Norwegian University of Life Sciences
Faculty of
Department of

Master Thesis 2014
60 credits

Migration of trace metals and radionuclides in terrestrial ecosystem; Serule case study

Department of Environmental Sciences/CERAD CoE

Norwegian University of Life Sciences

Thapelo Nthokana

UMB

Master of Science in Radioecology

Migration of trace metals and radionuclides in terrestrial ecosystem, Serule case study

Thapelo Nthokana

Department of Environmental Sciences/CERAD CoE

Norwegian University of Life Sciences

Aas, Norway

15.05.2014

Supervisor:

Professor Lindis Skipperud

Co-supervisor:

Professor Brit Salbu

Acknowledgements

I would like to thank Botswana government through Radiation Protection Inspectorate for the scholarship and giving me the opportunity to be a part of the team collecting background data for trace metals and ^{210}Po concentrations at the proposed uranium mining site in Serule village located in north-east of Botswana.

My gratitude also go to Professors Lindis Skipperud and Brit Salbu for their guidance, support, patience and supervision during the laborious analysis and interpretation of data.

Lene Valle, I appreciate all the help you accorded me in the isotope laboratory together with Marit Pettersen and Tove Loftaas.

I will also like to than Karl Andreas Jensen for his insight in relation to mass spectroscopic analysis. Your enthusiasm to share your special knowledge was amazing

Tebogo Carson Ranko, thank for working hard as you jumped through all bureaucratic hoops to see to it that analytical samples were sent to me at the laboratory, all of this would not have been possible without them.

Ministry of Infrastructure, Science and Technology, Mr Lopang Pelekekae, thank you for seeing to it that my welfare was taken care of while away from home. And radiation Protection Inspectorate director Mr Thapelo Otukile, thank for resolving all the administrative issues, allowing me to concentrate on academics.

Last but by no means least; I thank my wife for understanding my absence when she had to take care of our first baby on her own with me half way across the world but never complaining.

Summary

Anthropogenic activities like mining disturb the natural cycles of chemical elements between the biosphere and the geosphere. Most of these activities lead to environmental pollution which is defined as the elevation of a particular substance above its background concentration. The chemicals especially persistent ones like trace metals, can end up in soils which acts as long term sinks for environmental pollutants.

Soil is also a substrate on which plants grow, and plants extract most of their nutrients from their substrates. Therefore the importance of the health of the substrate soil cannot be overemphasised because polluted soils can have adverse effects on the yield of both crops and pastures imparting negatively on both arable and pastoral farming as well as wildlife survival chances i.e. whole ecosystems can be affected by soil pollution. In an effort to reap the rewards of mining but not forgetting the benefits of unpolluted soils, mining sites are to be rehabilitated at the end of their economic life. Feasible rehabilitation options for waste sites are identified through determining the state of the mining site in relation to chemicals of concern before mining activities commence. Rehabilitation of mining sites is particularly important where the target mineral is naturally occurring radioactive material like uranium due to its radiological and chemical toxicity.

This master thesis is a part of comprehensive study of potential environmental impacts at a proposed uranium mining site in north-east Botswana in order to provide sound environmental management during mining and feasible remediation alternatives post mining. The thesis was co-sponsored by government of Botswana through Radiation Protection Inspectorate and Norwegian University of Life Science's Department of Plant and Environmental Sciences. The main objectives of the thesis were to quantify trace metals; Cr, Co, Cu, Fe, Mn, Ni, Zn, As, Cd, Pb, Th, and U and radioactive ^{210}Po in soil, in leaves of *Colospermum mopane* tree and in the larval stage of *Imbrasia belina* emperor moth. Then, the concentration of the trace metals in different environmental compartments was used to calculate transfer factor –(concentration in *C. mopane leaves*/concentration in soil)- and concentration factor – (concentration in *I. belina* larvae/concentration in *C. mopane* leaves) from the proposed mining site. This data was compared to reference data collected 100km from the proposed mining site and analysed in parallel to the samples of interest to give background trace metal levels an uncontaminated area north-east Botswana.

Total trace metals concentrations were obtained using established methods based on inductively coupled plasma-mass spectroscopy at the Department of Plant Science of the University of Life Sciences, Ås, Norway. All the metals prove to have concentration levels below the maximum permissible soil load, for example, for Pb and Cr the highest concentration of (19.20 ± 5.20) mg/kg and (71.80 ± 10.52) mg/kg respectively, were well below permissible limit of 200 mg/kg. The maximum concentrations of Cu and Cd were (30.80 ± 7.43) mg/kg and (0.45 ±0.017) mg/kg respectively, were lower than the permissible limit of 100 mg/kg in soils. Soil concentration of trace metals was always higher than that in plants growing on the soil meaning that *C. mopane* does not accumulate the metals. Hence all soil to plant metal transfers factors were below unity. The transfer factors were of the order Zn > Cd >Cu >Mn > Ni >As > Cr > Co > U > Pb > Fe > Th. Most of the transfer factors varied with soil Mn, Fe and pH characterised by very high correlation coefficients. Thus, implying that the uptake of trace metals by plants via root uptake depends on the soil properties.

In contrast to transfer factor, the concentration factor was higher than unity for all trace metals as *Imbrasia belina* caterpillars tended to accumulate trace metals more than leaves of *Colospermum mopane*. Zn appeared to have the highest concentration factor, probably due to its essential physiological function in animals. Next is As although As has no known physiological function. Then follows Cr, Fe and Mn, and finally a number of trace metals which also lack beneficial function to organisms; U, Th and Pb. All these metals have standard deviations at least equivalent to 50% of their mean values, which means they are not inhomogeneously distributed in the samples. Cu is more homogeneous as it probably due to *I. belina's* effort to regulate Cu concentration because Cu is an oxygen transport vehicle in arthropods.

