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The Accumulation of Trace Metals in Rodent Tissues along the TAZAMA Pipeline and TANZAM Highway in MINAPA, Tanzania.

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Natural Resources Management
Submission date: July 2018
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Declaration

I hereby declare to the Norwegian University of Science and Technology (NTNU) that, with exception to the references from other people's work that were appropriately cited within texts, the rest part is my own work which has never been presented elsewhere or being published.

Wambura Mashauri Mtemi

Dedication

I dedicate this thesis to my beloved father (Mashauri Wambura Mtemi) and mother (Agnes Mashauri) for their supports, prays and encouragement during my entire academic journey. May almighty God bless you all.

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List of Abbreviations

Ag	Silver element
Cd	Cadmium element
Cr	Chromium element
Cu	Copper element
DW	Distilled water
E	East direction
Fe	Iron element
gm	Grams
GPS	Geographic Positioning System
Hg	Mercury element
kg	Kilograms
Km^2	Square kilometers
MINAPA	Mikumi National Park Authority
Mn	Manganese element
MWT	Microwave temperature
Ni	Nickel element
PAHs	Polycyclic Aromatic Hydrocarbons
Pb	Lead element
S	South direction
Sn	Tin element
TANAPA	Tanzania National Parks Authority
TANZAM	Tanzania-Zambia highway
TAZAMA	Tanzania-Zambia Mafuta (Mafuta being the Swahili name for oil)
TAZARA	Tanzania-Zambia Railway Authority
Vn	Vanadium element
Zn	Zinc element

Acknowledgment

I humbly express my gratitude to Almighty God for keeping me healthy, strong, and focused since the beginning of master's studies until today when I am finishing my master's studies at the Norwegian University of Science and Technology (NTNU).

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Abstract

Small mammalian' species, specifically rodents, have been used as bioindicators in estimating the level and magnitude of disturbances caused by anthropogenic activities in the environments. Herein, several toxic compounds including trace metals have been analyzed from either the whole body or specific organs including liver, kidney, spleen, heart, and muscles. The study was performed in the Mikumi National Park (MINAPA), Tanzania with the general objective of comparing the accumulation of trace metals in the rodent' tissues and soils, along a distance gradient from four study sites including the Tanzania-Zambia Mafuta (Mafuta being the Swahili name for oil) pipeline (TAZAMA), Tanzania-Zambia highway (TANZAM), the intersection of TAZAMA pipeline and TANZAM highway, as well as a control site. Small mammals were collected by traps during a 6-night period from each site using Sherman live traps, baited with a mixture of peanut butter, coconut, and sardines, while soil samples were collected at depth of 0-15 cm using an auger. Four different small mammalians' species were collected from all four study sites including *Mastomys natalensis* (81.2%), *Crocidura* spp. (16.8%), *Lemniscomys griselda maculosus* (1.3%), and *Acomys cahirinus* (1%). The concentrations (mg/kg) of trace metals such as Fe, Zn, Cr, Mn, Pb, Cu, Ni, and Cd, in soils, were analyzed using the Atomic Absorption Spectrophotometer (AAS) while in rodent tissues the Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) method was used. The results showed that; TAZAMA pipeline, TANZAM highway, and TAZAMA pipeline-TANZAM highway intersection, had the highest concentrations (mg/kg) of trace metals in soils, livers as well as kidneys than those from a control site. Meanwhile, the results from all four study sites showed that soils had the highest concentrations (mg/kg) of trace metals than rodent tissues.

However, based on the research findings, this study recommends that conservation education regarding environments should be provided to both investors and road users including drivers, tourists, and passengers who come across with the Mikumi National Park. For the already thrown waste materials in the park, the park authority should take them out and dispose of them in recommended areas as this will help to reduce the rate of pollution in the environment. Lastly but not least, more research is needed on *Crocidura* spp. that were found having abnormality behind their femurs.

Keywords: Bioindicators, Environmental quality, Infrastructures, Mikumi National Park, Small mammal's, Soil, the gradient of distance, Trace metals.

Introduction

Background Information

Small mammals are referred to as any flightless mammal with an adult body weight of less than one kilogram (Barnett & Dutton 1995). However, there are some individuals from other mammal species, apart from small mammal species, with body weights smaller than some of the larger rodents. Examples of such species include the Mustelids (such as ferrets, weasels), and some ungulate small deer (such as water chevrotain (*Hyemoschus aquaticus)*, and mouse deer (*Tragulus* spp.). In addition, the term small mammals apply only to "rodents, marsupials, insectivores and elephant shrews" (Barnett & Dutton 1995).

Small mammals specifically rodents and shrews, prefer to feed on plant materials as well as invertebrates. However, they are being predated by various organisms including snakes, raptors and of small to medium-sized carnivores (Venance 2009). In addition, the species richness and abundance of small mammals are governed by the presence of food together with the structure of vegetation in a given habitat.

The variation in communities of small mammal species is being influenced by; 1) the rate of predation, 2) the presence of non-native species within the habitat, as well as 3) the intensity and nature of disturbance in their natural habitats mostly due to the burning of vegetation, animal trampling and grazing pressure (Avenant 2011). The impacts of the above-mentioned activities on an individual small mammal species depend on general body condition (i.e. health status), plasticity, as well as anti-predator behavior.

Small mammals especially rodents, have been used as bioindicators in estimating the level and magnitude of disturbances caused by anthropogenic activities in the environments (Avenant 2011; Mukhacheva et al. 2010; Phelps & McBee 2009; Rodriguez-Estival et al. 2015). Anthropogenic activities such as use of different means of transportation, mining, petroleum exploration and exploitation, military activities, burning of fossil fuels, agriculture (using agrochemicals such as fertilizers and pesticides), have been reported to produce a substantial amount of trace metals in soils and water (Alleva et al. 2006; He et al. 2015; Onojake & Okonkwo 2011; Opaluwa et al. 2012; Pereira et al. 2006). The above-mentioned activities are usually associated with deforestation which obviously results in habitat destruction and fragmentation that are in turn confounded by global climate change. Thus, these produce structural changes in species composition and proportion of individual small mammal species, as well as changes in the abundance of small mammals (Mukhacheva et al. 2010).

In addition, the degree and magnitude of the above-named changes depend on the natural habitat and intensity of the impacts, as well as on different species' ability to adapt to the changes within its habitat. Several individuals from different species are exposed to toxic compounds, especially trace metals, through eating contaminated foods and water (Topolska et al. 2004). Small mammals, such as black rats (*Rattus rattus* L.), domestic mouse (*Mus spretus* L.), multimammate mouse *(Mastomys natalensis),* and others, have been considered as good indicators of contaminant near polluted areas because of the following reasons;

1. They can be obtained in both polluted and non-polluted habitats and are widely distributed geographically (Phelps & McBee 2009; Reynolds et al. 2006; Tovar-Sanchez et al. 2012);
2. They can coexist with people while eating their food which makes them right candidates for human exposure (Pereira et al. 2006);
3. They are part of some ecosystems found only on landforms and occupying a central part in various food chains (Pereira et al. 2006);
4. They are in close contact with land resources (soil and vegetation) during their whole life whereby they get exposed to pollutants through feeding on polluted food or soil as well as through skin engrossment (Pereira et al. 2006; Phelps & McBee 2009; Tovar-Sanchez et al. 2012);
5. They exist within a small habitat usually, less than 90 m^2 which makes them good indicators for the presence of pollutants in a specific habitat (Pereira et al. 2006);
6. They are easy to be collected and study (Avenant 2011; Damek-Poprawa & Sawicka-Kapusta 2003; Phelps & McBee 2009; Tovar-Sanchez et al. 2012);
7. They are abundant and thus, are sufficient to catch without causing negative effects on their population level (Avenant 2011; Pereira et al. 2006).

Furthermore, soils, vegetations, water, as well as air, are the main recipients of contaminants such as trace metals, of which small mammals, and wildlife in general, living in those areas are vulnerable of being exposed to, and thus dangers may occur to wildlife and human health (Rodriguez-Estival et al. 2015; Wuana & Okieimen 2011).

Moreover, small mammals specifically rodents, have been used as sentinels, in which the number of toxic compounds is being analyzed within the whole body or from targeted organs, such as the liver, kidney, heart, spleen or muscles (Schleich et al. 2010; Shore 1995). In most cases, the liver and kidney have been regarded to accumulate high levels of toxicity compared to other organs. This is because their cells can keep a high level of metal ions without harming the organism (Miska-Schramm et al. 2014).

Trace metals have an impact on various body physiological functions such as reproductive and immune functions in vertebrates, which have made them endocrine interrupters. Nonetheless, trace metals may lead to mutation and cancer to the exposed organisms, and thus, elements such as As, Pb, Hg, Cd, as well as PAHs compound, have been ranked as the "First, second, third, seventh, and ninth, respectively in the list of hazardous substances by the United States Agency for Toxic Substances" (Rodriguez-Estival et al. 2015). Therefore, it is vital to be familiar with the impacts associated with anthropogenic activities on wildlife, especially small mammal's species (Schleich et al. 2010).

Problem Statement and Justification

Despite the potential benefits of TAZAMA (Tanzania-Zambia Mafuta) pipeline and TANZAM (Tanzania-Zambia) highway to the rural and national economy in Tanzania, the presence of these infrastructures inside the Mikumi National Park (MINAPA), have been thought to cause environmental problems in the park (World-Bank 1985, 1991). The reason for this is because of deforestation within the park during the construction of roads, installations, and maintenance of oil pipelines, that have led to habitat change, loss, and fragmentation. On top of that, oil spills and leakages from damaged pipes have polluted water sources, vegetation, and soils, thus posing dangers to wildlife and humans health. Nevertheless, the above-named activities may interrupt animal movements and behaviors, and thus, negatively affect species richness and their population size (Fiori & Zalba 2003).

On the other hand, littering from the casual laborer's of the TAZAMA pipeline, increased rate of illegal hunting, wildfires within the park caused by workers during the dry season, non-native species being introduced in the park by the casual laborer's and workers of TAZAMA pipeline from the Cathodic Power Stations (CPS), and injuries of many animals due to the open trenches left after the installation and repair of pipes in the park, all of which might affect the small mammals and wildlife in general (MINAPA presentation on 30 and 31 August 2017 workshop in Mikumi). The park is being traversed by five infrastructures such as TAZAMA pipeline, TANZAM Highway, High voltage power lines, TAZARA (Tanzania-Zambia) railway, as well as Optic fiber cables, which have made the park to be of a special concern regarding the conservation of wildlife.

Studies have been done along the TANZAM highway in the MINAPA some reporting on the number of waste materials deposited in the park by road users (Nyahinga et al. 2016); and others on road kills of animals in the park due to over-speed and careless driving (Drews 1995; Newmark et al. 1996; Nyahinga et al. 2016). However, none of them have focused on the influence of these infrastructures regarding toxic compounds, such as trace metals. The highway connects Tanzania and Zambia and connects the Morogoro, Iringa, and Mbeya regions, in Tanzania, whereby it traverses the Mikumi National Park by 50 km of which several effects have been reported. Road kills being the first effect in which several animals including small mammals are dying because of collisions with vehicles (Drews 1995; Newmark et al. 1996). There is also littering of different wastes such as water bottles, cans, aluminium foils, glass bottles, pieces of tins, jars, food remains, etc. of which with time may contaminate the water sources, soils, vegetations, as well as causing the changes in wildlife feeding behavior because of the thrown wastes in the park (Nyahinga et al. 2016).

Generally, the presence of roads within the park has shown to cause various ecological impacts such as habitat loss, habitat change, and habitat fragmentation, which in turn affects species abundance and richness, as well as leading to population fragmentation (Fiori & Zalba 2003). Moreover, access roads in the park favor introduction of non-native species, high rate of predator movements, increase rate of illegal hunting and thus rising their pressure on the prey's population such as small mammals, birds, and reptiles (Fiori & Zalba 2003). Furthermore, the presence of roads in the parks may be an obstacle for some species, especially small mammals, that might fail to cross them and thus be confined in limited home range sizes. In addition, disturbances due to noise and frequency of people movement and vehicles due to access roads may facilitate changes in the behavior, species richness, and abundance (Fiori & Zalba 2003).

