tbarsmg−1). Prior studies carried outby Bo-Mi et al. (2014) on the copepod Tigriopus japonicus also recorded Cu with a highly oxidative effect at concentrations of 0.01 and 0.1 mg L−1 [37]. Likewise, we observed that Cd induces lipid peroxidation. This was previously reported by Wang and Wang (2009) who tested concentrations of 80.01 to 0.1 mg L−1and reported that the degree of lipid peroxidation was positively correlated with Cd concentration in assays with T. japonicus [64]. Likewise, Bo-Mi et al. (2014) observed that Cd induced the generation of ROS in T. japonicus tissues [37].

Our results indicate that Ni did not cause any rise in lipid peroxidation levels in A. americanus. This agrees with previous reports by Wang and Wang (2010) on tests with T. japonicus [64].

Another biochemical response evaluated in this study was the activity of the AchE enzyme. Thetests with A. americanus showed that Cd, Cr, Cu, Hg, Mn and Pb had an inhibitory effect on the activity of this enzyme, with average inhibition percentages of 38%, 54%, 60%, 36%, 26% and 41% respectively, making it possible to consider these metals as neurotoxic agents.

Studies on aquatic organisms have shown that a 20% decrease in AchE activity affects important physiological functions such as feeding and swimming [66]. When inhibitions greater than 25% occur, the life expectancy of the affected organisms is reduced [67,68].

Nickel did not decrease AchE activity after 8 days of exposure in the tests with A. americanus, as Wang and Wang (2010) recorded for the marine copepod Tigriopus japonicuswhen exposed to Ni [65].

Finally, according to the results obtained in this study, the Cd, Cu and Hg concentrations recorded for the Lerma River basin, and those of Cd, Cr, Cu and Ni recorded for the Xochimilco canals, constitute a risk to A. americanus, since they are greater than the concentrations tested inthe sublethal bioassays with A. americanus that had an oxidative and neurotoxic effect on these organisms.

It should also be mentioned that the LC50 values for Cd and Cu recorded in this study (LC50 Cd = 0.041 ± 0.03 mg L−1; Cu = 2.004 ± 1.5 mg L−1) are lower than the values established as Maximum Permissible Limits in the NOM-001-SEMARNAT for the Protection of Aquatic Life (Cd = 0.1 to 0.2 mg L−1; Cu = 4 to 6 mg L−1) [14]. This may be considered a possible risk to organisms that come incontact with discharges that contain these metals. Thus, it is important to continue carrying outstudies to evaluate the degree of the effects on the A. americanus populations in the short and long terms.

5. Conclusion

A. americanus is an organism with greater sensitivity to Cd and Cr metals compared to other freshwater and marine copepods species. A.americanus may be proposed as a test organism to evaluate the presence and effect of metals, especially Cd, Mn and Pb, in toxicity studies, as well asfor monitoring purposes. However, more studies need to be done to propose this species as an indicator organism.

Acknowledgments

This research was supported by UAM Research Funds allocated by the CBS Division of UAM-Iztapalapa. It is a contribution to the project “Physiological, Biochemical and Molecular Responses of Aquatic Organisms Under Stress” (Respuestas Fisiológicas, Bioquímicas y Moleculares de Organismos Acuáticos Sometidos a Estrés).

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. De La Vega-Salazar Y (2003) Situación de los peces dulceacuícolas en México. Revista Ciencias UNAM 72: 20–30.

2. SEMARNAT (2016) Informe de la situación del medio ambiente en México. Compendio de estadísticas ambientales, indicadores clave de desempeño ambiental y crecimiento verde. Mexico:

3. Naranjo EJ, Dirzo R (2009) Impacto de los factores antropogénicos de afectación directa a las poblaciones silvestres de flora y fauna. In: CONABIO, Capital natural de México, Vol. II: Estado de conservación y tendencias de cambio. México:CONABIO 247–276.

4. Paulín-Vaca R, Hernández-Silva G, Lugo de la Fuente J (1997) Extracción secuencial de Cd, Co, Cu, Mn, Ni, Pb y Zn en sedimentos de la cuenca alta del Río Lerma. Actas INAGEQ 4:85–96.

5. Avila-Perez P, Zarazua G, Tejeda S, et al. (2007) Evaluation of distribution and bioavailability of Cr, Mn, Fe, Cu, Zn and Pb in the waters of the upper course of the Lerma River. X-Ray Spectrom 36: 361–368.

6. Carrion C, Ponce-de Leon C, Cram S, et al. (2012) Potential use of water hyacinth crassipes) in Xochimilco for metal phytoremediation. Agrociencia 46: 609–620.

7. Zarazúa G, Avila-Pérez P, Tejeda S, et al. (2013) Evaluación de los metales pesados Cr, Mn, Fe, Cu, Zn y Pb en sombrerillo de agua (Hydrocotyle ranunculoides) del curso alto del río Lerma, México. Rev Int Contam Ambie 29: 17–24.

8. Mercado-Borrayo BM, Heydrich SC, Rosas PI, et al. (2015) Organophosphorus and organochlorine pesticides bioaccumulation by Eichhornia crassipes in irrigation canals in an urban agricultural system. Int J Phytorem 17: 701–708.

9. Aldana G, Hernández M, Cramb S, et al. (2018) Trace metal speciation in a wastewater wetland and its bioaccumulation in tilapia Oreochromis niloticus. Chem Speciat Bioavailab 30: 23–32.

10. Tchounwou PB, Yedjou CG, Patlolla AK, et al. (2012) Heavy metals toxicity and the environment. EXS 101: 133–164.

11. Wu X, Cobbina SJ, Guanghua M, et al. (2016)A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ Sci Pollut Res

12. Brito EMS, De la Cruz M, Barróna C, et al. (2015) Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: Investigation of hydrocarbon degradation potential. Sci Total Environ 521: 1–10.

13. Bojórquez L. Esquivel-Herrera A (2017) Contaminación química de la zona lacustre de Xochimilco. In: Bojórquez L. Contaminación química y biológica de la zona lacustre de Xochimilco. Mexico: UAM, 85–169.

14. Norma Oficial Mexicana NOM-001-Semarnat. Que establece los Límites Máximos Permitidos de contaminantes en las descargas de aguas residuales en aguas y bienes en el Diario Oficial de la Federación el 6 de enero de 1997, México.

15. Golovanova IL (2008) Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biol 1: 93–101.

16. Barata C, Varo I, Navarro JC, et al. (2005) Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comp Biochem Physiol C 140:

17. Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101: 13–30.

18. Chang LW, Suzuki T (1996) Toxicology of Metals. Lewis Publishers, 432–567.

19. Pulido MD, Parrish AR (2003) Metal-induced apoptosis: mechanisms. Mutat Res 533: 227–241.

20. Forget J, Pavillon JF, Beliaeff B, et al. (1999) Joint action of pollutant combinations (pesticides and *metals) on survival (LC50 values) and acetylcholinesterase activity of Tigriopus brevicornis (copepoda, harpacticoida). Environ Toxicol Chem 18: 912–918*

21. Tsangaris C, Papathanasiou E, Cotou E (2007) Assessment of the impact of heavy metal pollution from a ferro-nickel smelting plant using biomarkers. Ecotoxicol Environ Saf 66:232–43.

22. Enríquez-García C, Nandini S, Sarma SSS (2011) Demographic characteristics of the copepod Acanthocyclops americanus (Sars, 1863), (Copepoda:Cyclopoida) fed mixed algal (Scenedesmus acutus) rotifer (Brachionus havanensis) diet. Hydrobiologia 666: 59–69.

23. Elías-Gutiérrez M, Suárez Morales E, Silva Briano M, et al. (2008) Cladocera y Copepoda de las aguas continentales de México. Guía ilustrada. México: UNAM, ECOSUR, SEMARNAT-CONACYT, CONABIO. 57:

24. Azad M, Agard JBR (2006) Comparative sensitivity of three tropical Cladoceran species (Diaphanosoma brachyurum, Ceriodaphnia rigaudii and Moinodaphnia macleayi) to six chemicals. J Environ Sci Health 41: 2713–2720.

25. NMX-AA-087-SCFI-2010 Análisis de agua - Evaluación de toxicidad aguda con Daphnia magna, Straus (Crustacea -Cladocera) - Método de prueba, México, 1–44.

26. USEPA (US Environmental Protection Agency) (2002) Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Washington DC: U.S: Environmental Protection Agency, EPA-821-R-02-012.

27. FAO (1986) Manual of methods in aquatic environment research. Part 10. Short-term static bioassays. FAO Fisheries Technical Paper. 247: 1–62.

28. APHA, AWWA y WPFC (1994) Métodos estándar para el examen de aguas y aguas de desecho,64º México: Ed. Interamericana, 124–278.

29. Diz FR, Araújo CV, Moreno-Garrido I, et al. (2009) Short-term toxicity tests on the harpacticoid copepod Tisbe battagliai: lethal and reproductive endpoints. Ecotoxicol Environ Saf 72: 1881–1886.

30. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Meth Enzym51: 302.

31. Ellman L, Courtey KD, Andreas VJr, et al. (1961) A new rapid calorimetric determination of cholinesterase activity. Biochem Pharmacol 7: 88–98

32. Guilhermino L, Lopes MC, Carvalho AP, et al. (1996) Inhibition of acetylcholinesterase activity as effect criterion in acute tests with juvenile Daphnia magna. Chemosphere 32: 727–738.

33. Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Annals Biochem 72: 248–254.

34. Hintze JL (1997) NCSS 97: Statistical System for Windows. NCSS. Kaysville, UT.

35. Raisuddin S, Kwok KWH, Leung KMY, et al. (2007) The copepod Tigriopus: A promising marine model organism for ecotoxicology and environmental genomics. Aquatic Toxicol 83:161–173.

36. Kulkarni D, Gergs A, Hommen U, et al. (2013) A plea for the use of copepods in freshwater ecotoxicology. Environ Sci Pollut Res 20:

37. Bo-Mi K, Jae-Sung R, Chang-Bum J, et al. (2014) Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (hsp) modulation in the intertidal copepod Tigriopus japonicus. Comp Biochem Physiol Part C 166: 65–74.

38. Brown RJ, Rundle SD, Hutchinson TH, et al. (2005) A microplate freshwater copepod bioassay for evaluating acute and chronic effects of chemical. Environ Toxicol Chem 24: 1528–1531.

39. Soto E, Oyarce G, Inzunza B, et al. (2003) Acute toxicity of organic and inorganic compounds on the freshwater cyclopoid copepod Eucyclops neumani neumani (Pesta, 1927). Bull Environ Contam Toxicol 70: 1017–1021.

40. Devdutt K, Gergs A, Hommen U, et al. (2013) A plea for the use of copepods in freshwater ecotoxicology. Environ Sci Pollut Res 20: 75–85.

41. Baudouin MF, Scoppa P (1974) Acute toxicity of various metals of freshwater zooplankton. Bull Environ Contam Toxicol 12: 741–751.

42. WongCK, Pak AP (2004) Acute and Subchronic Toxicity of the Heavy Metals Copper, Chromium, Nickel, and Zinc, Individually and in Mixture, to the Freshwater Copepod Mesocyclops pehpeiensis. Bull Environ Contam Toxicol 73: 190–196.

43. Lalande M, Pinel-Alloul B (1986) Acute toxicity of cadmium, copper, mercury and zinc to tropocyclops prasinus mexicanus (cyclopoida, copepoda) from three quebec lakes. Environ Toxicol Chem 5: 95–102.

44. Abbasi SA, Nipaney PC, Soni R (1988) Studies on environmental management of mercury (II), Chromium (VI) and zinc (II) with respect to the impact on some arthropods and protozoans. Inter J Environmental Studies 32: 181–187.

45. Ecotox Database. EPA Available at https://cfpub.epa.gov/ecotox/

46. Moraitou-Apostolopoulou M, Verriopoulos G (1982) Individual and combined toxicity of three heavy metals, Cu, Cd and Cr for marine copepod Tisbe holothuriae. Hydrobiologia 87: 83–87

47. Lee KW, Raisuddin S, Hwang DS, et al. (2007) Acute Toxicities of Trace Metals and Common Xenobiotics to the Marine Copepod Tigriopus japonicus: Evaluation of Its Use as a Benchmark Species for Routine Ecotoxicity Tests in Western Pacific Coastal Regions. Environ Toxicol 22:532–538

48. Emadeldeen HM (2017) Acute Toxicity of Cadmium and Nickel to Three Marine Copepods. Sci J King Faisal Univer 18: 9–16.

49. Pane L, Mariottini GL, Lodi A, et al. (2008) Effects of heavy metals on laboratory reared Tigriopus fulvus Fischer (Copepoda: Harpacticoida). In: Brown SE, Welton WC Heavy Metal Pollution. Nova Science Publishers, Inc, 158–165.

50. Hutchinson TH, Williams TD, Eales GJ (994) Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (Cyprinodon variegatus) and copepods (Tisbe battagliai). Mar Environ Res. 38: 275–290.

51. Barka S, Pavillon JF, Amiard JC (2001) Influence of different essential and nonessentialmetals on MTLP levels in the copepod Tigriopus brevicornis. Comp Biochem Physiol C 128: 479–493.

52. Mance G(1987) Pollution threat of heavy metals in aquatic environments. London: ElsevierAplied Science, 45–123.

53. Traudt EM, Ranville JF, Meye JS (2017) Effect of age on acute toxicity of cadmium, copper, nickel, and zinc in individual-metal exposures to Daphnia magna neonates. Environ Toxicol Chem 36: 113–119.

54. Moldovan OT, Melega IN, Leveib E, et al. (2013) A simple method for assessing biotic indicators and predicting biodiversity in the hyporheic zone of a river polluted with metals. Ecol Indic 24: 123–136.

55. El-Shabrawy GM, Elowa SE, Rizk ST, et al. (2005) Impact of industrial pollution on zooplankton community structure in Rosetta Nile Branch at Kafr El-Zayat area Egypt. Afr J Biol Sci 1: 1–14.

56. Abdel-Halim AS, Waheed ME, El-ShabrawyGM, Fawzia MF (2013)Sewage pollution and zooplankton assemblages along the Rosetta Nile branch at El Rahawy area, Egypt. Int J Environ Sci Engin 4: 29–45.

57. Krupa EG (2007) Structural characteristics of zooplankton of the Shardarinskoe reservoir and their use in water quality assessment. Water Resour 34: 712–717.

58. Gagneten AM, Paggi JC (2009) Effects of heavy metal contamination (Cr, Cu, Pb, Cd) and eutrophication on zooplankton in the lower basin of the Salado River (Argentina). Water Air Soil Pollut 198: 317–334.

59. United Nations (2009) The globally harmonized system of classification and labelling of chemicals.

ST/SG/AC.10/30/Rev.3. Available from: https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

60. Vasseur P, Cossu-Leguille C (2006) Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 62: 1033–42.

61. Hagger J, Malcolm JB, Lowe D, et al. (2008) Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: A case study. Mar Poll Bull 56: 1111–1118.

62. Broeg K, Westernhagen H, Zander S, et al. (2005) The bioeffect assessment index (BAI) a concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar Poll Bull 50: 495–503.

63. Volodymyr I L (2011) Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicol 101: 13–30.

64. Wang MH, Wang GZ (2009) Biochemical response of the copepod Tigriopus japonicusMoriexperimentally exposed to cadmium. Arch Environ Contam Toxicol 57: 707–717

65. Wang MH, Wang GZ (2010) Oxidative damage effects in the copepod Tigriopus japonicusMoriexperimentally exposed to nickel. Ecotoxicol 19: 273–284.

66. USEPA (1998). SCE policy issues related to the food quality protection act. Office of pesticide programs science policy on the use of cholinesterase inhibition for risk assessment of organophosphate and carbamate pesticides. Office of Pesticide Programs. Washington DC: US Environmental Protection Agency 20460.

67. Dailianis S, Domouhtsidou GP, Raftopoulou E, et al. (2003). Evaluation of neutral red retention assay, micronucleus test, acetylcholinesterase activity and a signal transduction molecule (cAMP) in tissues of Mytilus galloprovincialis (L.), in pollution monitoring. Mar Environ Res56: 443–470.

68. Stefano B, Ilaria C, Silvano F (2008). Cholinesterase activities in the scallop Pecten jacobaeus: Characterization and effects of exposure to aquatic contaminants. Sci Total Environ 392: 99–109.

·ISSN 2372-0352

Effect of macro-and micro-plastics in soil on growth of Juvenile Lime Tree (Citrus aurantium)

Andrew Wirnkor Verla1, Christian Ebere Enyoh 1,*, Isiuku Beniah Obinna2, Evelyn Ngozi Verla3, Wang Qingyue4, Md. Akhter Hossain Chowdhury5, Emmanuel Chinedu Enyoh6 and Tanzin Chowdhury

[1]1 Group Research in Analytical Chemistry, Environment and Climate Change (GRACE&CC), Department of Chemistry, Imo State University (IMSU), Imo State, Nigeria

2 Department of Chemistry, Imo State University (IMSU), Imo State, Nigeria

3 Department of Environmental Technology, School of Environmental Science, Federal University of Technology, Owerri, P.M.B.1526, Imo State, Nigeria

4 Graduate School of Science and Engineering, Saitama University, Saitama, Japan

5 Dept. of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

6 Quality Assurance and Control Department, Intercontinental Distillers Limited, Ota, Ogun State, Nigeria

A B S T R A C T

Plastics in agricultural soils are of current concern to environmental scientist due to potential effects on soil-plant system and food security. In this study, the effect of macro- and microsized plastic types of low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) in soil on growth of juvenile lime tree were studied using a pot experiment under ambient field conditions. To determine the effect, seven exposure patterns (single: LDPE, PP, PS and mixture: LDPE+PP, LDPE+PS, PP+PS, LDPE+PP+PS) in soil was tested. The results showed that macroand micro- plastic residues affected the plant during vegetative growth with LDPE single exposure had the strongest negative effects (inhibition of 0.26%). However, LDPE+PP+PS had some positive effects by improving growth higher than the control. Overall, microplastics showed more negative effects than macroplastics, but the lime tree showed strong tolerance (with tolerance index > 70 %) to the different treatments. Without doubt, more research is urgently needed in order to fully understand the effect and mechanism of macroplastics and microplastics in soil-plant system. When understood remediation methods could be formulated to minimize the effects of plastics which are now ubiquitous in the environment.