The most potent radio-toxin from uranium mining is alpha emitting ^{210}Po. At 138 day half-life and alpha particles characterised by 5.34 MeV of energy, ^{210}Po represents a risk to animals especially when incorporated in the organisms. To assess this risk, ^{210}Po was quantified in soil, plant and larvae by alpha spectrometry analysis at Isotope Laboratories, NMBU.

Significant difference was found in soil ^{210}Po activity concentration between the samples collected from proposed mining site and reference site except for sample 1and 3. Sample 1 had activity concentration lower that reference site while sample 3 had a range that overlapped with the range of the reference sample. The rest of the sample were well above the reference level implying that the proposed mining site had more polonium that the reference site. The variations of ^{210}Po mirrored that of U in soil in support of the expectation that below 0.2 m below the soil surface, all ^{210}Po is due to decay of U which consists primarily ^{238}U.

^{210}Po activity concentration in *C. mopane* was at its highest in sample 5 at 1250 ± 65 Bq/kg which also had the highest U concentration and there was little variation for the rest of the samples with a range, 47 ± 4 to 66 ± 6 Bq/kg. Based on the close activity concentration range of ^{210}Po at the proposed mining site, a significant difference between ^{210}Po concentration at the proposed mining and the reference sites was obtained. Furthermore, the linearity between ^{210}Po and U was reduced, thus reflecting that at low soil concentrations, the root intake was of minor importance for the ^{210}Po accumulation by plants compared to atmospheric deposition. However, sample 5, with its ^{210}Po concentration at least twice that measured in the remaining samples indicated that at high soil concentrations, root uptake plays a significant role in ecological transfer of ^{210}Po.

Beside the fact that *I. belina* caterpillars fed sorely on *C. mopane* leaves, no correlation between ^{210}Po in the two trophic levels was found. This could indicate that the age of the caterpillars has an effect on their ability to accumulate ^{210}Po because the caterpillars were harvested at different life stages.

Sammendrag

Menneskeskapte aktiviteter som gruvedrift forstyrrer naturlige sykluser av kjemiske elementer mellom biosfæren og geosfæren. De fleste av disse aktivitetene fører til miljøforurensning, som er definert som den heving av et bestemt stoff over sin bakgrunnskonsentrasjon. De mest motstandsdyktige kjemikaliene som tungmetaller, kan ende opp i jord som fungerer som langsiktige beholdere for miljøgifter.

Jord er også et substrat hvor plantene vokser, og planter trekket ut mesteparten av sine næringsstoffer fra sine substrater. Derfor viktigheten av helsen til jorden veldig høy fordi forurenset jord kan ha negative effekter på utbyttet av både avlinger og beite formidles negativt på både dyrkbar og pastorale oppdrett samt dyrelivs overlevelse sjansene dvs. hele økosystemer kan bli påvirket av jord forurensning. I et forsøk på å høste gevinsten av gruvedrift, men ikke glemme fordelene av ikke-forurenset jord, skal gruve ommråder bli rehabilitert på slutten av sin levetid. Gjennomførbare rehabiliteringsmuligheter for deponier er identifisert gjennom å bestemme tilstanden til gruveområdet i forhold til kjemikalier av bekymring før gruvedriften starter. Rehabilitering av gruve områder er spesielt viktig hvor ønsket mineral er naturlig forekommende radioaktivt materiale som uran på grunn av den radiologiske og kjemisk toksisitet.

Denne masteroppgaven er en del av omfattende studie av mulige miljøeffekter i en foreslått uran mine i nord -øst Botswana for å gi god miljøledelse i løpet av gruvedrift og gjennomføre utbedring av alternativer til etter gruvedriften . Avhandlingen ble co - sponset av regjeringen i Botswana gjennom strålevern Datatilsynet og Norsk University of Life Science avdeling for plante-og miljøvitenskap. De viktigste målene med avhandlingen var å kvantifisere spormetaller ; Cr , Co , Cu , Fe , Mn , Ni , Zn , As, Cd , Pb , Th og U og radioaktivt 210Po i jord , i blader av Colospermum mopane treet og i larvestadiet av Imbrasia Belina keiser møll . Deretter ble konsentrasjonen av spormetaller i forskjellige deler av miljøet som brukes til å beregne overføringsfaktor- (konsentrasjon i C. mopane blader / konsentrasjon i jord) - og konsentrasjonsfaktor - (konsentrasjon i I. Belina larver / konsentrasjon i C. mopane blader) fra det foreslåtte gruveområdet . Denne informasjonen ble sammenlignet med referansedata samlet inn 100 km fra det foreslåtte gruveområdet og analysert parallelt med prøver av interesse å gi bakgrunns spor metallnivåene fra et ikke-forurenset område i nord -øst Botswana .