Nevertheless, Trombulak and Christopher (2000) reported that access roads in the park be changing animal behaviors. The changes may be through; changing of home range sizes, alteration feeding habits, alteration of movement behavior, changing of reproductive rates, as well as changing response behavior to strange events.

However, according to Maltby et al. (1995), access roads might be the sources of pollution in the environments because of several pollutants produced by vehicles including trace metals, and hydrocarbons, that come from lubricating oils, grease, and fuels, brake linings as well as vehicle tires.

Lastly but not least, studies along the TAZAMA pipeline, TAZARA railway, and High voltage power lines, are lacking, and the influence of these infrastructures on the survival of wildlife, and deposition of trace metals in the MINAPA is not documented. Therefore, this study aims at using small mammals specifically rodents, as bio-indicators for the presence of toxic compounds such as trace metals in the MINAPA due to the presence of TAZAMA pipeline and TANZAM highway.

Objectives of the Study

The general objective of the study was to investigate and understand, to which extent the accumulation of trace metals, differs between tissues of small rodents and soils along a gradient of distance from TAZAMA pipeline and TANZAM highway, and more specifically;

i. To determine the richness and abundance of small mammal's species along the gradient of distance from TAZAMA pipeline and TANZAM highway

ii. To analyze the accumulation of trace metals in soils along a gradient of distance from TAZAMA pipeline and TANZAM highway

iii. To analyze the environmental and body burden (i.e. accumulation) of trace metals in rodent tissues from both immediate, intermediate and distant populations.

iv. To compare the accumulation of trace metals between the rodent tissues and soils along the gradient of distance from TAZAMA pipeline and TANZAM highway.

Hypotheses

i. Richness and abundance of small mammal's species differ along the gradient of distance from TAZAMA pipeline and TANZAM highway

ii. The accumulation of trace metals in soils differs along the gradient of distance from TAZAMA pipeline and TANZAM highway

iii. The accumulation of trace metals in rodent tissues differs along the gradient of distance from TAZAMA pipeline and TANZAM highway

iv. The accumulation of trace metals in soils and rodent tissues differs along a gradient of distance from TAZAMA pipeline and TANZAM highway.

Materials and Methods

Description of the Study Site

The study was performed in Mikumi National Park, the fourth biggest national park in Tanzania after Serengeti National Park, Ngorongoro Conservation Area, and Manyara National Park. The park was established in 1964 and it is managed by the Tanzania National Parks Authority (TANAPA). It is in the Kilosa District, within the Morogoro Region (7°12′S 37°08′E). Its size is 3,230 km^2, bordering Selous Game Reserve to the South (Nyahinga et al. 2016; Venance 2009). It is also bordered by the Udzungwa Mountains and the Uluguru Mountains in North together with the alluvial plain of the river basin Mkata to the northwest.

It consists of over 200 plant species, more than 60 mammalian species including; hippopotamus (*Hippopotamus amphibious*), lion (*Panthera leo*), Maasai giraffe (*Giraffa camelopardalis*), African elephant (*Loxodonta africana),* yellow baboon (*Papio cynocephalus*), eland (*Taurotragus oryx*), greater kudu (*Tragelaphus strepsiceros*), leopard (*Panthera pardus*), impala (*Aepyceros melampus*), Burchell zebra (*Equus burchellii*), wildebeest *(Connochaete staurinus*), African wild hunting dog (*Lycaon pictus*), black-backed jackal (*Canis mesomelas*), and many others (Nyahinga et al. 2016).

Nevertheless, the park also consists of small mammals such as rodents (*Rodentia*), shrews (*Soricidae*), and over 400 bird species including both resident and migratory birds. Moreover, the park has a single rainy season which lasts from November to May, and the dry season which lasts from June to October as well as the temperature of between 16 ^{o}C-28 ^{o}C (Nyahinga et al. 2016).

Within the park, there are several public infrastructures such as TAZAMA pipeline, TANZAM highway, High voltage power lines, TAZARA railway, and Optic fiber cables, which are traversing the park (Figure 1). Each of the above-mentioned infrastructures has its own effects on the flora, fauna and an ecosystem in general and thus, makes the park to be of a special concern.

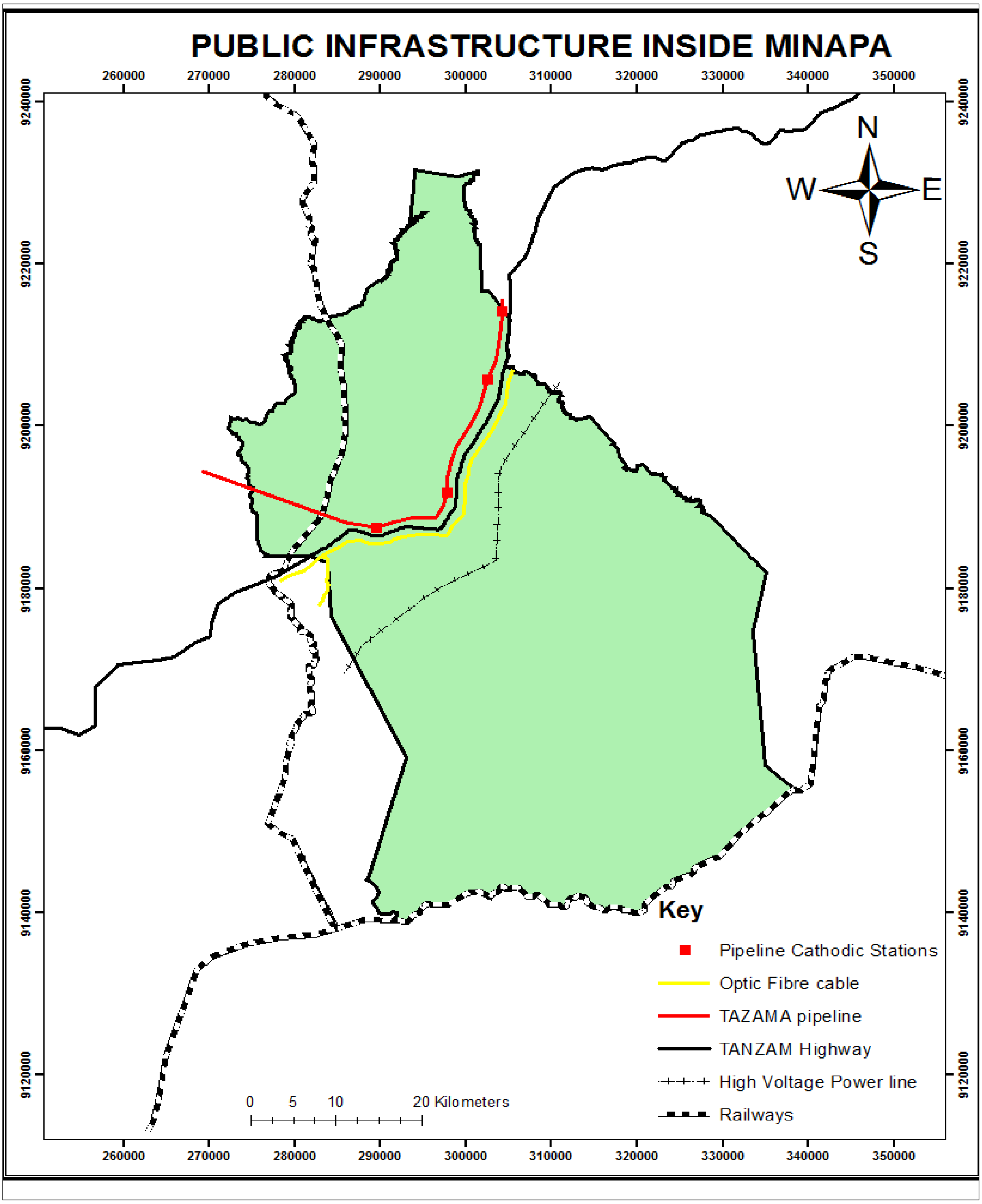


Figure 1: *Map of Mikumi National Park showing Public infrastructures traversing the Park*

Source: MINAPA

Reconnaissance Survey

A field survey was done during January 2018, to determine areas of which soil samples were taken and where traps were set. The study involved the location of TAZAMA (Tanzania-Zambia Mafuta (Mafuta being the Swahili name for oil)) pipeline, TANZAM (Tanzania-Zambia) highway, TAZAMA pipeline-TANZAM highway intersection, and finally a control site. The purpose of identifying the intersection site of TAZAMA pipeline and TANZAM highway was to study and gather knowledge about the combined effects of these two infrastructures on the species richness, abundance, as well as their influence on the deposition of toxic compounds such as trace metals. The first three study sites were located 3 km apart from each other and 6 km away from the control site. Finally, the locations for both sites were marked in GPS and recorded to ensure easy accessibility of the sites during the study. Furthermore, traps were set in similar habitat structure (wooded grassland) in all four study sites.

Animal Sampling

Small mammals were trapped for 6- trap-nights at each sampling site such as the TANZAM highway, TAZAMA pipeline, TAZAMA pipeline-TANZAM highway intersection, and from the control site. The term ‘trap-night’ indicates one trap having a period of 24-hours (Avenant 2011). Each site had three transects of nine plots arranged perpendicular to the site. The transects were spaced at 1 km interval from each other, having three plots of 100 x 100 m size each. The first plot (immediate area) was within 100 m, the second plot (intermediate area) was at 500 m and the third plot (the distant area) was at 1000 m, both arranged perpendicular from the sampling sites (Figure 2).

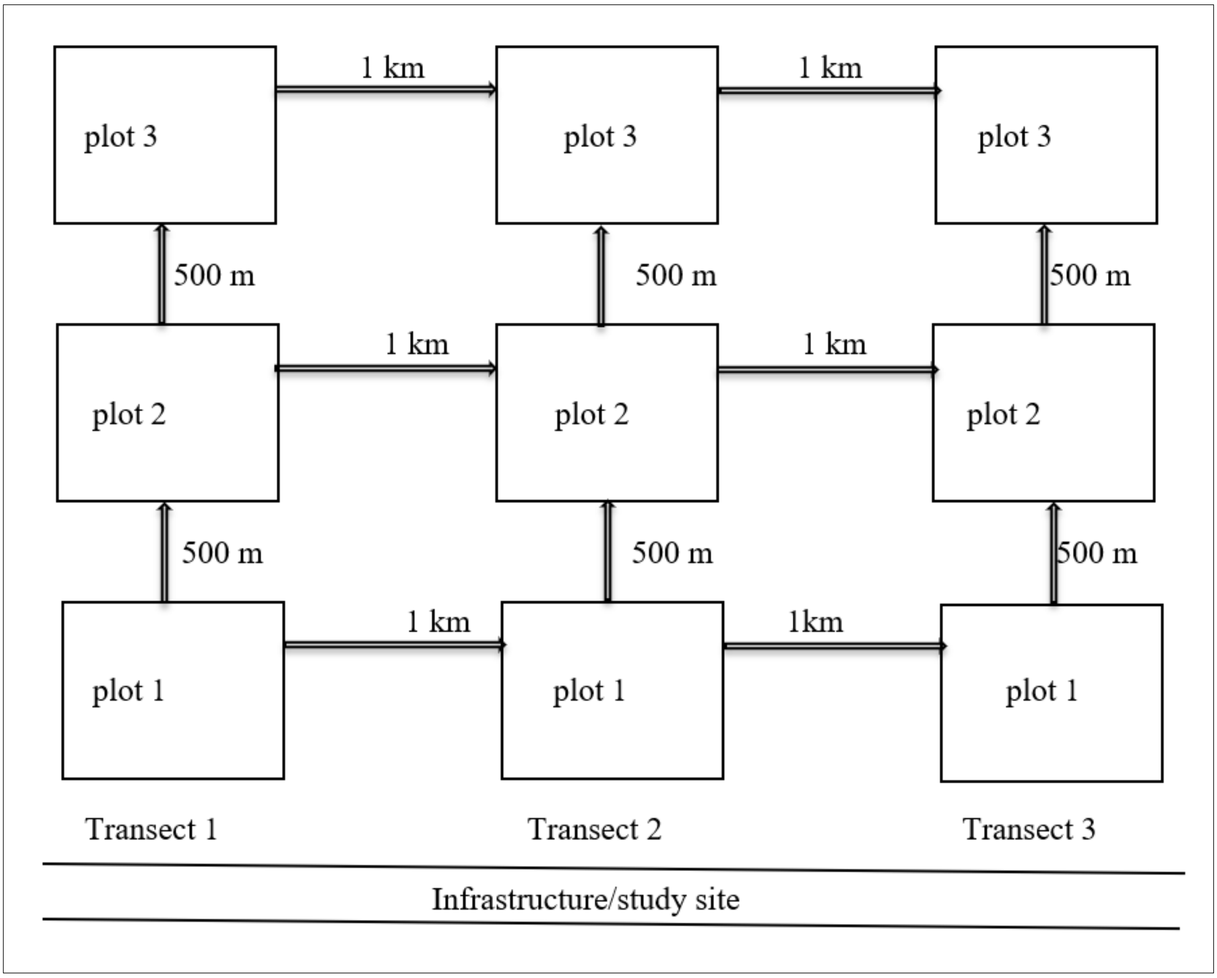


Figure 2: *Arrangement of transects and plots from all four study sites in which small mammal's individuals were trapped in Mikumi National Park, Tanzania.*

Each plot had two trap lines of 10 Sherman live traps each (23 cm x 9.5 cm x 8 cm) size, spaced at 10 m interval and baited by a mixture of peanut butter, coconut, and sardine. A mixture of several baits was used because it is thought to be effective bait in catching diverse small terrestrial mammal's species due to attraction (Plumptre et al. 2015). Trap lines were set on the ground 50 m apart from each other.