Keywords: agroecosystem; food security; lime tree; microplastics; plastic residues; soil

1. Introduction

In the last one and half decades, the pollution and impact of plastics in the environment has been the major focus of study for many scientists globally. Studies have demonstrated that presence of macro- and micro- plastics in aquatic ecosystems and atmospheric compartments posing serious threat to aquatic organism, marine ecosystem and human health [1–3]. Over the years, soil has become a major sink for microplastics [4,5] and are also part of microplastics contamination cycle involving both atmosphere and aquatic ecosystems [6]. According to Horton AA et al. [7] the soil contains more plastic waste than the ocean. In 2017, an estimated 34.8 million metric tons of plastics ended up as waste in landfills globally [8].

More than half of previous research regarding the occurrence and fate of microplastics in the environment has been focused on aquatic ecosystem [9,10] while soil ecosystems especially agricultural soil has been less studied [1,3,5]. In agriculture, it is a common practice to use shredded vegetable matter and/or plastics (e.g. polyethylene) as mulch material to improve soil quality by retaining the moisture, discouraging weed growth and insulating the soil thus making it more beneficial to desired plant growth [11]. These practices are currently on the rise globally [12–14]. According to Brodhagen M et al. [13], the application of plastic mulch, improved plants and economic growth. However, the down side of this practice is that the plastic is left in the soil after harvest due to the difficulties in removing them and coupled with high cost of recycling [13,15].Over the years the residual plastics accumulates resulting in large quantity of plastics in the soil [15,16]. Unfortunately, the rate of disintegration of these plastic residues in soil is currently unknown [17], therefore the accumulation is undesirable and could affect quality of soil, cropproduction [16,18] and potentially affect human health [1,3,6,8]. Due to UV radiation, mechanical abrasion, and interaction with fauna, macro plastics (> 5 mm) in the soil become brittle and then fragments into smaller particles, then microplastics (MPs, 0.1μm to 5 mm) [19]. Apart from the application of mulch film, microplastics could enter agricultural soil from application of sludge and organic fertilizer on land, waste water irrigation, littering and surface runoff as well as deposition of airborne microplastics [1,4,5,20]. The long-term presence of plastics in agricultural soils could cause stunted plants growth due to the ability to uptake them and negatively affect biodiversity. Previous reports have shown that plants can accumulates micro and nano size plastics, through their cell wall and membrane [5,21,22] and could uptake toxic pollutants as well [1]. Microplastics have the ability to convey toxic pollutants with endocrine disrupting ability within soil-plant system, whether by adsorption or from additives contained in the plastic [1,3]. This has aroused the interest of environmental analytical scientists regarding the soil-plant system.

Literature reveals that there has been limited experimental research focusing on macroplastics and microplastics in soil-plant system.Enyoh et al. [1] in their review identified a knowledge gap and called for deliberate studies focusing on the effects of microplastics concentrations and type on plant. Qi et al. [14] already took the first steps towards filling the knowledge gaps by studying the effects of macro- and micro- plastics in presence and absence of earthworm in soil on wheat plant in a climate chamber. Following the study of Qi Y et al. [14], the current research is aimed to further fill knowledge gaps by testing the effects of macro- and micro- plastics of three types, low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) in soil on the growth of juvenile lime tree (Citrus aurantium) under ambient field conditions. Citrus fruits are highly cultivated in Nigeria.

Currently, Nigeria is the 9th major global producer and largest in Africa [23]. As at 2008, Food and Agriculture Organization placed annual local production to be about 3.4 million metric tonnes from an estimated 3 million hectares of land [24]. Production has grown ever since at an average rate 3.21 % reaching 4.09 million metric tonnes in 2017 [25] due to increasing demand, mainly from the composition and many usefulness of its different parts. The pulp and peel of citrus fruits are being used as livestock feeds, produce citric acid and feed yeast among others. The peels also contain essential oils, a valuable material in the international market, the bark and leaves are well known to have phytochemicals that are of pharmacological importance. While the cultivation is seemingly rampant, to the best of our knowledge studies focusing on the impact of macro- and micro- plastics in terrestrial ecosystem and particularly agricultural soils in Nigeria is non-existent [26,27]. In Nigeria, current estimate revealed that 114.8 kt of plastics is in the environment as waste and it is predicted to reach 147.8 kt in 2023 [27]. Therefore, the study also provides first assessment of macro- and micro- plastics in agricultural soils on plant in Nigeria. Furthermore, the present study will encourage similar researches that will lead to a proper understanding of the plastic-soil-plant system.

2. Materials and methods

2.1. Experimental design

2.1.1. Soil and plant materials

A pot experiment was adopted to evaluate the effects of different types and sizes of plastic fragment on lime tree (Citrus aurantium) in a field condition. The soils used for the study were collected from a farmland in Orji, Owerri Imo State Nigeria (Lat. 5o30′56.778″ N, Lon. 7o2′59.574″ N and above sea level 123.40 m). A simple random sampling technique was adopted for sample collection, to spread sampling point objectively. Top soils (0–5 cm) were collected using a soil auger and transferred into black plastic bags. The sampling materials used were previously soaked and rinsed in 10% HNO3 overnight. The bags were tied tightly and transported to the laboratory for analysis. In the lab, soil samples were air-dried and debris removed. The soil was grounded and sieved with 2 mm mesh-sieve and kept for further use and analysis. The farmlands were used to grow the Citrus aurantium (nurseries) prior to the study. The nurseries collected from the farmland had an average height of 7 ± 2 cm and have been growing for more than a year. The soils collected were analyzed in triplicate for physicochemical characteristics as described previously [28,29]. Temperature was determined in situ using the soil gardeners thermometer while pH using Jenway 3510 pH meter. HANNA HI 8733 EC Meter was used in determining soil electrical conductivity in µS/cm. Moisture contents and soil organic matter were determined based on weight loss on ignition at 105 oC and 440 oC respectively. Particle size distribution (% sand, % silt and % clay) was determined using hydrometer. The soil colour was dark brown, which consisted of 51 ± 0.02 % clay, 13.6 ± 0.49 % sand and 4.6 ± 0.51% silt with an organic matter and moisture content of 4.21 ± 1.12 % and 18.23 ± 2.14 % respectively. The pH, temperature and electrical conductivity were 6.62 ± 0.04, 28.2 ± 1.34 oC and 371.62 ± 3.42 µS/cm respectively. The soil properties are consistent with tropical soils of this region [28,29]. The soil samples were then air-dried under room temperature, removed debris, crushed and sieved through a 2 mm steel sieve for homogeneity before use.

2.1.2. Plastic materials

Three types of plastic were evaluated in this experiment: (1) low-density polyethylene (LDPE), (2) polypropylene (PP) and (3) polystyrene (PS) (the sources of these plastic types were from household and containers for personal care products, presented in Figure 1). These plastic types were identified based on their SPI (Society of Plastic Industry) codes [30]. LDPE had code number 4, with density of 0.92 g/cm3, tensile strength of 600–2300 psi, melting temperature of 98–115 °C and resistant to solvent below 60 °C; PP had code number 5, with density: 0.90 g/cm3, tensile strength of 4500–5500 psi, melting temperature of 175 °C and resistant to solvent below 80 °C while PS had code number 6, with density of 1.05 g/cm3, tensile strength of 5000–7200 psi and melting temperature of 100 °C.

Figure 1. Source of plastic types used in the study.

The macroplastics (Ma) and microplastics (Mi) fragments used were obtained manually as described previously [14]. For Ma, the pieces of plastics were cut on a hard-wooden board using sharp blades and scissors and size of about 5cm were used. After cutting, some pieces of plastic were randomly collected from each sort. To obtain Mi (< 5 mm), the remaining unused macroplastics were further cut into pieces and then was frozen and grounded into a powder using a high speed blender. After grinding, the resulting powder was sieved through 1 mm sieves, to obtain microplastics of homogenous sizes. 2.1.3. Treatments Three factors were taken into consideration while planning the experiment: single (LDPE/PP/PS), mixture (LDPE+PP/ LDPE+PS/ PP+PS/ LDPE+PP+PS) and sizes of plastic residues (Ma/ Mi) (Table 1). Furthermore, eleven control treatments without any plastic residues were also examined. Overall, the experiment consisted of eight treatments with 165 pots x 3 replicates making a total of 495 pots.

Table 1. Treatments setting for the study.

Days	Control	Treatment													
		LDPE		PP		PS		LDPE+PP		LDPE+PS		PP+PS		LDPE+PP+PS	
0	Control 1	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
10	Control 2	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
30	Control 3	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
60	Control 4	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
90	Control 5	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
120	Control 6	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
150	Control 7	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
180	Control 8	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
210	Control 9	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
240	Control 10	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi
270	Control 11	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi	Ma	Mi

Notes: Ma- Macroplastics; Mi- Microplastics.

2.2. Set up and growth conditions

For the experimental set up, with slight modifications, we followed a procedure described previously [14]. For each pot (height = 7 cm, top diameter = 6 cm and bottom diameter = 4 cm), we added the sieved soil of weight 3000 g. Then accurately weighed plastic material (30 g) was mixed manually with water and the mixture poured in the pot containing the soil (except for the control which was free from plastic contamination) to make a treatment of 1 % w/w. The plastic mixture treatment also followed the same procedure except that the plastics (either Ma or Mi) added were 15 g + 15 g for two mixtures (also 1 % w/w for LDPE+PP, LDPE+PS and PP+PS) and 10 g + 10 g + 10 gfor three mixtures treatment (1 % w/w for LDPE+PP+PS). Similar treatments have been applied other studies [14,31,32]. After the set-up, moisture contents of the pots were unified to 18 % similar to the soil field capacity. All the pots were allowed to settle down for a period of one week before lime juvenile plants were transplanted from the field into pots. The cultivation of the plant was done under ambient field conditions (relative humidity: 72.70 ± 10.37 % and temperature: 29 ± 4.82 oC. Both parameters were measured with CO2 gas analyzer AZ77535 as described by [33]. The pots were placed randomly (and position shifted once a month) and watered weekly with tap water and the soil moisture was kept at around 12% to 18% with respect to weight [14].