Totale spormetaller konsentrasjoner ble oppnådd ved hjelp av etablerte metoder basert på induktivt koplet plasma - massespektroskopived Institutt for plante- vitenskap ved Universitetet for miljø og biovitenskap , Ås , Norge . Alle metallene viste seg å ha konsentrasjonsnivåer under den maksimalt tillatte jordbelastning, for eksempel, for Pb og Cr den høyeste konsentrasjonen av (19.20 ± 5.20) mg / kg og (71.80 ± 10.52) mg / kg , var godt under tillatte grense på 200 mg / kg. De maksimale konsentrasjonene av Cu og Cd var (30,80 ± 7,43) mg / kg og henholdsvis mg (0,45 ± 0,017) / kg , var lavere enn den tillatte grensen på 100 mg / kg i jord . Jord konsentrasjon av spormetaller var alltid høyere enn i planter som vokser i jord som betyr at C. mopane ikke akkumuleres metallene . Alle jord å plante metall overføringer faktorene var under enhet .Overførings faktorene var i størrelsesorden Zn > Cd > Cu > Mn > Ni > Som > Cr > Co > U > Pb > Fe > Th . De fleste av overføringsfaktorenevarierte med jord Mn, Fe , og pH kjennetegnet ved svært høye korrelasjonskoeffisienter . Det innebar at utvinning av spormetaller i planter via røttene , avhenger av egenskapene i jorden .

I kontrast til å overførings faktoren, konsentrasjonsfaktoren var høyere enn enhet for alle spormetaller. Imbrasia Belina larvene hadde en tendens til å akkumulere spormetaller flere blader av Colospermum mopane . Zn syntes å ha den høyeste konsentrasjons faktoren, sannsynligvis på grunn av sin avgjørende fysiologiske funksjon hos dyr. Neste er As. As har ingen kjent fysiologisk funksjon . Deretter følger Cr, Fe og Mn, og til slutt en rekke spormetaller som også mangler gunstig funksjon for organismer ; U , Th og Pb . Alle disse metallene har standardavvik på minst tilsvarende 50 % av gjennomsnittsverdiene , noe som betyr at de er heterogeniske distribuert i prøvene . Cu er mer homogen som det sannsynligvis på grunn av *I*. Belinas forsøk på å regulere Cu konsentrasjon fordi Cu er en oksygen transport kjøretøy i leddyr.

Den sterkeste radio - toksinen fra uran gruvedrift er alfa emitting 210Po . Ved 138 dagers halveringstid og alfa-partikler karakterisert ved 5,34 MeV energi, 210Po representerer en risiko for dyr , spesielt når innlemmet i en organismene. For å vurdere denne risikoen , ble 210Po kvantifisert i jord, planter og larver av alfa spektrometri analyse ved Isotoplaboratoriene , NMBU .

Signifikant forskjell ble funnet i jord 210Po aktivitetskonsentrasjon mellom prøver fra det foreslåtte gruveområdet og referanse området med unntak av prøven 1og 3. Prøve 1 hadde aktivitetskonsentrasjon lavere enn referanse området mens prøve 3 hadde et område som overlappes med utvalget fra referanseprøven . Resten av prøven var godt over.

referansenivået innebærer at det foreslåtte gruveområdet hadde mer polonium enn referanse stedet. Variasjonene av 210Po avspeilet at av U i jord til støtte for en forventning om at under 0,2 m under jordoverflaten , er alt 210Po skyldes nedbrytning av U som hovedsakelig består 238U .

210Po aktivitetskonsentrasjonen i C. mopane var på sitt høyeste i prøve 5 ved 1250 ± 65 Bq / kg , som også hadde den høyeste U -konsentrasjon , og det var liten variasjon for resten av prøvene med en rekke , 47 ± 4 til 66 ± 6 Bq / kg . Basert på den nære aktivitetskonsentrasjons spekter av 210Po på det foreslåtte gruveområdet , en signifikant forskjell mellom 210Po konsentrasjon på det foreslåtte gruve og referanse områder ble innhentet . Videre ble linearitet mellom 210Po og U redusert , og dermed reflekterte at ved lave jord konsentrasjoner , rot inntaket var av mindre betydning for den 210Po opphopning av planter i forhold til atmosfærisk nedfall . Imidlertid prøve 5 , med sin 210Po konsentrasjon på minst to ganger så stor som på de gjenværende prøvene indikerte at ved høye konsentrasjoner jord , spiller rotopptak en betydelig rolle i økologisk overføring av 210Po.

Forutenom det faktum at *I.* Belina larver fôret sårt på C. mopane blader , ble ingen sammenheng mellom 210Po i de to trofiske nivåer funnet . Dette kan tyde på at alderen på larvene har en effekt på deres evne til å akkumulere 210Po fordi larvene ble høstet på ulike stadier i livet.

Contents

1. Introduction

The economy of Botswana has been based on agriculture during the colonial era. A decade post-colonial era saw discovery of minerals particularly diamonds which transformed Botswana's economic landscape. The proceeds from diamond mining catapulted the country's economy from one of the world's poorest country to a medium income country over three decades (Leith, 2009). While mining offered more in terms of revenue than its predecessor (van Engelen, Malope, Keyser, & Neven, 2013), mining and processing of minerals proved to be more potent environmental polluters compared to agriculture. Mineral mining and processing are highlighted as the main anthropogenic sources of contamination of surface soils, with contribution of metals from overburden and from mine waste. Open pit mining which is earmarked for use in the envisioned uranium mine that are assumed to be established in Serule area of Botswana is expected to bring to the surface tonnes of uranium ore waste (A Cap Resources, 2011).