The traps were set in the late evening (1700 hours) and checked the next morning at (0730 hours) once a day to avoid temperature-related trap mortalities. In addition, Barnett and Dutton (1995) reported that most of the small mammal's species are active at night (nocturnal) or active during sunset and sunrise (crepuscular), and that is why the checking of traps was done once a day. Furthermore, the temperature was recorded every day during trap checking and the average temperature from all sites for 24 trap nights was 22.8 ^{o}C. For each trapped individual, parameters such as the species, sex, age, and general body condition, were recorded. Species were identified based on their morphological characteristics using identification keys provided by Kingdon (1997). Meanwhile, the general body condition of individuals was identified as normal or abnormal depending on the appearance of their morphological characteristics.

All adult individuals of the most common species (*Mastomys natalensis*) from both sites, were taken and put into cages before being sent to the laboratory for dissection. Other captured species such as *Crocidura* spp., *Lemniscomys griselda maculosus*, and *Acomys cahirinus* were toe clipped to avoid multiple identifications once recaptured, then being released at the site of capture. The caught non-targeted species such as millipedes were recorded then being released. Within the cages, cotton beddings, food, and water ad libitum were put to ensure a good environment for the individuals inside as described by Nunes et al. (2001).

Small Mammals Aging

Aging of the small mammals was done by considering their body sizes, fur colors, and textures. The animals were divided into three age categories such as young, juvenile and adult. Smaller individuals having greyer, softer and down like baby fur were determined to be young's; while those individuals which were no longer resembled the young', but which were too small to be adults were determined as juveniles. Those individuals which were at reproductively active and/ or were the same size as individuals which were reproducing were determined as adults (Barnett & Dutton 1995).

Animal Sexing

Sexing of the animal was done by assessing the presence of testes, the status of nipples, number of urogenital openings, and the distance between the reproductive organs and excretory organs (Barnett & Dutton 1995).

Tissue Collection and Preservation

After being caught, the most common species (*Mastomys natalensis*) were taken to the laboratory at Sokoine University of Agriculture (SUA) at the department of animal science of which dissection was carried out. Prior to tissues collection, every individual was anesthetized by being put into a plastic jar for 60 seconds which inside had a cotton wool soaked with diethyl ether. Thereafter, a blood sample was taken from blood vessels behind the eye using heparinized capillaries, then after being anesthetized individuals were ready for dissection (Barnett &

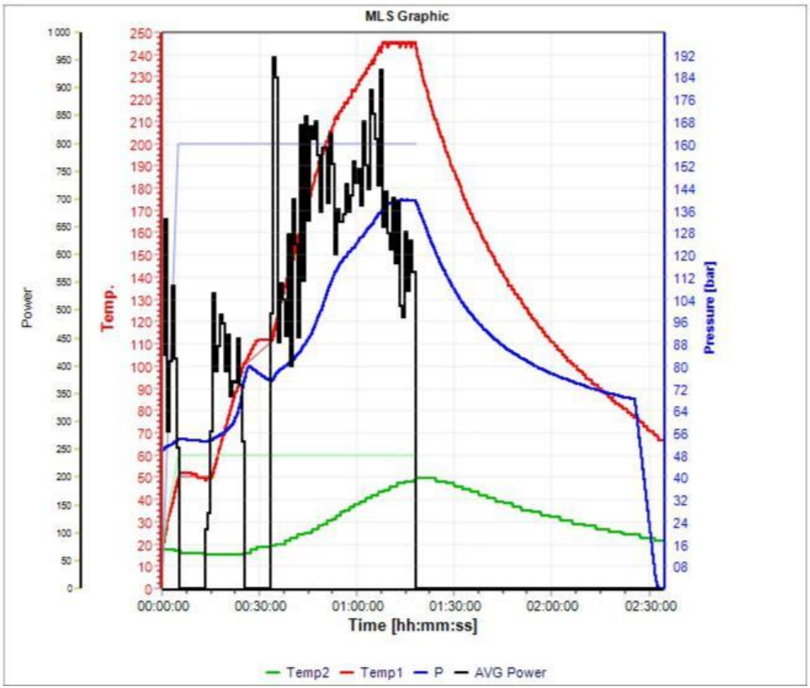
Dutton 1995). The dissection was aseptically done by removing the kidney, liver, heart, spleen, and femur tissues. Individual tissues were blotted with filter papers, weighed and stored at -80 ^{o}C prior to metals analysis.

Metals Analysis in Tissues

Tissue samples such as livers and kidneys were freeze-dried for 24 hours in the Vacuum Freeze Dryer until constant weight was attained, then the dry weight was recorded. Meanwhile, other tissues such as the spleen, heart, femur, and blood samples were stored at -80 ^{o}C for future use. Thereafter, dried tissue samples were put into 18 ml Teflon bottles followed by addition of 6 ml 50% v/v HNO_3 then being digested in the Milestone UltraClave machine according to the shown temperature profile (Figure 3 and Table 1). The digested sample solutions were diluted to between 57 to 63 gm with distilled water and finally being transferred into a 15 ml vial. Then the concentrations of trace metals were determined using the Inductive Coupled Plasma-Mass Spectrometry (ICP-MS).

Vacuum Freeze Dryer and Milestone UltraClave machine were used in freeze-drying and digestion of tissue samples because they prevent the loss of analytes specifically the ones that are volatile. All the procedures for tissues preparation and the determination of trace metals were done at the Norwegian University of Science and Technology (NTNU) at the department of a chemistry laboratory. The procedures for the analysis of trace metals in tissues were checked using the bovine liver -1577b as a standard reference material. Meanwhile, sample blanks were prepared in the same way as tissue samples to determine the detection limits (Torres et al. 2006).

MLS Microwave Report **System test: MWT AG**



MWT AG

Parameter

Method filename

Stand-profile-245ºC-10-min-oper

Create run : Administrator

Microwave

Power : pulse

Load pressure :50.0 bar

Release temp. :78.0 °C

Release pressure:10.0bar/min

Cooling : ON

Auto open : OFF

Cooling on

Temperature :40.0 °C

Ventilation time: 01:16:21

Rotor :40 positions

Vessel-Type :18ml teflon and Quarts

Ground load:

300 ml DW + 2 ml H_2SO_4+30 ml H_2O_2

Figure 3: *Milestone UltraClave temperature profile during the digestion of tissue samples.*

Table 1: *Milestone Microwave temperature (MWT) profile during the digestion of tissue samples.*

Step	Time (hh:mm:ss)	Temperature 1 (ºC)	Temperature 2 (ºC)	Pressure (bar)	Energy (Watt)
1	00:05:00	50	60	160	1000
2	00:10:00	50	60	160	1000
3	00:10:00	100	60	160	1000
4	00:08:00	110	60	160	1000
5	00:15:00	190	60	160	1000
6	00:05:00	210	60	160	1000
7	00:15:00	245	60	160	1000
8	00:10:00	245	60	160	1000

Soil Sampling and Preparation

Soil samples were collected from four different sites such as; the TAZAMA pipeline, TANZAM highway, TAZAMA-TANZAM highway intersection, and from the control at the depth of 0-15 cm using an auger. Soils were taken from each plot that had traps for catching small mammals. An equal volume of 15 sub-samples was collected from each plot and being mixed together to make one composite (representative) soil sample. A composite sample was spread into aluminium foil and mixed thoroughly to break the large masses into smaller particles and other materials which were not part of the soil such as stones, roots, and gravels were removed. Quartering of the sample was done, and soils were discarded diagonally on the aluminium foil to ensure thorough mixing of the soil sample; this procedure was repeated several times until 1kg of the composite soil sample was obtained. Finally, the sample was put into a clean sampling bag, labeled and stored prior to the preparation for the laboratory analysis (Opaluwa et al. 2012). The same procedures were repeated until all study sites were finished. Thereafter, both composite soil samples were air dried, ground and sieved with a 2 mm sieve made of stainless steel, then labeled and stored again prior to metals analysis. All soil preparation procedures were done at Sokoine University of Agriculture (SUA) in the department of soil science laboratory, while the analysis of trace metals was done at the University of Dar Es Salaam, Tanzania.

Metals Analysis in Soils

5 gm of soil sample was put into a 25 ml conical flask followed by the addition of 5 ml of concentrated H_2SO_4, 25 ml of concentrated HNO_3, as well as 5 ml of concentrated HCl. Then, the mixture in the conical flask was heated at 200 ^oC for 1 hour in a fuming cupboard and allowed to cool until room temperature was attained. 20 ml of distilled water was added to the mixture after cooling, then being filtered to complete the digestion. Thereafter, the filtered mixture was put into a 50 ml volumetric flask and being filled up to the mark, then allowed to stand for at least 15 hours. Finally, the filtrate was analyzed for such as Cadmium (Cd), Nickel (Ni), Copper (Cu), Lead (Pb), Zinc (Zn), Iron (Fe), Manganese (Mn), and Chromium (Cr) using the Atomic Absorption Spectrophotometer (AAS) (Adu et al. 2012).

Statistical Analysis of Species Richness, Abundance, and Trace Metals

The percentage of species abundance along a gradient of distance and among all the four study sites was calculated as the ratio between the number of individuals per species (n) in each site and the total number of individuals of all species (N) recorded in the study site. Nevertheless, species richness was determined by counting the number of species collected along a gradient of distance (i.e. between plots) and among all four study sites. Differences in species richness and abundance between plots and among all four study sites were analyzed using contingency tables and a Pearson's Chi-squared test (χ^2) was used to indicate statistical significance (Mukhacheva et al. 2010). On the other hand, one-way analysis of variance (ANOVA) with the help of R software version 3.3.3 was used to compare the mean concentrations (mg/kg) of trace metals and the significant differences were determined by the post hoc Tukey HSD test. To compare the mean concentrations of trace metals between soils and tissues, the Pearson correlation coefficient (r) was used. For all results, $p \leq 0.05$ was used to indicate statistical significance.

Results

Abundance and Richness of Small Mammal's Species along a Gradient of Distance and among all four Study Sites.

A total of 149 individuals (148 adults, 1 juvenile), 76 males and 73 females, belonging to four species were collected from the four different sites such as TAZAMA pipeline, TANZAM highway, TAZAMA pipeline-TANZAM highway intersection, as well as the control site (Table 2). The most abundant species from both sites was *Mastomys natalensis* (81.2%), followed by *Crocidura* spp (16.8%). The rare species were *Lemniscomys griselda maculosus* (1.3%), and *Acomys cahirinus* (1%).