2.3. Measurements of growth parameters

Plant heights were measured from the root to the tip of the plant using a tape rule. The number of branches and leaves were counted manually and recorded. Plants dry biomasses were recorded after drying at 70 °C (using DHG-9023A Oven by B. Bran Scientific and Instrument Company, to a constant weight. Leaf areas were measured using a plastic ruler as shown in Figure 2. The length (L cm) of the leaf was measured at the apex and width measured at the center (W cm). Leaf areas were then calculated as the product of the length and the width (L* W) in cm2. The growth parameters were measured for all treatments at 30 days interval for 270 days (between January 2019 and October 2019, covering both the dry and rainy period).

(a) **(b)**

Figure 2. Plant height (a) and leaf area (b) measurements.

2.4. Statistical and data analysis

Statistical data analyses were performed using IBM SPSS Statistics 20. Mean values from replicate observations were recorded for each plant and were expressed as mean ± standard deviation. Line plots were made for 60, 120, 180, 240 and 270 days for clearer view of trend while box-plot was created to summarize data obtained over 270 days. One-way analysis of variance (ANOVA) was carried out to compare the different treatment and differences were considered significant at $P < 0.05$. Chemometric models such as relative growth rate (RGR), tolerance index (TI) and % inhibition were employed to assess the effects of treatment on vegetative growth of the juvenile lime tree. RGR value is an important index for estimating growth trends of plants exposed to the different treatment groups. It was proposed by Fisher in 1921 [34,35] and computed using Eq 1, where: ln(m1): logarithm of the final dry mass (g); ln(m0): logarithm of the initial dry mass (g); t0: initial time (d); t1: final time (d).

$$RGR = \left({In(m_1) - In(m_0)} / {t_1} - t_0 \right) * 1000 \quad (1)$$

The tolerance index (TI) was proposed by [36]. In the context of this study, it provides information regarding the tolerance of the plant to plastic contamination of the soil. It was calculated using Eq 2 as the ratio of growth rate of the plant in the plastic treated soil to growth rate of the plant in the control soil [34]. In the equation, RGR is the growth rate of the plant in the solution contaminated while RGRc is the growth rate of the plant in the control soil, without treatment. The % inhibition (Eq 3) provides information on the extent by which the contaminations of plastic in the soil inhibit/reduce growth of the plant with respect to the control. The variables for % inhibition issame as the ones in Eq 2.

$$TI = \left({RGR} / {RGR_c} \right) * 100 \quad (2)$$

$$\% \text{ Inhibition} = 1 - \left({RGR} / {RGR_c} \right) \quad (3)$$

3. Results

3.1. Lime tree development and vegetative growth

3.1.1. Plant height

The effects of different treatment for both macroplastics and microplastics in comparison to control are presented in Figure 3. Over 270 days, the effect trend for macroplastics on plant height was; LDPE+PP+PS (10.21 ± 5.36 cm) > LDPE+PS (8.58 ± 5.40 cm) > Control (8.42 ± 5.19 cm) > PS+PP (8.36 ± 5.64 cm) > PP (7.61 ± 4.26 cm) > PS (7.29 ± 4.33 cm) > LDPE+PP (7.20 ± 5.15 cm) > LDPE (7.17 ± 4.44 cm) while for microplastics the effect trend was; LDPE+PP+PS (10.45 ± 6.00 cm) > Control (8.42 ± 5.19 cm) > PS+PP (8.20 ± 5.33 cm) > PP (7.70 ± 4.44 cm) > LDPE+PP (6.94 ± 4.44 cm) > LDPE (6.64 ± 5.71 cm) > PS (6.59 ± 3.46 cm) > LDPE+PS (6.18 ± 4.22 cm) respectively. This therefore showed that only Ma-LDPE+PP+PS, Ma-LDPE+PS and Mi-LDPE+PP+PS increased the height of the plant than the control over 270 days. However, all treatments assessed by One-way ANOVA showed significant differences ($P < 0.05$) from the control (Figure 4). Between the two plastic sizes, there was also a significant difference ($P < 0.05$), with addition of microplastics having greater negative effects (reducing) on plant height than the addition of macroplastics (Figure 4).

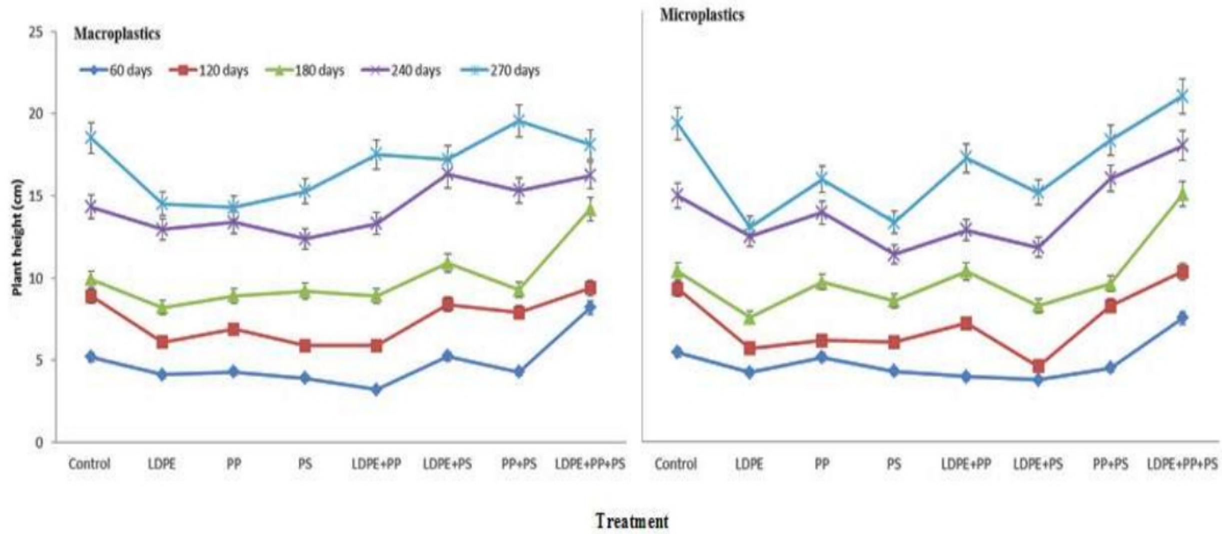

Figure 3. Plant height for different macroplastics and microplastics treatments in 60, 120, 180, 240 and 270 days. Bars indicate standard error (± SE) at 0.05.

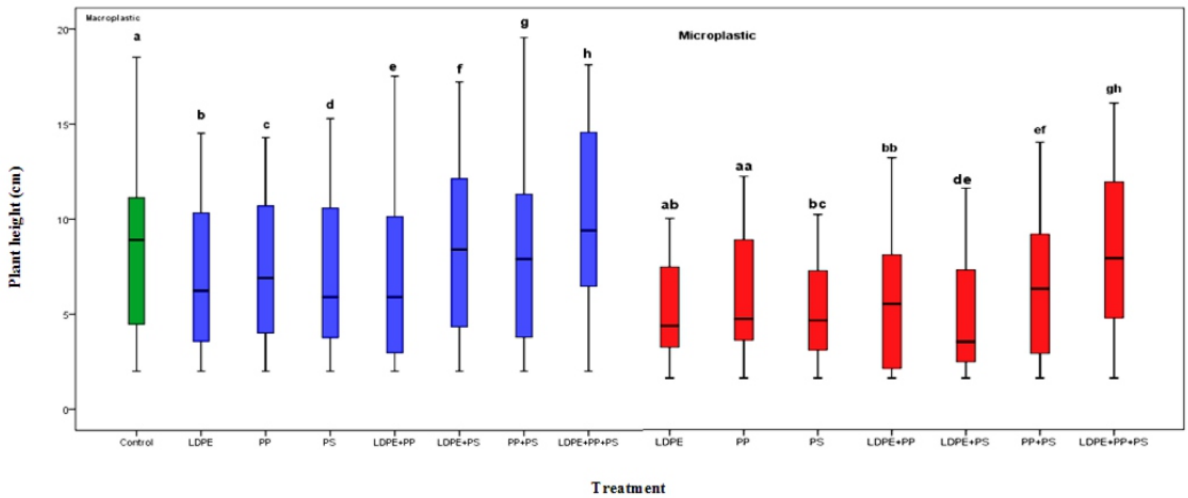

Figure 4. Box plot for effect of different macroplastics and microplastics treatments on plant height over 270 days. Different alphabet in plot indicate significant differences at $P < 0.05$.