While uranium is a ubiquitous part of the environment and hence found in a series of minerals, U has never been found in metallic form in nature (Choppin, Liljenszin, & Rydberg, 2002). Uranium is mined from ore bodies, i.e., rocks with impure, but economically valuable, minerals of interest (Dill, 210). These rocks are weathered by natural and artificial weathering activities, which can be physical or chemical in nature, to form soil. The concentrations of metals in soils vary laterally and horizontally as a function of mineral composition of parent rocks from which they are formed and minerals' migration patterns (Sheppard, Sheppard, Ilin, Tait, & Sanipelli, 2008). Natural weathering of rocks provides background concentration of metals in soils whereas anthropogenic activities cause pollution since they elevate the metal concentrations above background levels (Roberts & Johnson, 1978; Walker, Silby, Hopkin, & Peakall, 2012). Metals pollution is perpetual as they have a high latency period in the environment owing to their non-degradable nature (Volpe, et al., 2009), making control at the source the most effective intervention strategy.

Effective intervention strategies are aimed at circumventing risk posed by these contaminants; hence the strategy is cognisant upon risk assessment. Risk assessment is defined by source term characterisation, transport in soil, uptake by plants and finally transfer to herbivores and carnivores (Salbu, 2007). The source term is represented by new and unstable soils deposited on the surface post mineral ore mining and processing. The sparsely cohesive soils are left susceptible to attack by water and wind erosion and thereby further spreading contamination occur due to erosion to surface soils and water bodies downstream the U mining site (Vega, Covelo, Andrade, & Marcet, 2004). However, the coarse nature of the new soil to "lock in" metals and hampering their migration from soil to plants difficult rather because trace metals enter plants through root uptake (Ernst W. H., 1996).

Plants are entry points for trace metals from soils transferred into terrestrial ecosystems from their substrate, (Golmakani, Vahabi Moghaddam, & Hosseini, 2008). However, the bioavailable fraction is a function of the mineral ore composition which includes secondary minerals in the form of trace metals and metalloid as trace inclusion (Dill, 210). In areas where acid leaching is employed to extract mineral of interest (IAEA, 19997) or in waste containing elevated concentration of sulphides which can be oxidised to sulphuric acid in contact with air and waters (Monte, 2010), trace metals in course mine waste soil can be leached into solution. This makes the metals more mobile and potentially bioavailable as aqueous metal cations that can easily cross membranes into plant roots (Salbu, 2009; Monte, 2010). Figure 1 summarises the dynamic nature of physico-chemical forms of metals with emphasis on mechanisms responsible for converting metals from one species to another and vice versa as well as methods for determining element species.

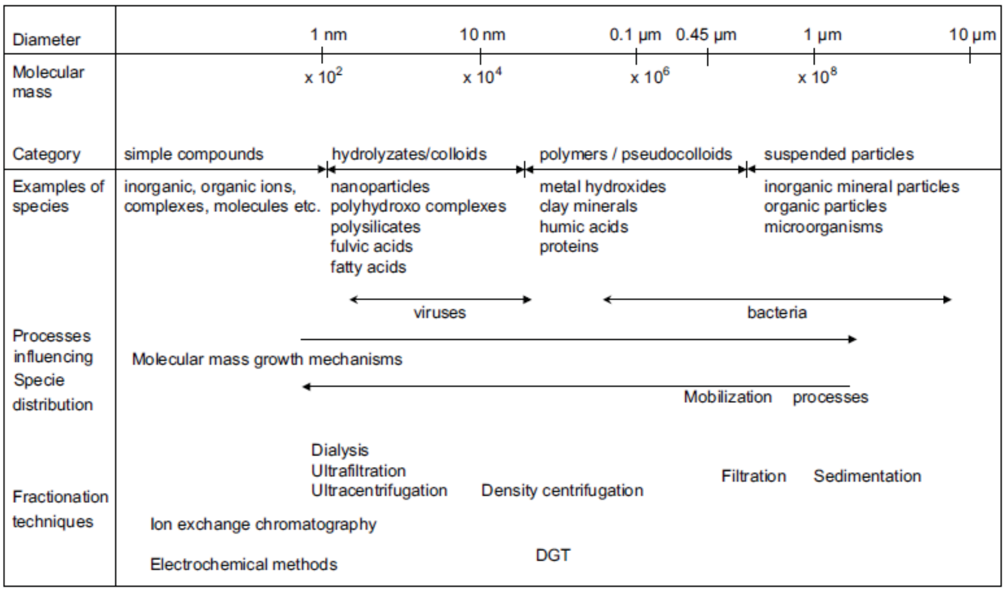

Figure 1; Size distribution, transformation processes, fractionation techniques of metal species in solution adapted from Salbu (2009)

Some of the trace metals ad/absorbed by plants are beneficial as they are essential nutrient and can also act as catalysts, relays for electronic messages and form part of protein structure in living tissues (Ernst W. G., 2012). Still other trace element have no known physiological function Thus, the former are termed essential metals while the later are toxins(Walker, Silby, Hopkin, & Peakall, 2012). Essential metal have both a lower, below which organisms fail to function normally, and upper a threshold, above which they become deleterious to biological systems (Ernst W. H., 1996). Toxins only have the upper limits, hence most epidemiological investigations related to the health of living systems concentrate on these elements (Volpe, et al., 2009). Figure 2 (a) and (b) are schematic diagrams showing responses of organisms to non-physiological metals and their essential counterparts, respectively.