Table 2: *Species abundance of rodents and shrews captured in Mikumi National Park, from four sites such as; TAZAMA pipeline, TANZAM highway, TAZAMA pipeline-TANZAM highway intersection, and Control.*

	Abundance in each study site				Total	% = (N/149) *100
Species name	TAZAMA pipeline	TANZAM highway	TAZAMA-TANZAM highway intersection	Control		
Mastomys natalensis	23	37	31	30	121	81.2
Crocidura spp.	3	16	3	3	25	16.8
Lemniscomys griselda maculosus	1	1	0	0	2	1.3
Acomys cahirinus	1	0	0	0	1	1

No significant difference in the abundance of small mammal species was found along a gradient of distance from TAZAMA pipeline ($\chi^2 = 7.3$, df = 6, p = 0.296; Table 3). Likewise, the other three sites such as TANZAM highway, TAZAMA pipeline-TANZAM highway intersection, and control, did not differ statistically significant in the abundance of small mammal's species along a gradient of distance ($\chi^2 = 7.2$, df = 4, p = 0.127; $\chi^2 = 3.3$, df = 2, p = 0.192; $\chi^2 = 0.7$, df = 2, p = 0.693; Table 3) respectively. Furthermore, no statistically significant difference was found in the abundance of small mammal species among the four study sites ($\chi^2 = 12$, df = 9, p = 0.213; Table 3).

Nonetheless, no statistically significant difference was found in species richness along a gradient of distance from TAZAMA pipeline ($\chi^2 = 4.9$, df = 6, p = 0.562; Table 3). Likewise, the TANZAM highway, did not differ statistically significantly in species richness between plots ($\chi^2 = 1.6$, df = 4, p = 0.817; Table 3). Likewise, TAZAMA pipeline-TANZAM highway intersection, did not differ in species richness ($\chi^2 = 0.8$, df = 2, p = 0.659). Finally, the same trend was found in the control site ($\chi^2 = 0.8$, df = 2, p = 0.659; Table 3).

Generally, among all four study sites, TAZAMA pipeline was rich in species, although not statistically significant so ($\chi^2 = 8$, df = 6, p = 0.238), with other sites (TANZAM highway, TAZAMA pipeline-TANZAM highway intersection, and Control) (Table 3).

Trace Metal Concentrations (mg/kg) in Soils

The mean concentrations of trace metals (mg/kg) in soils obtained along a gradient of distance from all four study sites are shown in Table 4. Along a gradient of distance from TAZAMA pipeline, the ranking of mean concentrations of trace elements (mg/kg) was Fe>Zn>Cr>Mn>Pb>Cu>Cd>Ni. Only the mean concentrations Cu were found statistically significant (ANOVA; F = 17.2, df = 2 and 6, p = 0.003; Table 4). The post-hoc analysis showed that the means of Cu differed statistically between intermediate and immediate areas, and between distant and immediate areas (Tukey HSD test, p = 0.014; p = 0.003) respectively while the means difference of Cu between the distant and intermediate area was not statistically significant **(**Tukey HSD test, p = 0.346; Appendix 1).

Nonetheless, along a gradient of distance from TANZAM highway, the sequence of occurrence for the mean concentrations of trace metals (mg/kg) in soil was Fe>Cr>Mn>Pb>Cu>Zn>Ni>Cd. Only mean concentrations of Fe and Mn were found significant along a gradient of distance (ANOVA; F = 83.7, df = 2 and 6, p < 0.001; ANOVA;

F = 50.1, df = 2 and 6, $p < 0.001$; Table 4) respectively. The post-hoc analysis showed that means of Fe differed statistically between the intermediate and immediate areas, the distant and immediate areas as well as between the distant and intermediate areas (Tukey HSD test, p = 0.002; $p < 0.001$; p = 0.001; Appendix 1) respectively. Meanwhile, the post-hoc analysis showed the means of Mn to differ statistically between the intermediate and immediate areas as well as between the distant and immediate areas (Tukey HSD test, $p < 0.001$ each**)** while the means group between distant and intermediate areas did not differ statistically (Tukey HSD test, p = 0.944; Appendix 1).

Table 3: *Species abundance and richness along a gradient of distance and among the four study sites such as TAZAMA pipeline, TANZAM highway, TAZAMA pipeline-TANZAM highway intersection, and Control.*

Study site	Species richness				Species abundance (n)			
	Total	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Total (N)
TAZAMA pipeline	4	1	2	3	8	11	9	28
TANZAM highway	3	2	2	3	21	19	14	54
TAZAMA pipeline-TANZAM highway intersection	2	2	1	1	6	17	11	34
Control	2	2	2	1	15	15	3	33

Table 4: *Trace metals mean (SD) concentrations (in mg/kg) and their maximum permissible limits (mg/kg), in soils along a gradient of distance from all four study sites.*

Element	TAZAMA pipeline	TANZAM highway	TAZAMA pipeline-TANZAM highway intersection	Control	Maximum permissible limit (WHO 1993, 1996; WHO/FAO 2001)
Cu	42.43 (8.00) **	43.70 (12.89)	37.21 (12.02)	32.34 (17.34)	100
Zn	338.20 (891.63)	37.04 (49.16)	190.40 (442.51)	15.17 (7.12)	300
Ni	0.00 (0.00)	2.70 (5.53)	0.00 (0.00)	2.93 (4.60)	50
Fe	6601.20 (4706.09)	5254.10 (3744.88) ***	6289.70 (6586.42)	3229.40 (3991.07)	21,000
Mn	186.91 (65.57)	109.99 (120.65) ***	279.00 (89.77) *	135.16 (42.7)	80
Cd	0.04 (0.11)	0.04 (0.11)	0.00 (0.00)	0.34 (0.57)	3
Cr	204.40 (87.77)	136.17 (45.74)	178.93 (69.16)	137.92 (39.90)	400
Pb	79.69 (61.64)	87.84 (49.20)	99.49 (52.97)	60.70 (34.01)	50

**Significant ($p \leq 0.05$); **Significant ($p \leq 0.01$); and ***Significant ($p \leq 0.001$).*

Moreover, along a gradient of distance from TAZAMA pipeline-TANZAM highway intersection, the obtained mean concentrations of trace metals in soil followed this order; Fe>Mn>Zn>Cr>Pb>Cu>Cd=Ni. Only the mean concentrations of Mn were statistically significant (ANOVA; F = 5.6, df = 2 and 6, p = 0.042; Table 4). The post-hoc analysis showed the means of Mn differs statistically only between the distant and immediate areas (Tukey HSD test, p = 0.04) while between the intermediate and immediate areas as well as between the distant and intermediate areas no statistically significant was found (Tukey HSD test, p = 0.287; p = 0.292; Appendix 1) respectively.

Furthermore, along a gradient of distance from control site, the sequence of occurrence for the mean concentrations of trace metals in soil was Fe>Cr>Mn>Pb>Cu>Zn>Ni>Cd, and no statistically significant difference was found among the mean concentrations of these metals (Table 4).

Trace Metal Concentrations (mg/kg) in the Liver of *Mastomys natalensis*

Along a gradient of distance from TAZAMA pipeline, the ranking of mean concentrations of trace metals (mg/kg) in livers was Fe>Zn>Cu>Mn>Cr>Pb>Ni=Cd. Only the mean concentration of Mn was found statistically significant (ANOVA; F = 5.0, df = 2 and 15, p = 0.021; Table 5). The post-hoc analysis showed that the means of Mn differed statistically only between the distant and immediate areas (Tukey HSD test, p = 0.02) while the means difference of Mn between the intermediate and immediate area and between the distant and intermediate area were not statistically significant **(**Tukey HSD test, p = 0.763; p = 0.09; Appendix 2) respectively.

Nonetheless, along a gradient of distance from TANZAM highway, the sequence of occurrence for the mean concentrations of trace metals (mg/kg) in livers was Fe>Zn>Cu>Mn>Pb>Cr>Ni=Cd. Only mean concentration of Ni was found significant along a gradient of distance (ANOVA; F = 6.1, df = 2 and 17, p = 0.012; Table 5). The post-hoc analysis showed that means of Ni differed statistically between the distant and immediate areas as well as between the distant and intermediate areas (Tukey HSD test, p = 0.01; p = 0.05) respectively, while no significance in means Ni difference was observed between the intermediate and immediate area (Tukey HSD test, p = 0.771; Appendix 2).

Table 5: *Trace metals mean (SD) concentrations (in mg/kg) in the liver of Mastomys natalensis with their reference material (bovine liver-1577b) along a gradient of distance from all four study sites.*

Element	TAZAMA pipeline	TANZAM highway	TAZAMA pipeline-TANZAM highway intersection	Control	Bovine liver (1577b) as a reference material (Provided from NTNU)
Cu	9.12 (3.44)	9.74 (2.27)	9.89 (2.87)	4.88 (1.37)	164.18 (3.02)
Zn	69.50 (17.73)	72.81 (20.41)	86.34 (23.14)	38.93 (10.65)	133.59 (0.82)
Ni	0.02 (0.03)	0.02 (0.04) *	0.01 (0.02) *	0.06 (0.11)	0.32 (0.05)
Fe	525.90 (247.53)	394.00 (301.77)	597.90 (505.97)	309.64 (151.11)	188.66 (1.48)
Mn	2.74 (0.84) *	5.56 (1.56)	6.23 (2.99)	4.79 (1.35)	10.01 (0.12)
Cd	0.02 (0.02)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.46 (0.01)
Cr	0.12 (0.19)	0.12 (0.13)	0.32 (0.74)	0.04 (0.03)	0.34 (0.07)
Pb	0.05 (0.02)	0.35 (1.15)	0.13 (0.17)	0.03 (0.01)	0.11 (0.00)

**Significant ($p \leq 0.05$); **Significant ($p \leq 0.01$); and ***Significant ($p \leq 0.001$).*

Moreover, along a gradient of distance from TAZAMA pipeline-TANZAM highway intersection, the obtained mean concentrations of trace metals in livers followed this order; Fe>Zn>Cu>Mn>Cr>Pb>Cd>Ni. Only the mean concentration of Ni was found to be statistically significant (ANOVA; $F = 5.4$, df = 2 and 11, $p = 0.023$; Table 5). The post-hoc analysis showed that the means difference of Ni did differ statistically only between the distant and immediate area (Tukey HSD test, $p = 0.022$), while between the distant and intermediate area and between intermediate and immediate area no significant difference was found between the means differences of Ni (Tukey HSD test, p = 0.094; p = 0.681; Appendix 2) respectively.

Furthermore, along a gradient of distance from the control site, the sequence of occurrence for the mean concentrations of trace metals was Fe>Zn>Cu>Mn>Ni>Cr>Pb>Cd, and no statistically significant difference was found among the mean concentrations of these metals (Table 5).

Trace Metal Concentrations (mg/kg) in Kidneys of *Mastomys natalensis*

Along a gradient of distance from TAZAMA pipeline, the ranking of mean concentrations of trace metals (mg/kg) in kidneys was Fe>Zn>Cu>Mn>Pb>Cr>Ni>Cd. Only the mean concentration of Cr was found statistically significant (ANOVA; F = 5.8, df = 2 and 15, p = 0.014; Table 6). The post-hoc analysis showed that the means of Cr differed statistically only between the distant and intermediate areas (Tukey HSD test, p = 0.011) while the means difference of Cr between the intermediate and immediate area and between the distant and immediate area were not statistically significant (Tukey HSD test, p = 0.426; p = 0.102; Appendix 2) respectively.

Nonetheless, along a gradient of distance from TANZAM highway, the sequence of occurrence for the mean concentrations of trace metals (mg/kg) in kidneys was Fe>Zn>Cu>Mn>Pb>Cr>Cd>Ni. Only mean concentration of Cu was found significant along a gradient of distance (ANOVA; F = 5.1, df = 2 and 17, p = 0.019; Table 6). The post-hoc analysis showed that means of Cu differed statistically between the intermediate and immediate areas (Tukey HSD test, p = 0.015), while between the distant and immediate area and between the distant and intermediate area there were no significant differences in the mean Cu differences (Tukey HSD test, p = 0.183; p = 0.404; Appendix 2) respectively.