3.1.2 Branch number

The effects of the different treatment and plastic sizes on number of branches are presented in Figure 5. Similar trend was generally observed for both size group of the plastic. At day 60 and 120 all treatment showed equal number of branches with control (= 2 and 3 respectively). Over the entire study period, Ma-LDPE+PP+PS (3.72 ± 2.33) and Mi-LDPE+PP+PS (3.63 ± 2.15) had the highest number of branches while Ma/Mi-LDPE (3.00 ± 1.79) had lowest respectively. All treatment showed no significant differences ($P > 0.05$, Figure 6) from the control (3.55 ± 2.11) over the study period.

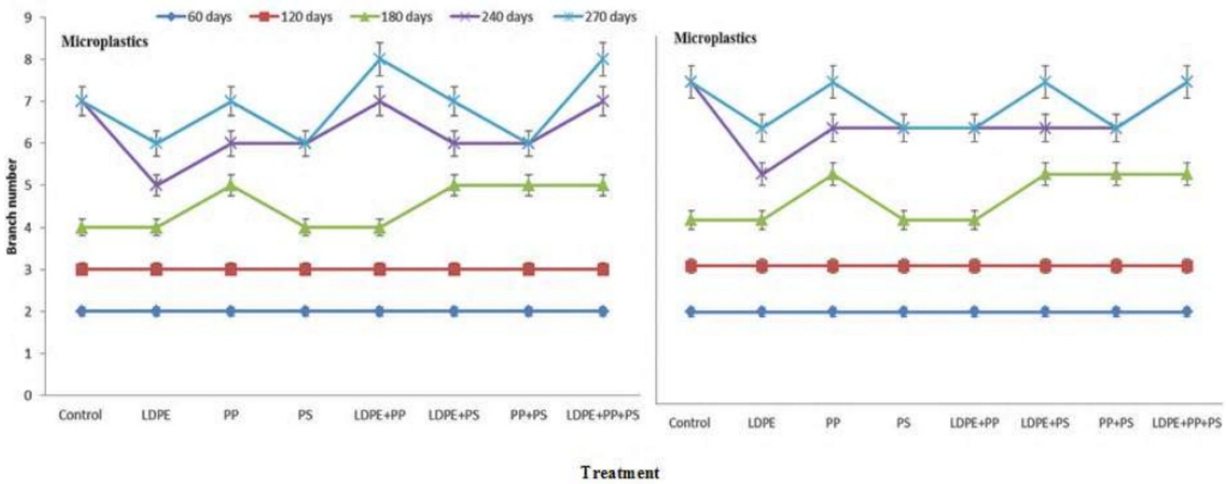

Figure 5. Number of branches for different macroplastics and microplastics treatments in 60, 120, 180, 240 and 270 days. Bars indicate standard error (± SE) at 0.05.

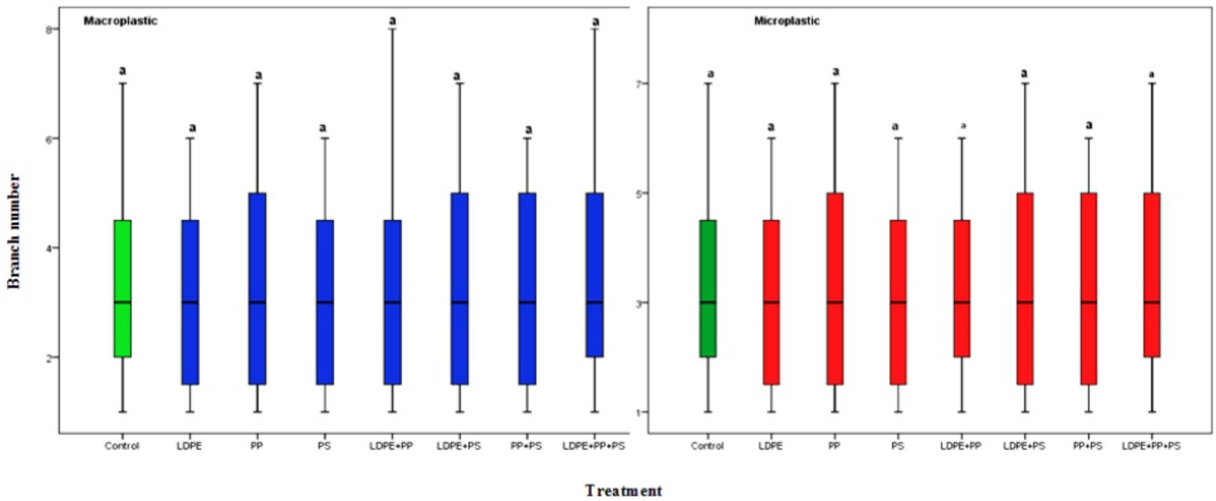

Figure 6. Box plot for effect of different for different macroplastics and microplastics treatments on number of branches over 270 days. Bars indicate standard error (± SE) at 0.05.

3.1.3 Number of leaves

The effects of the different treatment and plastic sizes on number of leaves are presented in Figure 7. Over the entire study period (270 days), the highest and lowest number of leaves was observed for LDPE+PP+PS treatment (Ma: 28.73 ± 25.06 > Mi: 28.18 ± 25.06) and PS treatment (Ma: 22.18 ± 18.72 < Mi: 22.73 ± 19.48) (Figure 8). The former only had higher number of leaves than the control (28.27 ± 24.23), but not significantly different ($P > 0.05$, Figure 8). However, comparing plastic size effects (Figure 7), microplastics treatment appeared to have stronger inhibition on the number of leaves, but they also showed no significant differences ($P > 0.05$) (Figure 8).

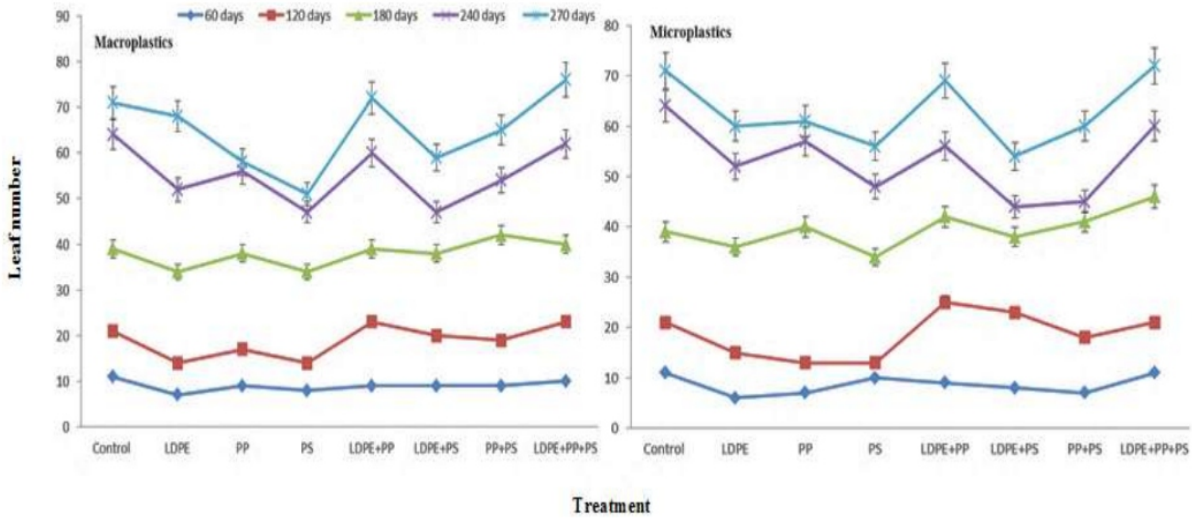

Figure 7. Leaf number for different macroplastics and microplastics treatments in 60, 120, 180, 240 and 270 days. Bars indicate standard error (± SE) at 0.05.

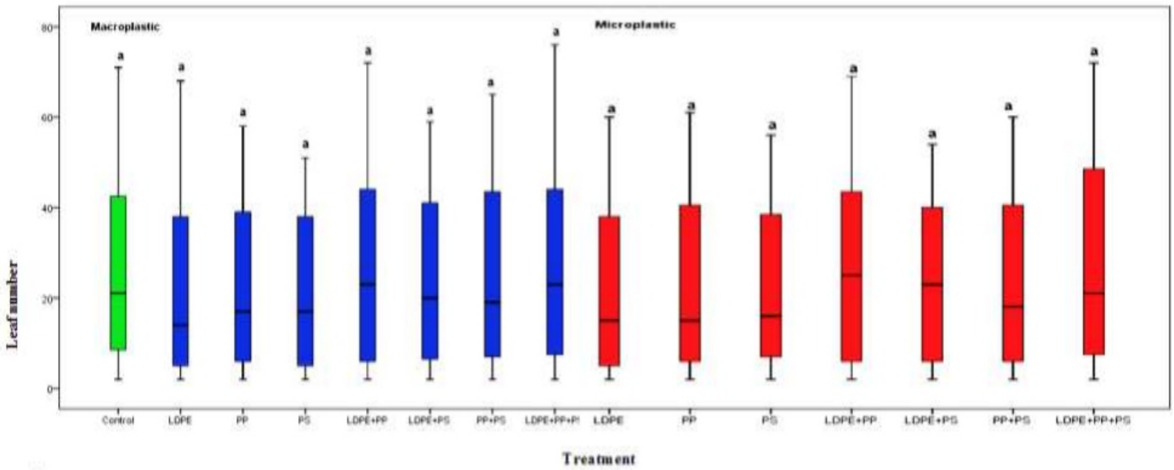

Figure 8. Box plot for effect of different macroplastics and microplastics treatments on leaf number over 270 days. Different alphabet in plot indicate significant differences at P < 0.05.