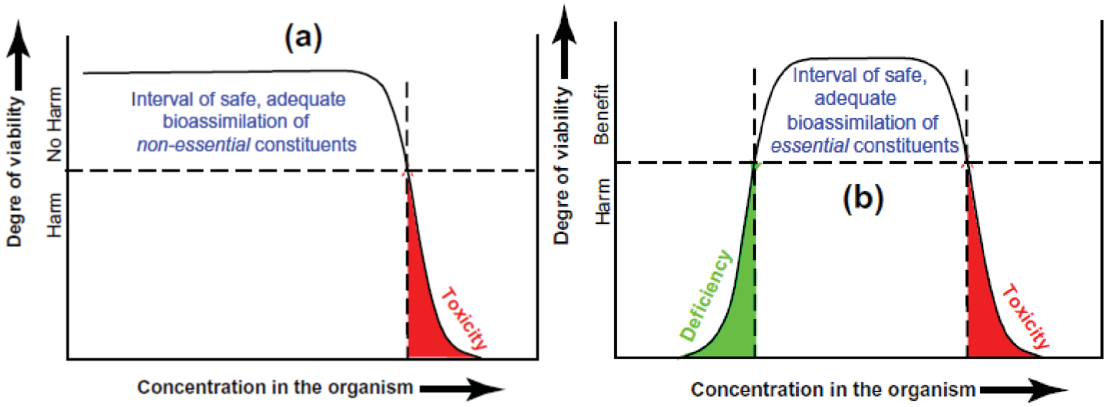

Figure 2; Schematic representation of dose-response relationship for non-essential and essential metals in living organisms (Ernst, 2012)

Metal toxicity varies from nephrotoxicity, carcinogenicity, to brain and liver damage in animals among others. These effects can be synergic, additive or antagonistic when the metals exist in the same medium and at the same time (Spurgeon, et al., 2010). Another hazard commonly associated with uranium mining is environmental contamination with normally occurring radioactive materials, NORM. NORM contributes more that 80 % of radiation exposure doses to humans with the uranium decay chain including especially radium and daughters (Matthews, Kim, & Martin, 2007). Uranium ore also contains other minerals and after mining the ore is crashed and uranium extracted using selective techniques, waste rich in secondary minerals is left in the area. (Aoun, El Samrani, Lartiges, Kazpad, & Saad, 2010; International Atomic Energy Agency, 1997). The presence of multi elemental contaminants and their migration to plants has been observed in mining areas in Botswana (Nkoane, Sawula, Wibetoe, & Lund, 2005; Ekosse, Ngila, & Forcheh, 2005;

Mogopodi, Mosetlha, Torto, & Wibetoe, 2008) and radioactivity in Botswana soils (Murty & Karunakara, 2008). However, there is no data on heavy metals and radionuclide migration from soil through plants to herbivoures in Botswana.

1.1. Hypothesis

It is hypothesised that surface soils in the Serule area, Botswana, have high concentrations of a series of trace metals, arsenic and uranium and its decay products. The elements are believed to migrate from soil to plants by root uptake, and further transfer through trophic levels in to the food chains as part of nature's nutrient cycle (Kabata-Pendias, 2004). Therefore, in Serule area transfer of trace metals and radionuclides to *Colospermum mopane* and to *Imbrasia belina* which is eaten by men is expected.

1.1.1. Objectives

To test the above hypotheses, the aim of the study is to establish background concentrations and transfer factors from soils to plants for Cu, Fe, As, Mn, Cr, U, Pb, Co, Ni, Zn, and ^{210}Po before uranium mining is established in the area. The above hypothesis will be tested by quantifying the ecosystem transfer by

1. Establishing metal concentrations and ^{210}Po activity concentrations
    a. in soil as substrate
    b. in *Colospermum mopane* as primary producer
    c. in *Imbrasia belina* as herbivore and
2. Calculating
    a. transfer factors (TF) for each trace metal and radionuclide from soil to plant on a dry weight basis
    b. concentration factor (organism/leaves) of each trace metal and radionuclide for the caterpillar, *I. belina* feeding on *C. mopane* leaves on a wet weight basis

The results will serve as benchmark for the U mining industry to be established and should be highly valuable for future evaluation of potential contamination associated with the industrial activities

2. Theory

2.1. Uranium industries

Uranium is the sole fuel for the current generation of nuclear power plants; hence it is the most extensively mined of the naturally occurring radioactive materials. The use of uranium as a fuel has led to the nuclear power production being named the uranium fuel cycle (Tripathi, Sahoo, Jha, Khan, & Puranik, 2008). The uranium fuel cycle comprises ore mining and milling, fuel enrichment and fabrication, nuclear power plant (reactor) operation, spent fuel storage and transport, fuel reprocessing and radioactive waste management. All activities preceding the reactor form the front end of the nuclear fuel cycle while those that take place after this demarcating line are referred to as backend (Choppin, Liljenszin, & Rydberg, 2002; Yassi, Kjellstrom, De Kok, & Guidotti, 2001).

The frontend of uranium fuel cycle poses the greatest radiological risk to man and the environment (O'Brien & Cooper, 1998), when reactor accidents are excluded. The scope of this study will be limited to mining and milling because they are the only activities planned for in Botswana’s uranium industry (A-Cap Resources, 2012).

2.1.1. Mining and milling

Uranium fuel cycle starts with exploration, and mineral ores with uranium abundance ranging from 0.3 to 20% (w/w) are usually mined. The depth of the ore bodies informs the type of mining to be employed; open pit is preferred for near surface ores, while deep laying ores are brought to the surface through underground mining. The latter is more costly hence it is applied to ores which are richer to justify the economics of the mining venture. The ore is hauled to a milling facility where it is crashed and uranium oxide extracted chemically into solution through acid or alkaline leaching depending on the chemistry of the ore. The uranium laden solution is concentrated by liquid/liquid extraction into yellow cake which is purified by burning of other inclusions.