Table 6: *Trace metals mean (SD) concentrations (in mg/kg) in kidneys of Mastomys natalensis with their reference material (bovine liver-1577b) along a gradient of distance from all four study sites.*

Element	TAZAMA pipeline	TANZAM highway	TAZAMA pipeline-TANZAM highway intersection	Control	Bovine liver (1577b) as a standard reference material (Provided from NTNU)
Cu	10.77 (1.22)	11.32 (1.07) *	11.79 (1.60)	10.34 (1.09)	164.18 (3.02)
Zn	67.11 (7.12)	69.52 (5.50)	70.26 (7.54)	62.49 (4.53)	133.59 (0.82)
Ni	0.16 (0.66)	0.02 (0.01)	0.02 (0.01)	0.03 (0.05)	0.32 (0.05)
Fe	389.40 (207.42)	320.51 (71.41)	321.68 (68.15)	305.90 (73.19)	188.66 (1.48)
Mn	4.29 (0.79)	4.20 (0.58)	4.47 (0.74)	4.01 (0.69)	10.01 (0.12)
Cd	0.08 (0.03)	0.10 (0.09)	0.06 (0.01)	0.03 (0.01)	0.46 (0.01)
Cr	0.16 (0.09) *	0.16 (0.06)	0.16 (0.06)	0.16 (0.06)	0.34 (0.07)
Pb	0.27 (0.15)	1.68 (4.74)	0.81 (1.26)	0.21 (0.09)	0.11 (0.00)

**Significant ($p \leq 0.05$); **Significant ($p \leq 0.01$); and ***Significant ($p \leq 0.001$).*

Moreover, along a gradient of distance from TAZAMA pipeline-TANZAM highway intersection, the obtained mean concentrations of trace metals in kidneys followed this order; Fe>Zn>Cu>Mn>Pb>Cr>Cd>Ni. None of the mean concentrations of the trace elements was found to be statistically significant.

Furthermore, along a gradient of distance from the control site, the sequence of occurrence for the mean concentrations of trace metals in kidneys was Fe>Zn>Cu>Mn>Pb>Cr>Cd=Ni, and no statistically significant difference was found among the mean concentrations of these metals (Table 6).

Comparison of the Mean Concentrations (mg/kg) of Trace Metals in Soils and *Mastomys natalensis* Tissues

Table 7: *Pearson correlation coefficients (r) for trace metals in soils and Mastomys natalensis tissues such as livers and kidneys along a gradient of distance from all study sites.*

Study sites		Cu	Zn	Ni	Cd	Cr	Pb	Mn	Fe
1; TAZAMA pipeline	Soil-liver	-0.41	-0.98	0.00	0.00	-0.21	-0.55	-0.77	-0.99
	Soil-kidney	-0.05	0.93	0.00	0.50	0.81	-0.57	-0.43	-0.15
2; TANZAM highway	Soil-liver	0.37	-0.99	-0.84	0.50	0.17	0.34	0.55	0.83
	Soil-kidney	-0.85	0.40	0.24	1.00***	0.68	0.31	-0.72	-0.84
3; TAZAMA pipeline-TANZAM highway intersection	Soil-liver	-0.82	0.87	0.00	0.00	-0.99	0.94	0.96	0.97
	Soil-kidney	0.97	0.78	0.00	0.00	0.71	0.61	0.95	-0.90
4; Control	Soil-liver	-0.70	0.60	0.51	0.69	-0.12	-0.01	-0.71	-0.99
	Soil-kidney	0.99	-0.79	0.19	0.69	1.00*	0.51	0.51	1.00*

**Significant ($p \leq 0.05$); **Significant ($p \leq 0.01$); and ***Significant ($p \leq 0.001$).*

Along a gradient of distance from TAZAMA pipeline, the ranking of Pearson correlation coefficient in the mean concentrations of trace metals (mg/kg) between soils and livers was Fe>Zn>Mn>Pb>Cu>Cr>Cd=Ni, while between soils and kidneys the correlation was Zn>Cr>Pb>Cd>Mn>Fe>Cu>Ni. None of the correlations between soils and livers and between soils and kidneys were statistically significant (Table 7).

Nonetheless, along a gradient of distance from TANZAM highway, the sequence of Pearson correlation coefficient in the mean concentrations of trace metals (mg/kg) between soils and livers was Zn>Ni>Fe>Mn>Cd>Cu>Pb>Cr, while between soils and kidneys the correlation was Cd>Cu>Fe>Mn>Cr>Zn>Pb>Ni. None of the correlations between soils and livers were statistically significant while only the correlation of Cd was found statistically significant between soils and kidneys ($t = \text{Inf}$, $df = 1$, $p < 0.001$; Table 7).

Moreover, along a gradient of distance from TAZAMA pipeline-TANZAM highway intersection, the Pearson correlation coefficient in the mean concentrations of trace metals (mg/kg) between soils and livers followed this order; Cr>Fe>Mn>Pb>Zn>Cu>Cd=Ni, while between soils and kidneys the correlation was Cu>Mn>Fe>Zn>Cr>Pb>Cd=Ni. None of the correlations between soils and livers and between soils and kidneys were statistically significant (Table 7).

Furthermore, along a gradient of distance from the control site, the sequence of the Pearson correlation coefficient in the mean concentrations of trace metals (mg/kg) between soils and livers was Fe>Mn>Cu>Cd>Zn>Ni>Cr>Pb, while between soils and kidneys the correlation followed this order; Fe=Cr>Cu>Zn>Cd>Pb=Mn>Ni. None of the correlations between soils and livers were statistically significant while only the correlations of Fe and Cr were found statistically significant between soils and kidneys ($t = 18.5$, $df = 1$, $p = 0.034$; $t = 35.7$, $df = 1$, $p = 0.018$; Table 7) respectively.

Discussion

Abundance and Richness of Small Mammal's Species

Four different small mammal's species such as *Mastomys natalensis, Crocidura* spp., *Acomis cahirinus,* and *Lemniscomys griselda maculosus* were found along a gradient of distance and among all four study sites. Among all four-caught species, only *Lemniscomys griselda maculosus* is a diurnal species (i.e. active during the day) and sometimes a crepuscular species (i.e. active at sun rise and sun set) (Cassola 2016), while the remaining species such as *Mastomys natalensis*, *Crocidura* spp. and *Acomys cahirinus*, are all nocturnal species (i.e. active at night) (Choate 1972).

Generally, along a gradient of distance from all four study sites, there was no satisfactory number of individuals and species. This could be confounded by several reasons including; catching of non-targeted species which entered the traps either accidentally or because they were attracted by the smell of baits. For example, in the field, the most non-targeted species that was caught in the traps were millipedes (about 11 individuals). Nevertheless, 9 Sherman traps were destroyed by elephants as well as monkeys and baboons, especially when they found closed traps and failed to open them.

Furthermore, according to Barnett and Dutton (1995), the abundance and richness of small mammal's species may be reduced because individuals have changed their natural feeding behavior due to anthropogenic disturbances such as littering, and thus they are probably no longer attracted by the smell of baits used. In addition, possibly alteration of home range area of small mammal's species due to anthropogenic disturbances is another reason for the low species richness and abundance, as individuals will be either widely scattered or confined in a specific habitat (Barnett & Dutton 1995; Trombulak & Christopher 2000).

Moreover, fieldwork was done during the rainy season which might have confounded the species abundance and richness because of unsuitable weather condition for the targeted species. This point is being supported and evidenced by Barnett and Dutton (1995) that mostly small mammals species, specifically rodents, and shrews, are abundant during the dry season as this is a favorable weather condition for them and it is a time of which they are most active. Nevertheless, number of days spent during trapping could be another reason for the low species richness and abundance as each site was trapped for only 6-nights. This is also being supported by Barnett and Dutton (1995) that the longer the site is trapped the higher the species richness and abundance will be.

From the study, the results show that the multimammate mouse *(Mastomys natalensis)* was the most abundant in all study plots, and more abundant than any other species (such as *Crocidura* spp., *Lemniscomys griselda maculosus, and Acomys cahirinus*). The reason is probably that of its higher reproductive rate (Avenant 2011). On top of that, *Mastomys* spp. have been reported by Avenant (2011) to be the first small mammal to colonize and recover quickly in case of a disturbance in their natural habitats possibly due to the burning of vegetation, overgrazing, and during prolonged drought or rainy period. Nevertheless, the species has been shown not to escape its natural habitat even when there is burning or soon after burning, and thus, alive individuals after burning reproduce rapidly which recovers the population size (Avenant 2011).

On the other hand, some individuals of *Crocidura* spp. were observed to have an abnormality (the reddish-brown swellings) at the back of the femurs that possibly might have influenced to their low abundance. Out of 25 (18 females, 7 males) collected individuals, 10 of them had this reddish-brown swelling behind the femurs and were females. Until now it is not known if such abnormality is due to a disease or not, and the causes of such abnormality are not known either. Furthermore, it is not clear if this abnormality is the cause of the low abundance of the *Crocidura* spp. individuals in the Mikumi National Park. Therefore, we suggest more research to be done regarding such abnormality in *Crocidura* spp. individuals.

Figure 4: *Crocidura* spp. *with abnormality behind the femur in the Mikumi National Park, Tanzania (Photo: W. Mashauri).*

However, along a gradient of distance from TAZAMA pipeline, the abundance and richness of small mammal's species were low at the area close to the source of disturbance (i.e. oil pipeline), and the abundance and richness of species were increasing along a gradient of distance from the source of disturbance or pollution. This is probably because the immediate area might have been disturbed to the extent that there are few suitable microhabitats, and limited resources, to ensure the existence of many individuals from different species due to habitat loss, change, and fragmentation, due to vegetation clearance (Fiori & Zalba 2003; Mukhacheva et al. 2010). The clearance of vegetation is being done by casual laborers of the TAZAMA pipeline to enhance visibility and easy accessibility of the pipeline during maintenance.

In addition, Mukhacheva et al. (2010) reported that there is fluctuation in species richness and abundance of small mammals as the source of disturbance or pollution is approached, thus low richness and abundance in the immediate areas from the source of the disturbance. Although no statistically significant difference was found in species abundance and richness between plots, the obtained results only vaguely support my hypothesis that the richness and abundance of small mammal's species differ along a gradient of distance from the pipeline.

Furthermore, from TANZAM highway, species richness was low in the immediate area from the source of disturbance and richness of species was increasing along a gradient of distance from the source of the disturbance. This is probably because there is habitat heterogeneity with rich resources at the intermediate and distant areas from the source of disturbance due to less or absence of disturbance (Mukhacheva et al. 2010; Phelps & McBee 2009). On contrary to species richness, the abundance of small mammal's species was higher in the immediate (close) area to the source of disturbance, and the number was decreasing along the gradient of distance from the source of the disturbance. No doubt this is possibly due to the number of waste materials such as food remains, water bottles, jars, tubes, tires, etc., that are being thrown by road users in the park along the TANZAM highway, as these make the availability of both food and microsites for small mammal's individuals (Nyahinga et al. 2016).

Nonetheless, there might be clumps of seeds being dropped by vehicles that are transporting cereals and other types of food from one region to another via the TANZAM highway in Tanzania. Moreover, Mukhacheva et al. (2010) reported that, sometimes within the disturbed or polluted site, there are still favorable microhabitats with higher clumps of food as well as water to ensure the survival of many individuals and species, so this reason could possibly as well apply to the TANZAM highway to have such a high number of individuals in the immediate area from the source of disturbance. On the other hand, individuals of small mammal's species perhaps have changed their feeding behavior due to the cheap availability of food along the TANZAM highway because of littering (Nyahinga et al. 2016; Trombulak & Christopher 2000) and thus results into many individuals in the immediate area from the highway. In addition, Trombulak and Christopher (2000) reported that a larger number of individuals from different species including small mammal's species is being attracted near to the roadsides because some vehicles are spilling waste products that are edible. A none of the results were statistically significant, these results also, vaguely support my hypotheses that small mammal's species richness and abundance differ between plots (i.e. along a gradient of distance).

Nevertheless, from TAZAMA pipeline-TANZAM highway intersection, species abundance was low at the immediate area from the source of disturbance and the number of individuals was increasing along the gradient of distance from the source of the disturbance. Vegetation clearance done with TAZAMA pipeline and TANZAM highway workers probably have influenced to the low abundance because of the loss, change, and fragmentation of habitats which creates an unfavorable condition for individuals to reside the area (Fiori & Zalba 2003; Mukhacheva et al. 2010). In addition, possibly the availability of favorable microsites with rich resources at the intermediate and distant areas from the source of disturbance due to less or absence of disturbance might have influenced the increase in species abundance along the gradient of distance (Mukhacheva et al. 2010; Phelps & McBee 2009).