3.1.4. Leaf area

The leaf areas of the different treatment groups are presented in Figure 9. In size Ma, except for treatment LDPE+PP+PS (P < 0.05) and LDPE+PP (P < 0.05), all other treatment had no significant difference from the control (P > 0.05). Furthermore, only Ma-PS (19.82 cm2) and PP+PS (19.24 cm2) had lower leaf areas than the control (20.15 cm2). In size group Mi, all treatment (except for PP, PS, LDPE+PS and PP+PS) significantly reduced the leaf areas compared to macro sizes. The effect of microplastics showed significant differences from macroplastics, for LDPE, LDPE+PP and LDPE+PP+PS treatments. The effect trend over the entire period of study was LDPE+PP > LDPE+PS > PP+PS > PP > PS > LDPE > LDPE+PP+PS (Figure 9).

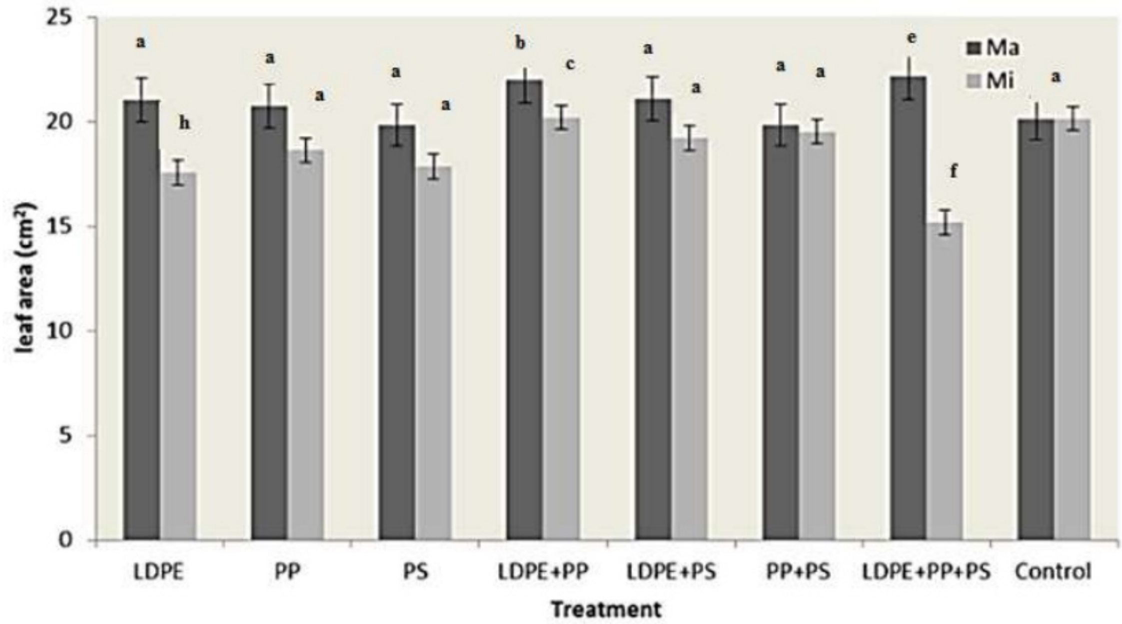

Figure 9. Mean leaf area for all treatment over 270 days. Bars indicate standard error (± SE) at 0.05.

3.1.5. Total plant biomass (TPB)

The trend for total plant biomass is presented in Figure 10. The TPB of the plant increased with time from 1.40 g from the initial day to as high as 9.22 g at the 270th day. Overall, for macroplastics, the control (5.00 ± 3.10 g) had higher biomass than all treatments except for LDPE+PP+PS (5.43 ± 2.39 g) while for microplastics, all treatments had lower biomass than the control. However, all treatments for both macroplastics and microplastics showed significant differences from the control ($P < 0.05$) also between the size categories ($P < 0.05$).

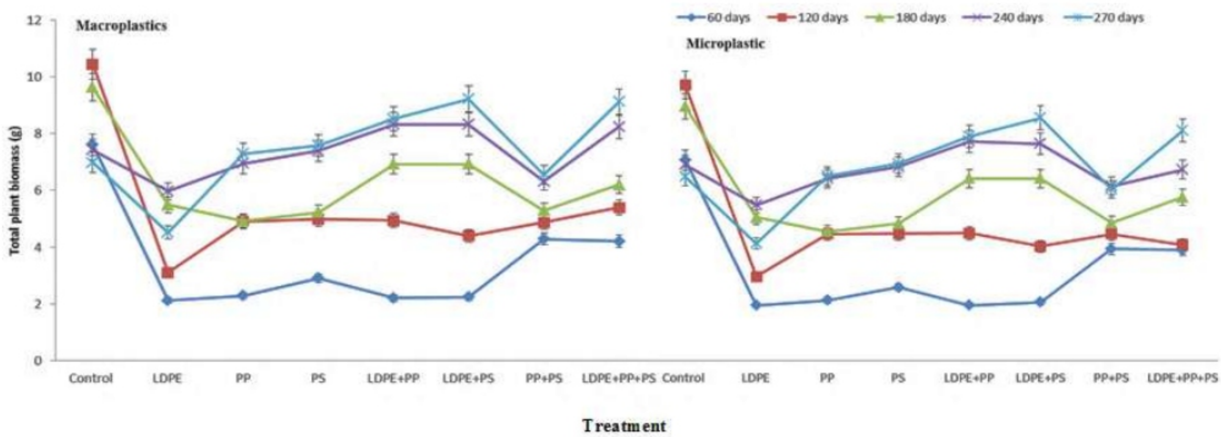

Figure 10. Total plant biomass for all treatments in 60, 120, 180, 240 and 270 days. Bars indicate standard error (± SE) at 0.05.

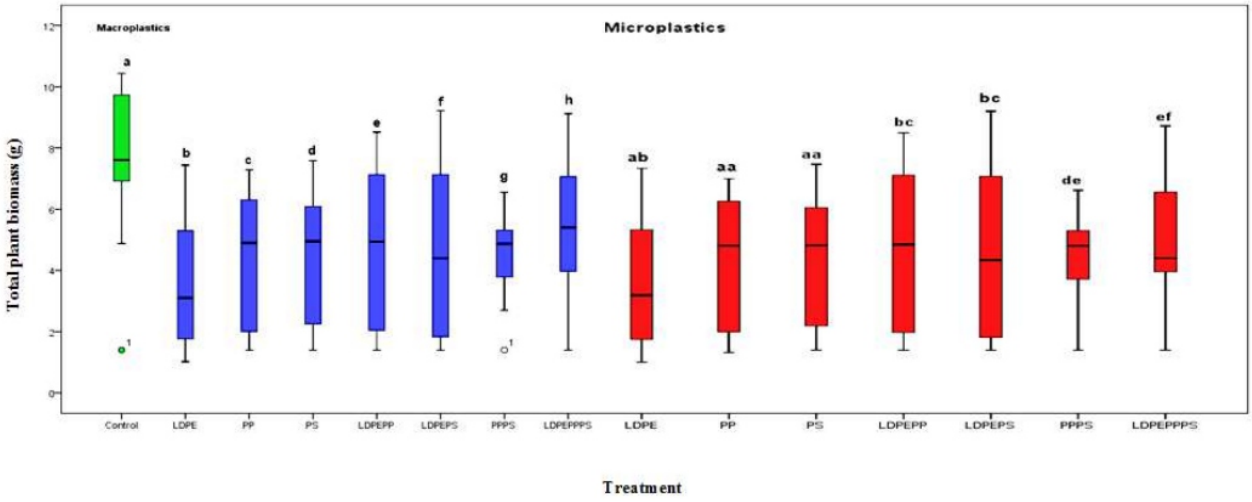

Figure 11. Box plot for effect of different macroplastics and microplastics treatments on plant height over 270 days. Different alphabet in plot indicate significant differences at P < 0.05.

3.2. Relative growth rate (RGR), % inhibition and tolerance index (TI) of juvenile lime tree

The relative growth rate of C. aurantium to the different treatment is presented in Table 2. The control had a better growth rate (10.29 ± 2.65) for all treatments except for Ma-LDPE+PP+PS. The lowest growth rate of the plant was observed for LDPE treatment. RGR of LDPE, PP+PS and LDPE+PS treatments for both plastic sizes were significantly different from the control (P < 0.05), while other treatment groups showed no significant differences from the control (P > 0.05).

	Treatments						
	LDPE	PP	PS	LDPE+PP	LDPE+PS	PP+PS	LDPE+PP+PS
	Relative growth rate (RGR)						
Control	10.29 ±2.65a	10.29 ±2.65a	10.29 ±2.65a	10.29 ±2.65a	10.29 ±2.65a	10.29 ±2.65a	10.29 ±2.65a
Macroplastics	7.62 ±2.22b	9.02 ±2.29aa	9.23 ±2.34a	9.78 ±2.75a	9.68 ±2.95bc	9.41 ±1.54de	10.97 ±2.39a
Microplastics	7.60 ±2.19b	8.91 ±2.26ab	9.14 ±2.32a	9.73 ±2.76a	9.61 ±2.94bc	9.23 ±1.71ef	10.13 ±2.25a
	% Inhibition						
Macroplastics	0.26a	0.12a	0.10a	0.05a	0.06a	0.09a	-0.07a
Microplastics	0.26a	0.13a	0.11a	0.05a	0.07a	0.10a	0.02b
	Tolerance index (%)						
Macroplastics	74.05a	87.66a	89.70a	95.04a	94.07a	91.45a	106.61a
Microplastics	73.85a	86.59a	88.82a	94.56a	93.39a	89.69a	98.45b

*Values in column with the same alphabet are not significantly different at 5% level of probability

Similar to relative growth rate, the highest inhibition (Table 2) was observed for the LDPE treatment (0.26) while other treatments were in the order of PP > PS > LDPE+PS > LDPE+PP > PP+PS > LDPE+PP+PS. Ma-LDPE+PP+PS had a negative % inhibition due its RGR being higher than that of control and was significantly different from other treatment (P < 0.05).