Different stages of the mining and milling of uranium presents a variety of potential hazards to man and environment; mine water, mill tailings and effluents which contain dissolved heavy metals and other impurities Figure 3. Processing of low grade ores, as much as 99% of the ore is discarded as tailings which comprise barren cake and liquor containing uranium series radionuclides (Tripathi, Sahoo, Jha, Khan, & Puranik, 2008).

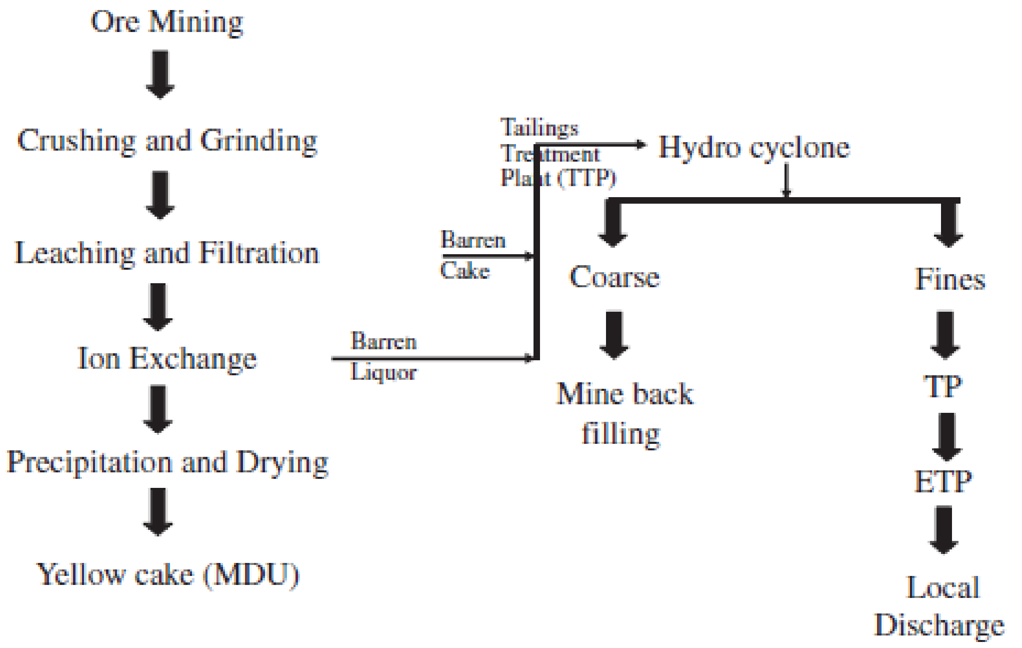

Figure 3: Processes involved in uranium extraction

2.1.2. Uranium

Uranium is the heaviest naturally occurring element and has an atomic number of 92. The heavy nucleus makes uranium unstable, decaying through a series Figure 4 of intermediate radionuclides to the stable lead isotopes (Choppin, Liljenszin, & Rydberg, 2002). The specific lead isotope attained at the end of a decay series is cognisant upon the parent uranium nuclide, and the most significant uranium isotopes are ^{238}U, ^{235}U and ^{234}U in descending order of abundance (El Aassy, et al., 2012; Ronald, 1998). ^{235}U and ^{238}U contribute equally to total activity of naturally occurring uranium besides difference in abundance at 0.72 % and 99.27 % respectively due radionuclides' differing specific activities. The specific activity depends on half-life and the shorter lived ^{235}U, $7.038*10^{8}$ a produces more disintegration per unit time than longer lived ^{238}U, $4.468*10^{9}$a (Sheppard S. C., Sheppard, Gallerand, & Sanipelli, 2005).

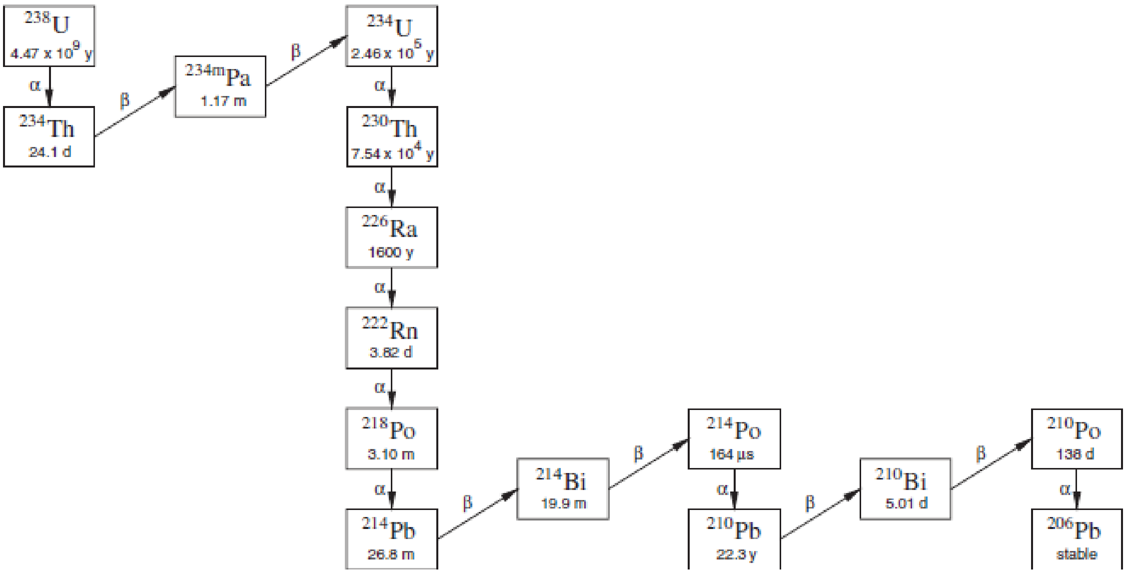

Figure 4: U^{238} decay series (Matthews, Kim, & Martin, 2007)