Moreover, the richness of species was high in the immediate area from the source of disturbance and species richness was decreasing along the gradient of distance from the source of pollution or disturbance. Possibly, this was influenced with the littering within the park along the TANZAM highway and TAZAMA pipeline done by road users and workers of the TAZAMA pipeline and TANZAM highway, which might have changed the feeding behavior of small mammal's species (Nyahinga et al. 2016; Trombulak & Christopher 2000). Like other study sites, the obtained results in this site also are vaguely supporting my hypotheses that richness and abundance of small mammal's species vary along a gradient of distance.

On contrary to other study sites, both immediate and intermediate areas from the control site had an equal number of individuals and species. This indicates that the site is probably not disturbed or polluted and thus, there is higher heterogeneity of habitats with rich resources to ensure the existence of many small mammal's individuals and different species (Mukhacheva et al. 2010). However, the abundance and richness of small mammal's species were low in the distant area compared to the immediate and intermediate areas. This decrease in species richness and abundance is probably due to high predation rate, animal trampling, the presence of non-native species as well as high grazing pressure of animals, that might have reduced the quality of habitat (Avenant 2011). In addition, according to Venance (2009), species abundance and richness are governed with resources availability and vegetation structure in a given habitat, thus, individuals might have vacated the area possibly because of the limited availability of resources.

Moreover, among all four study sites, TANZAM highway had higher species abundance and richness of small mammals than other sites possibly due to the availability of favorable microhabitats, much food, and water because of littering by road users which might have attracted many individuals and small mammal's species to reside the area. In addition, it could be due to the availability of more clumps of seeds that are dropped by vehicles transporting cereals and other kinds of food from different regions passing along the TANZAM highway in the park. Furthermore, possibly individuals might have changed their natural feeding behavior of feeding on plant materials and invertebrates and being habituated to feed on the remaining of cooked food and other wastes that are thrown in the park along the TANZAM highway by road users (Nyahinga et al. 2016). Lastly but not least, Trombulak and Christopher (2000) reported that a larger number of individuals from different species including small mammal's species is being attracted nearby the roadsides because some vehicles are spilling waste products that are edible. So, this also probably might be the reason for why TANZAM had a high number of richness and abundance of small mammal's species than other study sites.

Trace Metal Concentrations (mg/kg) in Soils

Trace elements or trace metals are defined as those metals or elements that have densities of greater than $5g/cm^3$ (Ekwumemgbo et al. 2006; Onojake & Okonkwo 2011). Trace metals or trace elements contamination in the environment is now of a great concern, as they are toxic compounds that have long residue effects once they get in the environment or organisms.

Meanwhile, they can neither be metabolized with living organisms (i.e. non-biodegradable) nor can be changed/altered once they get in the food chains (irreversible effect) (Ekwumemgbo et al. 2006; Onojake & Okonkwo 2011; Opaluwa et al. 2012; Wuana & Okieimen 2011). Some trace elements or metals such as Fe, Zn, Cu, Mn, Ni, Sn, and Vn are naturally occurring elements in the environment and are used as nutrients for plant growth depending on their concentrations. But some elements such as Pb, Cd, Cr, Hg, Ag, etc. occur indirectly in the environment due to the influence of several anthropogenic activities including agriculture, military activities, petroleum exploration and production, burning of fossil fuels, use of different means of transportation, etc., and they are very toxic at both concentrations (i.e. low or high) (Alleva et al. 2006; Ekwumemgbo et al. 2006; Onojake & Okonkwo 2011; Opaluwa et al. 2012; Schleich et al. 2010).

Generally, from the study, the results show that along a gradient of distance from all four study sites, the mean concentrations (mg/kg) of trace elements such as Fe, Cu, Cd, Cr, Zn, and Ni in soils were below the maximum permissible limits as described by (WHO 1993, 1996; WHO/FAO 2001) for soils. Meanwhile, the mean concentrations (mg/kg) of Pb and Mn were found to exceed the maximum permissible limits for soils as described by (WHO 1993, 1996; WHO/FAO 2001) between plots from all four study sites. Regardless of the mean concentrations of Fe being higher than other metals between plots from both four study sites, but still, these concentrations of Fe were below the recommended values. However, the mean concentrations of trace elements obtained in soils from the study vary among the sites, thus supporting my hypotheses that the accumulation of trace metals differs along a gradient of distance among the sites.

Moreover, with exception to TAZAMA pipeline-TANZAM highway intersection site, the obtained mean concentrations of Cd from other three sites are relative high regarding the public concerns together with the concentrations of Pb and Cr from all four study sites. This is because, both Pb, Cr, and Cd are toxic in all concentrations (low or high) and thus they are not needed in living organisms even for the trace amounts (Adeniyi & Afolabi 2002; Onojake & Okonkwo 2011; Opaluwa et al. 2012; Schleich et al. 2010; Wuana & Okieimen 2011). Likewise, the mean concentrations of Mn between plots from all four study sites were found to exceed the maximum permissible limit, and thus these concentrations of Mn are harmful in soils, wildlife as well as for public health. This is supported by Ekwumemgbo et al. (2006) that all trace elements regardless of whether they are naturally occurring or indirectly distributed with human activities, once they exceed their maximum permissible limits could damage an entire ecosystem as well as public health.

However, among all the four study sites, the control site had the lowest mean concentrations of trace metals compared to other three sites (i.e. control<TANZAM highway<TAZAMA pipeline-TANZAM highway intersection<TAZAMA pipeline) indicating that the site has probably a minimum rate of disturbance or pollution. Similar results were found by Adeniyi and Afolabi (2002) and Ekwumemgbo et al. (2006) who found the low accumulation of trace elements in soils obtained from the control sites compared to the accumulation of trace elements obtained from petroleum contaminated sites. This indicates that this slight variation in the mean concentrations of the obtained trace metals between the control site and other three study sites was probably due to the presence of TAZAMA pipeline and TANZAM highway in the park.

Furthermore, the observed differences in the mean concentrations of trace elements obtained such as Fe, Cu, Zn, Mn, Cd, Cr, Ni, and Pb between the control site and other three study sites, could possibly be influenced with the emissions from vehicles, oil spillage, littering, combustion of fossil fuels as well as maintenance of oil pipelines and roads. This is being supported by Maltby et al. (1995) that access roads might be the sources of pollution in the environments because of several pollutants produced by vehicles including trace metals, and hydrocarbons, that come from lubricating oils, grease, and fuels, brake linings as well as vehicle tires. In addition, Adu et al. (2012) and Trombulak and Christopher (2000) reported that emissions from vehicles and road maintenance contribute to a substantial amount of trace elements in the environment including Pb, Ni, Fe, Cd, Mn, Zn which are contained in the fuels as gasoline additives to prevent engine knocking.

Nonetheless, several authors e.g. (Allen-Gil et al. 2003; Ekwumemgbo et al. 2006; Rodriguez-Estival et al. 2015; Takáč et al. 2009; Wuana & Okieimen 2011), have reported that substantial amount of trace elements including Pb, Cr, Zn, Ni, Cd, Cu, Fe and Mn are being produced due to combustion of oils of which these metals are either emitted as particles in the environment or being concentrated in ashes that are then being transported by wind, water or gravity to contaminate the environment.

Throwing of different waste materials in the park such as food remains, tins, cans, water bottles, car tires, aluminium foils, metal materials, etc., along the TANZAM highway and TAZAMA pipeline as stated by Nyahinga et al. (2016) is possibly another reason that has contributed to the variations in the mean concentrations of the obtained trace elements such as Pb, Fe, Cu, Zn, Mn, Ni, Cd and Cr. This is strongly supported by Schleich et al. (2010) and Wuana and Okieimen (2011) that some of the waste materials that are being thrown are containing high levels of trace elements which then can be taken up by plants and later bioaccumulate in animal tissues and be harmful to small herbivores, as well as to the whole food chain.

Furthermore, the control site was found to have higher concentrations of Ni and Cd than other sites which could possibly be influenced indirectly by anthropogenic activities along the TAZAMA pipeline and TANZAM highway. This is probably because various trace elements including Ni and Cd may be transported in soils through leaching, runoff as well as through aerial drifts from a place of their occurrence to other places via wind, water, and gravity (Phelps & McBee 2009; Trombulak & Christopher 2000).

Moreover, all the mean concentrations of trace metals such as Fe, Cu, Zn, Cd, Cr, Pb, Ni and Mn found from the control site were not statistically significant while some trace metals such as Cu (from TAZAMA pipeline), Mn (from the intersection site), and Mn and Fe (from TANZAM highway), their concentrations differed statistically significant in soils. This strongly supports that the presence of these two infrastructures such as TAZAMA pipeline and TANZAM highway within the park has significantly contributed to the variations in the mean concentrations of the obtained trace elements in soils. Similar results were found by Adeniyi and Afolabi (2002) and Ekwumemgbo et al. (2006) where statistically significant was found in the mean concentrations of trace elements obtained from petroleum polluted areas in contrary to the control site.

Trace Metal Concentrations (mg/kg) in Livers and Kidneys of *Mastomys natalensis* and their Correlation in Soils

The obtained results from the study show that along a gradient of distance from all four study sites, the mean concentrations (mg/kg) of trace elements such as Mn, Cu, Cd, Cr, Zn, and Ni in livers and kidneys of *Mastomys natalensis* were below the value of the reference material (i.e. bovine liver-1577b provided by the Norwegian University of Science and Technology (NTNU)). Meanwhile, the mean concentrations (mg/kg) of Pb in kidneys and Fe in both livers and kidneys were found to exceed the value of the reference material (i.e. bovine liver-1577b provided by the Norwegian University of Science and Technology (NTNU)) between plots from all four study sites. However, the mean concentrations of trace elements obtained in the livers and kidneys from the study vary among the sites, thus supporting my hypotheses that the accumulation of trace metals differs along a gradient of distance among the sites.

While trace elements such as Cd, Cr, and Pb are toxic and non-physiological elements, Cu, Zn, Ni, Mn and Fe are useful elements as they play several physiological functions in living organisms (Damek-Poprawa & Sawicka-Kapusta 2003; Schleich et al. 2010). The results show that the mean concentrations (mg/kg) of Pb in kidneys exceeded that of the reference material while in livers, these concentrations were low than that of the reference material from all study sites. This is because chronic exposure of Pb concentration has been reported by Komarnicki (2000) to result in a high concentration of Pb in kidneys than livers. Despite the fact that Fe element is needed in the body of living organisms as a physiological element, the obtained mean concentrations in livers and kidneys from both study sites are high than that of the reference material (i.e. bovine liver-1577b) indicating that this amount of Fe may cause damages to the small mammal's species and an ecosystem in general (Damek-Poprawa & Sawicka-Kapusta 2003; Schleich et al. 2010).

The liver and kidney have been regarded as the primary targets of repeated dose toxicity of different pollutants due capability of their cells to store a substantial amount of metal ions without any damage to the organism (Miska-Schramm et al. 2014). However, when there is a high intake of these trace elements by the liver and kidney tissues, damages to the cells and organs are more likely to occur and thus impairing their functions.

High levels of trace metals in livers and kidneys have been found in the study sites having a high concentration of trace metals in soils such as TAZAMA pipeline-TANZAM highway intersection site, TANZAM highway as well as TAZAMA pipeline site on contrary to the control site. These results are in line with Adeniyi and Afolabi (2002) and Schleich et al. (2010) who found the considerable amount of trace metals in the tissues of small mammals inhabiting the petroleum and vehicle emissions contaminated sites than tissues of small mammals species obtained from the control site.