C. aurantium generally showed high tolerance to the different treatment groups (Table 2). For macroplastics, the tolerance index ranged from 74.05 % for LDPE to 106.6 % for LDPE+PP+PS while for microplastics group, the tolerance index ranged from 73.85 % for LDPE to 98.45 % for LDPE+PP+PS. Overall, high tolerance to macroplastics than microplastics contamination was shownby the plant.

4. Discussion

The growth of the lime tree was not severely affected by macroplastic and microplastic exposure in this study when in comparison with the control. Despite the observed toxicity symptoms as the decrease in total plant biomass, there was no unfavorable impact on the relative growth rate (RGR). In fact, there was better growth stimulation in one of the treatments (Ma-LDPE+PP+PS) than the control. This growth could also be due to the possibility of the plastic treatments improving soil biophysical properties such as bulk density, soil aggregation and water holding capacity [32,37] and other factors such as soil animals such as earthworms. Recent studies have suggested that earthworm presence in soil could reduce the deterioration made by plastic residues on growing plants [3,14]. It was explained further that the particles in the soil are eaten by earthworm [37], although unfavorable for the earthworm, but the endeavor will reduce plastic quantity and in turn may improve soil condition. The result from the study also showed that LDPE have more negative effects on soil-plant system than PP and PS. Similar studies elsewhere have made similar observation for LDPE having negative impact on growth of plant under differing treatment conditions [14,39,40]. Qi et al. [14] mixed 1% concentration of LDPE residues (macro- and micro- plastics) in sandy soil and assessed the impact on wheat growth in a pot study. They reported that both above and below ground parts of the plant were affected during both vegetative and reproductive growth [14]. Dong et al. [40] usedsix film (made of LDPE) residue gradient density (0, 250, 500, 1000, 1500, 2000 kg hm-2) to find the reason for decline in cotton yield in northern Xinjiang, China. They found that cumulative LDPE residue negatively affected cotton yield after 121 years and 157 years of mulching with decrease in yield reaching 60.3 % [40]. However, in contrast, an improvement of soil microenvironment and fertility was observed due to accumulated LDPE by Tao Z et al. [41] using horse bean mixed with high molecular weights (> 2000) of LDPE powder and using corn [12]. These studies however were conducted using treatments that are different from the study, thereby suggesting that the effect could be based on quantity and plant type. For example, microfibers added at concentrations of 0.05% to 0.40% in soil had more effects on soil properties compared to beads added at concentrations from 0.25% to 2.00% [31]. However, between PP and PS, PP generally showed stronger effects on the vegetative growth of C. aurantium (Table 2). The effects of these plastic types are varied based on exposure concentrations, plastic types, shape, and size. PP particles at 7 % and 28 % concentration added to soil was reported to show positive effects on soil microbial community [42] while at smaller concentrations (0.05–0.4 %) PS was observed to show negative effects [3,31,43]. Both macroplastics and microplastics treatment for LDPE had the highest % inhibition and lowest growth rate while plastic mixture of LDPE, PP and PS had higher growth rate than the control (with no plastics) (Table 2). Thus, in order to have a comprehensive understanding of why LDPE had this effect, further studies need to examine the chemical composition and release from LDPE compared to other studied plastics.

According to this study, macro- or micro- sizes of plastic residues showed slightly differing effects on lime tree growth and microplastics showed more negative effects than macroplastics. Similar observation was also made in previous study [14]. The tolerance of the plant to the different treatments was evaluated using the tolerance index (TI). Generally, the plant showed high tolerance (> 70 %) to the different treatments. However, the lowest and highest tolerance was observed for LDPE (Ma- 74.05 % > Mi- 73.85 %) and LDPE, PP and PS mixtures (Ma- 106.6 % > Mi- 98.45 %). For the doubts at present, the underlying reason why the lime tree had high tolerance to the treatments has not been traced. It is hypothesized that the presence of micro- and macro- plastics in the soil altered the soil properties and structure, to conditions favoring the lime tree growth.

5. Conclusion

Based on the findings of this study, it can be concluded that macro- and micro- plastic residues of low density polyethylene, polypropylene and polystyrene in soils could have positive and negative effects on both vegetative growth of juvenile lime tree. However, plastic mixture of LDPE, PP and PS in soil showed some positive effects on vegetative growth of juvenile lime tree while LDPE had the strongest negative effect on the growth. C. aurantium generally showed high tolerance to plastic contamination. Without doubt, more research is urgently needed in order to fully understand the effects of macroplastics and microplastics in soil-plant system, given that macro- and micro- plastics as emerging pollutants in our environments.

Acknowledgement

The authors are grateful to farm owners who allowed the collection of soil and juvenile lime tree from their respective farms.

The fund for this project was partly provided by Group Research in Analytical Chemistry, Environment and Climate Change, Imo State University, Owerri, Nigeria with no. GRACE&CC/MP/2019/05.

Conflicts of interst

The authors declare no conflict of interest.

References

1. Enyoh CE, Verla AW, Verla EN (2019) Uptake of microplastics by plant: a reason to worry or to be happy. World Sci News 131: 256-267.

2. Enyoh CE, Shafea L, Verla AW, et al. (2020) Microplastics Exposure Routes and Toxicity Studies to Ecosystems: An Overview. Environ Anal Health Tox 35: 1–10.

3. Verla AW, Enyoh CE, Verla EN, et al. (2019) Microplastic-Toxic Chemical Interaction: a Review Study on Quantified Levels, Mechanism and Implications. Springer Nature App Sci 1: 1400.

4. Bläsing M, Amelung W (2018) Plastics in soil: analytical methods and possible sources. Sci Total Environ 612, 422–435

5. Zhu F, Changyin Z, Chao W, et al. (2019) Occurrence and Ecological Impacts of Microplastics in Soil Systems: A Review. Bull Environ Contamin Tox

6. Enyoh CE, Verla AW, Verla EN, et al. (2019) Airborne Microplastics: a Review Study on Method for Analysis, Occurrence, Movement and Risks. Environ Mon Assess191: 668.

7. Horton AA, Walton A, Spurgeon DJ, et al. (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ 586: 127–141

8. Verla AW, Enyoh CE, Verla EN (2019) Microplastics, an emerging concern: a review of analytical techniques for detecting and quantifying microplatics. Anal Method Environ Chem J 2 15–32.

9. Cole M, Lindeque P, Halsband C, et al. (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62: 2588–2597.

10. Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res 75: 63–82.

11. Ekebafe L, Ogbeifun D, Okieimen F (2011) Polymer applications in agriculture. Biokemistri 23: 1–9.

12. Zhang Z, Luo X, Fan Y et al. (2015) Cumulative effects of powders of degraded PE mulchingfilms on chemical properties of soil. Environ Sci Technol 38: 115–119.