Natural uranium is not suitable for fuelling nuclear power reactors because of it has a low content of the fissile radionuclide, ^{235}U. ^{235}U readily undergoes thermal fission following capture of slow neutrons however, ^{235}U accounts only for 0.72 % of natural uranium with 99.27 % being ^{238}U (Ronald, 1998). Uranium with ^{235}U abundance of 3-5 % is needed to fuel Light Water Reactors (OECD Nuclear Energy Agency; IAEA;, 2011; Rothwell, 2010). Therefore, natural uranium need to be enriched in ^{235}U, and enrichment starts off by reducing U_3O_8 to UO_2 before re-oxidising it to gaseous UF_6 (Shayeganrad, 2013). The gas in spun at high velocities in centrifuges and the uranium isotopes are separated according to their masses. Due to small difference in masses of the radioisotopes, the separation is not very efficient hence lower enrichments are attained.

With more than a million years in half-life, specific activities of natural uranium is too low to cause any radiological concerns, however ^{226}Ra and its daughters, which are progeny of ^{238}U pose radiological risks. ^{226}Ra and its progeny have half-lives which are delicately poised to accumulate in the environmental while being short enough to cause cancer risk in organisms (Vesterbacka & Ikaheinmonen, 2005; Mora, Robles, Corbacho, Gasco, & Gazquez, 2011). Cancer risk is defined by the linear energy transfer of ionising radiation to matter which apportions most of the damage to the heavy alpha particles (Lin & Wu, 2009; Henricsson, Ranebo, Hansson, Raaf, & Holm E, A biokinetic study of polonium-210 in man, 2012). The most potent alpha emitter in the ^{238}U decay series is ^{210}Po.

2.1.3. Polonium

Polonium is a metalloid belonging to group 16 also known as chalcogens in the periodic table. It has many isotopes none of which are stable, but ^{208}Po, ^{209}Po and ^{210}Po which are of interest in environmental studies as they are the only ones with half–lives exceeding a day at 2.9 years, 102 years and 138 days respectively (Figgins, 1961). The first two are of anthropogenic origin while ^{210}Po is a daughter of ^{238}U decay series making it ubiquitous in the environment. ^{210}Po is second only to ^{241}Pu and five times more potent than its progenitor, ^{226}Ra in terms of radiotoxicity. Radiotoxicity of ^{210}Po is attributed to high linear energy transfer (LET) of its high energy alpha particle, 5.3 MeV coupled with short half-life culminating in high specific activity of 144 GBq/mg (Henricsson, Ranebo, Hansson, Raaf, & Holm, A biokinetic study of 209-Po in man, 2012). The high specific activity translates into a very low permissible body burden of 1.1kBq (Lee & Wang, 2013), translating into 0.05µg considered a deadly dose (Figgins, 1961).

Being a pure alpha emitter, ^{210}Po is strictly an internal hazard and it can be consumed with food and water, inhaled in air or enter through open wounds and assimilated with other chalcogens. The chalcogens form part of proteins making them essential except polonium which has no known biological function (Waska, Kim, Kim, Kang, & Kim, 2008). The diverse entry points are dependent on the source term of the pollutant with ingestion of food relying on the uptake of ^{210}Po from soil. In deep rocks and deep soil, ^{210}Po is distributed along the same pattern as its grand parent, ^{210}Pb where they are at equilibrium as evidence by their activity ratio reaching unity (Akyil, Gurboga, Aslani, & Aytas, 2008). Since ^{210}Pb migration is influenced by redox cycling of Mn and Fe, ^{210}Po has been found to follow this pattern (Wei & Murray, 1994). However polonium is particle reactive, attaching to organic matter and its uptake by plant roots is limited (Persson & Holm, 2011).

The sequestering of ^{210}Po in plant substrate leaves atmospheric deposition as the primary source of ^{210}Po contamination to above ground vegetative parts of plants (Akyil, Gurboga, Aslani, & Aytas, 2008). The deposition occurs when radium decays in porous soil and radon gas escapes into the atmosphere at a rate directly proportional to uncovered land masses. Once radon is in the atmosphere it decays via its 3.6 day half-life through a number of steps to ^{210}Pb and ^{210}Po both of which attach to atmospheric particles. The particles fall to the ground through gravitational pull in what is termed dry deposition but wet deposition characterised by precipitation washing out the particles is more efficient in scavenging ^{210}Pb and ^{210}Po from the atmosphere (Pham, Povinec, Nies, & Betti, 2013). Leafy plants intercept the deposition and are hence contaminated, offering radionuclides oral route of entry to herbivores (Brown, et al., 2011).

Since radiological detriment is based on the dose, length of exposure and proximity to radiation source, the short biological half-life of ^{210}Po 31 days (Henricsson, Ranebo, Hansson, Raaf, & Holm, A biokinetic study of 209Po in man, 2012) is expected to reduce its impact. Moreover, 138 days physical half-life of ^{210}Po suggests that it should be lost from ecological system in a short period. However, it is found to persist because its progenitor ^{210}Pb mimicking calcium and hence is deposited in bones where it continues to produce ^{210}Po.