On contrary to other study sites, the control site had the lowest mean concentrations of trace elements in the livers and kidneys indicating that the site is probably less disturbed or polluted and the trend of contamination was TAZAMA pipeline-TANZAM highway intersection>TANZAM highway>TAZAMA pipeline>control site. Similar results were observed in soils of TAZAMA pipeline-TANZAM highway intersection site, TANZAM highway as well as TAZAMA pipeline site to have remarkable mean concentrations of trace metals than soils of the control site. This indicates that the levels of trace metals accumulation in livers and kidneys of *Mastomys natalensis* were probably due to water pollution, air pollution and pollution of land resources such as soils, and plant materials or vegetations. The results are in line with Schleich et al. (2010) who found the mean concentration of trace elements in small mammals species specifically rodents to be correlated with air pollution, water pollution and pollution of land resources such as soils and vegetations.

Nevertheless, the results show three different types of correlations between soils and livers and between soils and kidneys. Zero (0) correlation indicates that there is no relationship or association in the mean concentrations (mg/kg) of trace metals between soils and livers and between soils and kidneys. On the other hand, negative correlation indicates that there was a decrease in the mean concentrations (mg/kg) of trace metals in livers and kidneys when the mean concentrations (mg/kg) of trace metals was increasing in soils (i.e. inversely proportional relationship). Meanwhile, the positive correlation indicates that there was an increase in the mean concentrations (mg/kg) of trace metals in livers and kidneys when the mean concentrations (mg/kg) of trace metals in soils were increasing (i.e. directly proportional relationship). Furthermore, the higher the Pearson correlation coefficient (r) value is, the higher the association is between the variables and vise verse is true (Ekwumemgbo et al. 2006).

The above-mentioned associations or correlations between soils and tissues such as livers and kidneys happen because small mammal's species, especially rodents, are in close contact with land resources (such as soil and vegetation) during their whole life whereby they get exposed to pollutants through feeding on polluted food, water or soils as well as through skin engrossment (Pereira et al. 2006; Phelps & McBee 2009; Tovar-Sanchez et al. 2012). Generally, the results show that soils had highest mean concentrations (mg/kg) of trace metals compared to liver and kidney tissues. This is because soils, vegetations, water, as well as air, are the main recipients of contaminants such as trace metals, of which small mammals, and wildlife in general, living in those areas are vulnerable of being exposed to, and thus dangers may occur to wildlife and human health in the entire food chain (Rodriguez-Estival et al. 2015; Sanchez-Chardi et al. 2009; Wuana & Okieimen 2011).

Moreover, all the mean concentrations of trace metals such as Fe, Cu, Zn, Cd, Cr, Pb, Ni and Mn in livers and kidneys found from the control site were not statistically significant, while some trace metals such as Mn in livers, Cr in kidneys (from TAZAMA pipeline), Ni in livers (from the intersection site), and Cu in kidneys and Ni in livers (from TANZAM highway), their concentrations differed statistically significant. This strongly supports that TAZAMA pipeline and TANZAM highway have significantly contributed to the variations in the mean concentrations of the obtained trace elements in the livers and kidneys. These results are in line with Sanchez-Chardi et al. (2009) who found the statistically significant in the mean concentrations of trace elements in small mammals livers and kidneys obtained from the landfill site on contrary to the control site.

Lastly but not least, all the negative and zero correlations between soils and livers and between soils and kidneys of all the mean concentrations of trace elements such Fe, Cu, Zn, Cd, Cr, Pb, Ni and Mn were not statistically significant while some positive correlations of few trace elements such as Cd (from TANZAM highway), and Cr and Fe (from the control site) between soils and kidneys were found statistically significant.

Conclusion and Recommendations

Conclusion

The obtained results from the study reveal that small mammal's species richness and abundance fluctuate as the sources of pollution or disturbance are approached. However, the number of individuals and species found from both four study sites was not satisfactory because of several reasons that might have confounded. The reasons include the catching of non-targeted species, weather condition variation, changing of home range sizes of small mammal's individuals, alteration of their natural feeding behavior, as well as the destruction of the Sherman traps by medium to larger sized animals including carnivores. Meanwhile, the findings also show that several anthropogenic activities including littering, oil spillage, combustion of fossil fuels, emissions from vehicles as well as maintenance of oil pipelines and roads have influenced to the deposition and variations of the obtained trace metals such as Fe, Cu, Zn, Mn, Cd, Cr, Ni, and Pb in soils and tissues such as livers and kidneys from all four study sites.

Nonetheless, my findings show that damages to small mammal's species and wildlife in general in Mikumi National Park are more likely to occur due to the relatively high concentrations of Pb, Cd, Cr, and Mn that were obtained in soils during the study. Nevertheless, the study has found that the presence of TAZAMA pipeline and TANZAM highway in the Mikumi National Park has significant effects on the small mammal's species as well as an ecosystem in general because of disturbances and pollution including vegetation clearance, littering, noise due to vehicles as well as through deposition of substantial amount of trace elements in the park.

Lastly but not least, my findings have revealed that small mammal's species specifically rodents are the right candidates to be used as bio-indicators for the presence of toxic compounds in given habitat types.

Recommendations

Based on the study findings from all four study sites, I recommend the following actions to be considered to ensure that Mikumi National Park in Tanzania continues to sustain and or exist in the future;

Conservation education regarding environments is of great importance to be taken into consideration by investors before investing their projects in any protected area (PA), as these areas are biodiversity-rich hotspots that need to be protected and conserved to ensure the functional ecosystem without impairing its ecosystem services with its associated cultural values. This should be applied to the road users too including tourists, drivers, and passengers passing through the protected areas.

Strict law enforcement should be put in action on careless drivers who over-speed the recommended driving speed, and those road users that throw waste materials in the park. For the already thrown waste materials in the park, the park authority should take them out and dispose of them in recommended areas as this will help to reduce the rate of pollution in the environment.

However, more research regarding the *Crocidura* spp. abnormality should be done to identify if such abnormality is due to disease or other factors and if such abnormality has an influence on the reproductive system and lifespan of *Crocidura* spp. that might possibly have led to the low population size of this species in the Mikumi National Park.

Lastly but not least, Mikumi National Park has several public infrastructures traversing it including Tanzania-Zambia Railway (TAZARA), High voltage power lines, and Optic fiber cables, apart from TAZAMA pipeline and TANZAM highway. So, more research in the Mikumi National Park regarding the effects of these infrastructures on the small mammal's species and wildlife, in general, is imperative to ensure a functional ecosystem.

References

Adeniyi, A. A., and J. A. Afolabi. 2002. Determination of total petroleum hydrocarbons and heavy metals in soils within the vicinity of facilities handling refined petroleum products in Lagos metropolis. Environment International **28**:79-82.

Adu, A. A., O. J. Aderinola, and V. Kusemiju. 2012. An Assessment of Soil Heavy Metal Pollution by Various Allied Artisans in Automobile, Welding Workshop and Petrol Station in Lagos State, Nigeria. Science Journal of Environmental Engineering Research:8.

Allen-Gil, S. M., J. Ford, B. K. Lasorsa, M. Monetti, T. Vlasova, and D. H. Landers. 2003. Heavy metal contamination in the Taimyr Peninsula, Siberian Arctic. The Science of the Total Environment **301**:119–138.

Alleva, E., N. Francia, M. Pandolfi, A. M. De Marinis, F. Chiarotti, and D. Santucci. 2006. Organochlorine and Heavy-Metal Contaminants in Wild Mammals and Birds of Urbino-Pesaro Province, Italy: An Analytic Overview for Potential Bioindicators. Arch Environ Contam Toxicol **51**:123-134.

Avenant, N. 2011. The potential utility of rodents and other small mammals as indicators of ecosystem 'integrity' of South African grasslands. Wildlife Research **38**:626.

Barnett, A., and J. Dutton. 1995. Expedition Field Techniques: Small Mammals (excluding bats).

Cassola, F. 2016. *Lemniscomys griselda*. The IUCN Red List of Threatened Species 2016: e.T11489A115102674.

Choate, T. S. 1972. Behavioural Studies on some Rhodesian Rodents. Zoological Africana **7**:103-118.

Damek-Poprawa, M., and K. Sawicka-Kapusta. 2003. Damage to the liver, kidney, and testis with reference to the burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. Toxicology **186**:1-10.

Drews, C. 1995. Road kills of animals by public traffic in Mikumi National Park, Tanzania, with notes on baboon mortality. African Journal of Ecology **33**:89-100.

Ekwumemgbo, A. P., O. N. Eddy, and K. I. Omoniyi. 2006. Heavy Metals Concentrations of Water and Sediments in Oil Exploration Zone of Nigeria. Heavy Metals in the Marine Environment.

Fiori, S. M., and S. M. Zalba. 2003. Potential impacts of petroleum exploration and exploitation on biodiversity in a Patagonian Nature Reserve, Argentina. Biodiversity and Conservation **12**:1261-1270.

He, Z., J. Shentu, X. Yang, V. C. Baligar, T. Zhang, and P. J. Stoffella. 2015. Heavy Metal Contamination of Soils: Sources, Indicators, and Assessment. Environmental Indicators **9**:17-18.

Kingdon, J. 1997. The Kingdon Field Guide to African Mammals. London: Academic Google Scholar.

Komarnicki, G. J. K. 2000. Tissue, sex and age-specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (*Talpa europaea* L.) in a central urban area. Chemosphere **41**:1593-1602.

Maltby, L., D. M. Forrow, A. B. A. Boxall, P. Calow, and C. I. Betton. 1995. The effects of motorway runoff on freshwater ecosystems: 1. Field study. Environmental Toxicology and Chemistry **14**:1079-1092.

Miska-Schramm, A., M. Kruczek, and J. Kapusta. 2014. Effect of copper exposure on reproductive ability in the bank vole (*Myodes glareolus*). Ecotoxicology **23**:1546-1554.

Mukhacheva, S. V., Y. A. Davydova, and I. A. Kshnyasev. 2010. Responses of Small Mammal Community to Environmental Pollution by Emissions from a Copper Smelter. Russian Journal of Ecology **41**:513-518.

Newmark, W. D., J. I. Boshe, H. I. Sariko, and G. K. Makumbule. 1996. Effects of a highway on large mammals in Mikumi National Park, Tanzania. African Journal of Ecology **34**:15-31.

Nunes, A. C., M. D. Mathias, and A. M. Crespo. 2001. Morphological and haematological parameters in the Algerian mouse (*Mus spretus*) inhabiting an area contaminated with heavy metals. Environmental Pollution **113**:87-93.

Nyahinga, T., J. Ringo, M. Allan, and M. Gabriel. 2016. Quantification of Solid Wastes along the Highway Crossing Mikumi National Park, Tanzania. International Journal of Modern Social Sciences **5**:140-152.

Onojake, M. C., and V. Okonkwo. 2011. Trace Metals Associated with Oil Spillage: A case study. Journal of Chemical and Pharmaceutical Research **3**:742-751.

Opaluwa, O. D., M. O. Aremu, L. O. Ogbo, K. A. Abiola, I. E. Odiba, M. M. Abubakar, and N. O. Nweze. 2012. Heavy metals concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. Advances in Applied Science Research **3**:780-784.

Pereira, R., M. L. Pereira, R. Ribeiro, and F. Goncalves. 2006. Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). Environmental Pollution **139**:561-575.

Phelps, K. L., and K. McBee. 2009. Ecological Characteristics of Small Mammal Communities at a Superfund Site. American Midland Naturalist **161**:57-68.

Plumptre, A. J., S. Ayebare, H. Mugabe, B. Kirunda, R. Kityo, S. Waswa, B. Matovu, S. Sebuliba, M. Behangana, R. Sekisambu, P. Mulondo, T. Mudumba, M. Nsubuga, S. Isoke, S. Prinsloo, and G. Nangendo. 2015. Biodiversity Surveys of Murchison Falls Protected Area. Wildlife Conservation Society.

Reynolds, K. D., M. S. Schwarz, C. A. McFarland, T. McBride, and B. Adair. 2006. Northern pocket gophers (*Thomomys talpoides*) as Biomonitors of Environmental Metal Contamination. Environmental Toxicology and Chemistry **25**:458-469.

Rodriguez-Estival, J., M. A. North, and J. E. G. Smits. 2015. Sublethal health effects in laboratory rodents from environmentally relevant exposures to oil sands contaminants. Environmental Toxicology and Chemistry **34**:2884-2897.