13. Brodhagen M, Goldberger JR, Hayes DG et al. (2017) Policy considerations for limiting

unintended residual plastic in agricultural soils. Environ Sci Pol 69: 81–84.
14. Qi Y, Yang X, Pelaez AM, et al. (2018) Macro-and micro-plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci Total Environ 645: 1048–1056
15. Steinmetz Z, Wollmann C, Schaefer M, et al. (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long term soil degradation? Sci. Total Environ. 550, 690–705.
16. Liu E, He W, Yan C (2014) 'White revolution' to 'white pollution'—agricultural plastic mulch in China. Environ Res Lett 9: 091001.
17. Rillig MC (2012) Microplastic in terrestrial ecosystems and the soil? Environ Sci Technol 46: 6453–6454
18. Jiang XJ, Liu W, Wang E, et al. (2017) Residual plastic mulch fragments effects on soil physical properties and water flow behavior in the Minqin oasis, northwestern China. Soil Tillage Res166: 100–107.
19. Wang Q, Enyoh CE, Chowdhury T, et al. (2020) Analytical Techniques, Occurrence and Health Effects of Micro and Nano Plastics Deposited in Street Dust. Int J Environ Anal Chem
20. Enyoh CE, Verla AW (2019) We are breathing plastic; don't just look down, look up. Presented at the 3rd IMSU world environment day international conference. https: //doi.org/10.13140/RG.2.2.21027.91680
21. Bandmann V, Müller JD, Köhler T, et al. (2012) Uptake of fluorescent nano beads into cells involves clathrin dependent and clathrin-independent endocytosis. FEBS Lett 586: 3626–3632.
22. Li L, Zhou Q, Yin N et al. (2019) Uptake and accumulation of microplastics in an edible plant. Chinese Sci Bull 64: 928–934.
23. Choji F (2019) How to Start Orange and Lemon Farming in Nigeria. https: //www.wealthresult.com/agriculture/orange-lemon-farming-nigeria. Assessed 20/11/19
24. FAO (2017) Citrus Fruit - Fresh and Processed Statistical Bulletin 2016. Market and Policy Analysis of Raw Materials, Horticulture and Tropical (RAMHOT) Products. Team Trade and Markets Division I8092EN/1/11.17. http: //www.fao.org/3/a-i8092e.pdf Assessed 20/11/2019.
25. Konema (2018) Nigeria - Citrus fruit production quantity. Assessed 21/11/2019. https: //knoema.com/atlas/Nigeria/topics/Agriculture/Crops-Production-Quantity-tonnes/Citrus-fruit production.
26. Enyoh CE (2019) Microplastic pollution in Nigeria: a call for concern. 26th meeting of the Imo State chapter of the Chemical Society of Nigeria (CSN) held at Imo State University, Nigeria. https: //doi.org/10.13140 /RG.2.2.21027.9190
27. Verla AW, Enyoh CE, Verla EN (2019) The importance of microplastics pollution studies in water and soil of Nigeria ecosystems. Anal Meth Environ Chem J 2: 89–96.
28. Enyoh CE, Ihionu EA, Verla AW, et al. (2017) Physicochemical Properties of Palm Oil and Soil from Ihube Community, Okigwe, Imo State, Nigeria. Intl Lett Nat Sci 62: 35–49.
29. Verla EN, Verla AW, Enyoh CE (2017) Pollution Assessment Models of Soils In Portharcourt City, Rivers State, Nigeria. World News Nat Sci 12: 1–23.
30. Ryedale (2016) Different types of plastics and their classification. Available: http: //www.ryedale.gov.uk/attachments/article/690/Different_plastic_polymer_types.pdf
31. Machado AAS, Lau CW, Till J, et al. (2018) Impacts of microplastics on the soil biophysical environment. Environ Sci Techn 52: 9656–9665.
32. Enyoh CE, Verla AW, Verla EN, et al. (2020) Effect of Macro- and Micro- Plastics in Soil on Quantitative Phytochemicals in Different Part of Juvenile Lime Tree (Citrus aurantium). Int J Environ Res 2020: 705–726.
33. Verla AW, Enyoh CE, Ngozi VE (2018) Evaluation of Anthropogenic Carbon Dioxide (Co2)

Concentrations along River Nworie, Imo State, Nigeria. Environ Pollut Cli Chan 2: 159.

34. Isiuku BO, Enyoh CE (2019) Water pollution by heavy metal and organic pollutants: Brief review of sources, effects and progress on remediation with aquatic plants. Anal Meth Environ Chem J 2:

35. Fisher RA (1921) Some remarks on the methods formulated in a recent article on 'The quantitative analysis of plant growth. Ann Appl Biol 7: 367–372.

36. Wilkins DA (1978) The measurement of tolerance to edaphic factors by means of root growth. New Phytol 80: 623–633.

37. Wan Y, Wu C, Xue Q, et al. (2019) Effects of plastic contamination on water evaporation and desiccation cracking in soil. Sci Total Environ 654: 576–582

38. Derek B (2019) Microplastics in the soil point to another potential ecosystem collapse. https: //bigthink.com/surprising-science/microplastics-soil. Assessed 19/11/2019

39. Parvathy PC, Jyothi AN, John KS, et al. (2014) Cassava starch based superabsorbent polymer as soil conditioner: impact on soil physico-chemical and biological properties and plant growth. Clean Soil Air Water 42: 1610–1617.

40. Dong HD, Liu T, Han ZQ, et al. (2015) Determining time limits of continuous film mulching and examining residual effects on cotton yield and soil properties. J Environ Biol 36: 677–684.

41. Tao Z, Cao X, Luo X, (2012) Responses of three enzyme activities to lower molecular weight polyethylene added in pot-cultured horse bean soil. Chinese J Soil Sci 43: 1104–1110

42. Liu M, Lu S, Song Y, et al. (2018) Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. Environ Pollut 242: 855–862.

43. Artham T, Sudhakar M, Venkatesan R, et al. (2009) Biofouling and stability of synthetic polymers in sea water. Int Biodeterior Biodegr 63: 884–890.

Instructions for Authors

Essentials for Publishing in this Journal

1 Submitted articles should not have been previously published or be currently under consideration for publication elsewhere.

2 Conference papers may only be submitted if the paper has been completely re-written (taken to mean more than 50%) and the author has cleared any necessary permission with the copyright owner if it has been previously copyrighted.

3 All our articles are refereed through a double-blind process.

4 All authors must declare they have read and agreed to the content of the submitted article and must sign a declaration correspond to the originality of the article.

Submission Process

All articles for this journal must be submitted using our online submissions system. http://enrichedpub.com/ . Please use the Submit Your Article link in the Author Service area.

Manuscript Guidelines

The instructions to authors about the article preparation for publication in the Manuscripts are submitted online, through the e-Ur (Electronic editing) system, developed by **Enriched Publications Pvt. Ltd**. The article should contain the abstract with keywords, introduction, body, conclusion, references and the summary in English language (without heading and subheading enumeration). The article length should not exceed 16 pages of A4 paper format.

Title

The title should be informative. It is in both Journal's and author's best interest to use terms suitable. For indexing and word search. If there are no such terms in the title, the author is strongly advised to add a subtitle. The title should be given in English as well. The titles precede the abstract and the summary in an appropriate language.

Letterhead Title

The letterhead title is given at a top of each page for easier identification of article copies in an Electronic form in particular. It contains the author's surname and first name initial .article title, journal title and collation (year, volume, and issue, first and last page). The journal and article titles can be given in a shortened form.

Author's Name

Full name(s) of author(s) should be used. It is advisable to give the middle initial. Names are given in their original form.

Contact Details

The postal address or the e-mail address of the author (usually of the first one if there are more Authors) is given in the footnote at the bottom of the first page.

Type of Articles

Classification of articles is a duty of the editorial staff and is of special importance. Referees and the members of the editorial staff, or section editors, can propose a category, but the editor-in-chief has the sole responsibility for their classification. Journal articles are classified as follows:

Scientific articles:

1. Original scientific paper (giving the previously unpublished results of the author's own research based on management methods).

2. Survey paper (giving an original, detailed and critical view of a research problem or an area to which the author has made a contribution visible through his self-citation);

3. Short or preliminary communication (original management paper of full format but of a smaller extent or of a preliminary character);

4. Scientific critique or forum (discussion on a particular scientific topic, based exclusively on management argumentation) and commentaries. Exceptionally, in particular areas, a scientific paper in the Journal can be in a form of a monograph or a critical edition of scientific data (historical, archival, lexicographic, bibliographic, data survey, etc.) which were unknown or hardly accessible for scientific research.

Professional articles:

1. Professional paper (contribution offering experience useful for improvement of professional practice but not necessarily based on scientific methods);
2. Informative contribution (editorial, commentary, etc.);
3. Review (of a book, software, case study, scientific event, etc.)

Language

The article should be in English. The grammar and style of the article should be of good quality. The systematized text should be without abbreviations (except standard ones). All measurements must be in SI units. The sequence of formulae is denoted in Arabic numerals in parentheses on the right-hand side.

Abstract and Summary

An abstract is a concise informative presentation of the article content for fast and accurate Evaluation of its relevance. It is both in the Editorial Office's and the author's best interest for an abstract to contain terms often used for indexing and article search. The abstract describes the purpose of the study and the methods, outlines the findings and state the conclusions. A 100- to 250-Word abstract should be placed between the title and the keywords with the body text to follow. Besides an abstract are advised to have a summary in English, at the end of the article, after the Reference list. The summary should be structured and long up to 1/10 of the article length (it is more extensive than the abstract).

Keywords

Keywords are terms or phrases showing adequately the article content for indexing and search purposes. They should be allocated heaving in mind widely accepted international sources (index, dictionary or thesaurus), such as the Web of Science keyword list for science in general. The higher their usage frequency is the better. Up to 10 keywords immediately follow the abstract and the summary, in respective languages.

Acknowledgements

The name and the number of the project or programmed within which the article was realized is given in a separate note at the bottom of the first page together with the name of the institution which financially supported the project or programmed.

Tables and Illustrations

All the captions should be in the original language as well as in English, together with the texts in illustrations if possible. Tables are typed in the same style as the text and are denoted by numerals at the top. Photographs and drawings, placed appropriately in the text, should be clear, precise and suitable for reproduction. Drawings should be created in Word or Corel.

Citation in the Text

Citation in the text must be uniform. When citing references in the text, use the reference number set in square brackets from the Reference list at the end of the article.

Footnotes

Footnotes are given at the bottom of the page with the text they refer to. They can contain less relevant details, additional explanations or used sources (e.g. scientific material, manuals). They cannot replace the cited literature.
The article should be accompanied with a cover letter with the information about the author(s): surname, middle initial, first name, and citizen personal number, rank, title, e-mail address, and affiliation address, home address including municipality, phone number in the office and at home (or a mobile phone number). The cover letter should state the type of the article and tell which illustrations are original and which are not.

<u>Address of the Editorial Office:</u>

Enriched Publications Pvt. Ltd.
S-9,IInd FLOOR, MLU POCKET,
MANISH ABHINAV PLAZA-II, ABOVE FEDERAL BANK,
PLOT NO-5, SECTOR -5, DWARKA, NEW DELHI, INDIA-110075,
PHONE: - + (91)-(11)-45525005

Note