Sanchez-Chardi, A., C. Penarroja-Matutano, M. Borras, and J. Nadal. 2009. Bioaccumulation of metals and effects of a landfill in small mammals Part III: Structural alterations. Environ Res **109**:960-967.

Schleich, E. C., O. M. Beltrame, and D. C. Antenucci. 2010. Heavy metals accumulation in the subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae) from areas with different risk of contamination. Folia Zoologica **59**:108-114.

Shore, R. F. 1995. Predicting Cadmium, Lead and Fluoride levels in small mammals from soil residues and by species-species extrapolation. Environmental Pollution **88**:333-340.

Takáč, P., T. Szabová, L. Kozáková, and M. Benková. 2009. Heavy metals and their bioavailability from soils in the long-term polluted Central Spiš region of SR. Plant Soil Environ **55**:167–172.

Topolska, K., K. Sawicka-Kapusta, and E. Cies'lik. 2004. The Effects of Contamination of the Krako'w Region on Heavy Metals Content in the Organs of Bank Voles (*Clethrionomys glareolus*, Schreber, 1780). Polish Journal of Environmental Studies **13**:103-109.

Torres, J., J. Peig, C. Eira, and M. Borras. 2006. Cadmium and lead concentrations in *Skrjabinotaenia lobata* (Cestoda: Catenotaeniidae) and in its host, *Apodemus sylvaticus* (Rodentia: Muridae) in the urban dumping site of Garraf (Spain). Environ Pollut **143**:4-8.

Tovar-Sanchez, E., L. T. Cervantes, C. Martinez, E. Rojas, M. Valverde, M. L. Ortiz-Hernandez, and P. Mussali-Galante. 2012. Comparison of two wild rodent species as sentinels of environmental contamination by mine tailings. Environmental Science and Pollution Research **19**:1677-1686.

Trombulak, C. S., and F. A. Christopher. 2000. Review of Ecological Effects of Roads on Terrestrial and Aquatic Communities. Conservation Biology **14**:18-30.

Venance, J. 2009. Small Mammal Communities in the Mikumi National Park, Tanzania. Hystrix It. J. Mamm. (n.s.) **20**:91-100.

WHO. 1993. Trace elements in human nutrition and health, World Health Organization, Geneva, Switzerland.

WHO. 1996. Trace elements in human nutrition and health. World Health Organization. Geneva.

WHO/FAO. 2001. Codex alimentarius commission. Food additives and contaminants. Joint FAO/WHO Food Standards Programme, ALINORM 10/12A.

World-Bank. 1985. TAZAMA Pipeline Rehabilitation Engineering Project (Credit 1627-ZA).

World-Bank. 1991. TAZAMA Pipeline Rehabilitation Engineering Project (Credit 1627-ZA).

Wuana, R. A., and F. E. Okieimen. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology **2011**:1-20.

Appendices

Appendix 1; Trace metals mean (SD) concentrations (in mg/kg) and their maximum permissible limits (mg/kg), in soils along a gradient of distance from all four study sites.

Sites		Means (SD) in (mg/kg)			Maximum permissible limit in soils (WHO 1993, 1996; WHO/FAO 2001)
1; TAZAMA pipeline	Element	Plot 1	Plot 2	Plot 3	
	Cu	51.90 (2.17)	39.90 (1.48)	35.50 (5.56)	100
	Zn	21.70 (1.32)	72.80 (82.23)	920.10 (1552.21)	300
	Ni	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	50
	Cd	0.11 (0.18)	0.00 (0.00)	0.00 (0.00)	3
	Cr	166.30 (58.16)	302.80 (60.03)	144.00 (40.59)	400
	Pb	79.00 (18.51)	84.50 (55.60)	75.50 (108.17)	50
	Mn	242.30 (16.44)	165.60 (82.70)	152.80 (55.34)	80
	Fe	5605.90 (4205.91)	9767.40 (6659.23)	4430.20 (1720.79)	21,000
2; TANZAM highway	Cu	56.80 (12.87)	38.60 (3.25)	35.74 (9.87)	100
	Zn	17.80 (6.00)	70.80 (83.90)	22.50 (2.67)	300

	Ni	6.35 (9.36)	1.75 (1.59)	0.00 (0.00)	50
	Cd	0.00 (0.00)	0.11 (0.19)	0.00 (0.00)	3
	Cr	121.70 (31.75)	166.10 (71.99)	120.70 (12.78)	400
	Pb	90.10 (59.24)	88.84 (58.54)	84.61 (52.16)	50
	Mn	266.20 (51.77)	27.50 (23.81)	36.31 (6.56)	80
	Fe	9487.80 (349.21)	5284.00 (1302.55)	990.50 (348.94)	21,000
3; TAZAMA pipeline-TANZAM highway intersection	Cu	41.20 (16.20)	42.30 (4.46)	28.10 (10.36)	100
	Zn	59.60 (20.36)	476.20 (773.15)	35.40 (29.77)	300
	Ni	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	50
	Cd	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3
	Cr	200.90 (110.63)	145.40 (62.51)	190.50 (19.49)	400
	Pb	59.64 (26.71)	147.20 (60.98)	91.70 (30.18)	50
	Mn	362.80 (58.35)	278.80 (79.52)	195.50 (38.92)	80
	Fe	11444.10 (8676.72)	7152.00 (1713.47)	273.00 (185.33)	21,000

4; Control	Cu	25.80 (9.24)	47.80 (23.14)	23.50 (8.39)	100
	Zn	22.00 (4.01)	9.10 (0.33)	14.43 (7.69)	300
	Ni	3.50 (4.51)	5.20 (6.55)	0.00 (0.00)	50
	Cd	0.60 (0.95)	0.30 (0.50)	0.20 (0.20)	3
	Cr	163.20 (66.13)	132.20 (17.52)	118.40 (10.55)	400
	Pb	80.00 (25.07)	60.40 (20.34)	41.70 (49.78)	50
	Mn	121.00 (42.69)	136.30 (46.24)	148.20 (52.70)	80
	Fe	5645.60 (4408.81)	3284.10 (5095.57)	758.60 (627.19)	21,000

Appendix 2; Trace metals mean (SD) concentrations (in mg/kg) in the livers and kidneys of *Mastomys natalensis* with their reference material (bovine liver-1577b) along a gradient of distance from all four study sites.

Study sites		Means (SD) (mg/kg) in livers			Means (SD) (mg/kg) in kidneys			Reference material (Bovine liver-1577b)
1; TAZAMA pipeline	Element	Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	
	Cu	8.88 (2.79)	7.92 (1.29)	10.57 (5.07)	10.52 (1.55)	11.63 (0.87)	10.20 (0.56)	164.18 (3.02)
	Zn	76.72 (23.96)	61.60 (9.88)	70.17 (15.56)	65.89 (9.13)	70.44 (7.01)	65.20 (3.69)	133.59 (0.82)

	Ni	0.03 (0.06)	0.02 (0.01)	0.02 (0.04)	0.42 (1.08)	0.02 (0.01)	0.01 (0.01)	0.32 (0.05)
	Cd	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.09 (0.04)	0.07 (0.01)	0.09 (0.03)	0.46 (0.01)
	Cr	0.03 (0.02)	0.12 (0.14)	0.24 (0.27)	0.17 (0.08)	0.20 (0.04)	0.10 (0.03)	0.34 (0.07)
	Pb	0.03 (0.00)	0.04 (0.02)	0.06 (0.02)	0.34 (0.20)	0.21 (0.07)	0.27 (0.14)	0.11 (0.00)
	Mn	2.26 (0.57)	2.54 (0.47	3.48 (0.96)	4.25 (0.60)	4.20 (1.23)	4.43 (0.52)	10.01 (0.12)
	Fe	560.42 (119.60)	436.74 (120.71)	574.66 (418.07)	269.98 (51.78)	403.94 (133.38	514.20 (308.16	188.66 (1.48)
2; TANZAM highway	Cu	10.11 (1.47)	8.92 (0.85)	10.13 (3.75)	10.50 (0.40)	12.05 (0.72)	11.41 (1.31)	164.18 (3.02)
	Zn	76.04 (21.20)	65.36 (6.34)	76.50 (28.68)	65.68 (1.50)	70.81 (5.83)	72.07 (6.16)	133.59 (0.82)
	Ni	0.00 (0.00)	0.01 (0.01)	0.05 (0.04)	0.02 (0.01)	0.01 (0.01)	0.02 (0.01)	0.32 (0.05)
	Cd	0.03 (0.01)	0.03 (0.03)	0.02 (0.01)	0.09 (0.03)	0.13 (0.16)	0.09 (0.04)	0.46 (0.01)
	Cr	0.09 (0.05)	0.13 (0.06)	0.15 (0.22)	0.14 (0.05)	0.18 (0.06)	0.17 (0.06)	0.34 (0.07)
	Pb	0.09 (0.05)	0.92 (1.95)	0.04 (0.02)	0.30 (0.12)	4.55 (7.78)	0.19 (0.08)	0.11 (0.00)
	Mn	6.07 (1.35)	4.49 (1.08)	6.02 (1.83)	4.15 (0.42)	4.27 (0.67)	4.18 (0.70)	10.01 (0.12)
	Fe	546.75 (433.00)	310.15 (92.33)	325.07 (258.00)	297.82 (48.94)	292.67 (51.40)	374.83 (87.45)	188.66 (1.48)

3; TAZAMA pipeline-TANZAM highway intersection	Cu	9.05 (2.33)	9.91 (3.43)	10.72 (3.15)	11.94 (1.49)	12.29 (2.20)	11.14 (1.01)	164.18 (3.02)
	Zn	84.30 (20.50)	88.57 (28.27)	86.19 (25.35)	71.01 (5.32)	73.15 (11.34	66.62 (3.82)	133.59 (0.82)
	Ni	0.00 (0.00)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.02 (0.01)	0.02 (0.01)	0.32 (0.05)
	Cd	0.01 (0.01)	0.02 (0.01)	0.02 (0.01)	0.06 (0.01)	0.07 (0.02)	0.06 (0.01)	0.46 (0.01)
	Cr	0.10 (0.04)	0.75 (1.27)	0.13 (0.05)	0.16 (0.04)	0.13 (0.03)	0.20 (0.13)	0.34 (0.07)
	Pb	0.05 (0.01)	0.20 (0.18)	0.15 (0.24)	0.82 (1.68)	1.20 (1.34)	0.41 (0.40)	0.11 (0.00)
	Mn	6.65 (3.34)	6.11 (4.24)	5.93 (1.35)	4.78 (0.96)	4.61 (0.45)	4.02 (0.61)	10.01 (0.12)
	Fe	701.38 (822.83)	659.19 (364.09)	433.24 (185.60)	311.10 (72.92)	308.23 (64.26)	345.70 (75.65)	188.66 (1.48)
4; Control	Cu	4.80 (1.25)	4.73 (1.20)	5.12 (1.81)	10.02 (1.20)	11.06 (0.87)	10.04 (1.00)	164.18 (3.02)
	Zn	39.56 (11.36)	36.02 (7.96)	41.11 (13.18)	61.20 (5.34)	65.49 (3.10)	61.00 (3.78)	133.59 (0.82)
	Ni	0.07 (0.17)	0.05 (0.07)	0.04 (0.06)	0.05 (0.08)	0.02 (0.01)	0.02 (0.01)	0.32 (0.05)
	Cd	0.01 (0.00)	0.01 (0.00)	0.00 (0.00)	0.04 (0.02)	0.04 (0.02)	0.03 (0.01)	0.46 (0.01)
	Cr	0.04 (0.03)	0.02 (0.01)	0.05 (0.03)	0.19 (0.07)	0.15 (0.07)	0.13 (0.03)	0.34 (0.07)
	Pb	0.03 (0.02)	0.04 (0.02)	0.03 (0.01)	0.24 (0.12)	0.18 (0.09)	0.21 (0.07)	0.11 (0.00)

Mn	5.52 (1.82)	3.89 (0.53)	4.95 (0.95)	3.84 (0.77)	4.34 (0.58)	3.89 (0.67)	10.01 (0.12)
Fe	243.57 (144.51)	297.33 (157.98)	399.03 (127.69)	345.54 (65.09)	309.42 (77.52)	262.76 (59.93)	188.66 (1.48)