2.2. Trace element associated with uranium mining

A lot of studies (Saha, Raychaudhuri, Chakraborty, & Sudarshan, 2010) have lent evidence to multi-stressor effects of radiation with trace metalsin a synergetic process observed when gamma irradiation of plant species increased concentration of iron and potassium. The same irradiation suppressed plant uptake of copper, calcium and manganese in an antagonistic relationship.

2.2.1. Manganese

Manganese is a transition metal and its multiple valences permit it to be involved in both reduction and oxidation reaction as it accepts or donates electrons respectively. The primary redox reaction is between Mn^{2+} and Mn^{3+} in biological systems with the latter being a fundamental component of metalloenzymes (Au, Benedetto, & Aschner, 2008). Due to its beneficial benefits to biological systems, manganese is classified as an essential nutrient albeit at minute concentrations as it can be neurotoxic at high concentrations. Concentration of manganese in living organisms is strictly controlled through balancing uptake and depuration rates as evidenced by increased liver metabolism and elevated Mn concentration in bile where large amounts of Mn were ingested (Au, Benedetto, & Aschner, 2008; Martinez-Finley, Gavin, Aschnar, & Gunter, 2013).

In abiotic environment, Mn exhibits a high affinity for oxygen, therefore occurs mostly as oxides. The oxides contain inter-convertible soluble and mobile Mn (II) or oxidised and sparingly soluble Mn (IV) (Meng, Zheng, Zhang, & He, 2009; Feng, Zhai, Tan, Liu, & He, 2007). Redox reactions between Mn species are a function of environmental factors such as temperature, pH, Eh among others. While oxidation is thermodynamically favoured, it progresses very slowly in nature (Mandernack, Post, & Tebo, 1995) unless when microorganisms are present to catalyse the oxidation (Battlefield, Saldotova, Lee, Spiro, & Tebo, 2013).

Oxidised Mn (IV) oxides mostly occur as anions with a very large surface area of highly reactive mineral phases. The reactive sites interact with aqueous ions of heavy metals like Pb, Zn, Co, Ni, As and Cr and hydrogen in soil pore water, adsorbing the cations and sequestering the affected heavy metals (Hennebel, De Gusseme , Boon, & Verstraete, 2009). Besides metals sequestering, Murray and Tebo (2007) observed Mn (IV) detoxifying As (III) and Cr (VI) by oxidising them to As (V) and Cr (III), respectively. Both interactions are based on rendering the heavy metal ions non-bioavailable.

2.2.2. Iron

Another transition element known to isolate metal from biological activity is iron even though its efficiency is outweighed forty times by manganese (Contin, Mondini, Leita, & De Nobili, 2007). Unlike manganese, iron only forms two cations, Fe (II) and Fe (III) with the latter being more stable in oxidising environment (Thompson, Chadwick, Rancourt, & Chorover, 2006). The oxidation is driven by the same factors as for manganese forming the gelatinous Fe (III) oxyhydroxide which represents a large mineral surface characterised by positive charge under acidic condition, and negative charge at high pH. At high pH, cations are adsorbed and immobilised in the Fe oxides only to be released if the acidic conditions or are reinstated (Akai & Anawar, 2013; Sen, Mahajan, & Khilar, 2002). More important characteristics for metal mobility are oxidation-reduction potential (Eh). When reductive conditions are formed, metal adsorbed to iron oxyhydroxides will be released with dissolution of the parent iron oxyhydroxide because Fe (II) is more soluble than Fe (III) (Thompson, Chadwick, Rancourt, & Chorover, 2006).

Iron importance is not limited to its role in biogeochemical cycle of other metals. In red blooded living organisms it is a fundamental part of the oxygen transporting vehicle, haemoglobin. Individuals with shortage of iron usually suffer from anaemia which can be life threatening condition requiring regular blood transfusions.

2.2.3. Arsenic

Arsenic is a moderately abundant chalcophilic metalloid of group IVB of the periodic occurring in more than 300 minerals on earth (Kyle, Breuer, Bunney, Pleysier, & May, 2011). Its abundance makes arsenic distribution to be influenced by exploiting mineral sulphides ores in which it is found in (IAEA, 1993). These anthropogenic processes could decrease or increase As bioavailability depending on the chemical environment particularly pH, redox potential and ionic strength (Akai & Anawar, 2013). In its natural environment arsenic exists predominantly as trivalent or pentavalent chemical species.

Both As (V) and As (III) are stable over wide range of pH and Eh which is not observed from most contaminants, but the presence of oxidants and reductants has profound effect on the prevalent species (Voigt, Brantley, & Rennet, 1996). In oxidising environment, as a chalcophile, arsenic occurs as insoluble arsenic (V) sulphide which decomposes to immobile arsenate oxyanions, AsO_4^{3-} upon contact with water (Ernst, 2012). The arsenate anion is readily reduced to mobile arsenite, AsO_3^{3-} under anoxic conditions rendering arsenic more bioavailable (Kyle, Breuer, Bunney, Pleysier, & May, 2011). Like most trace elements, bioavailable fraction of arsenic is scavenged from solution by iron, manganese and aluminium oxides as it exhibits a high affinity for the oxides' reactive surfaces (Hartley , Edwards, & Lepp, 2004). The reactive surfaces sequester As by strongly binding its anions, especially arsenate (Akai & Anawar, 2013).

The bioavailable and highly toxic inorganic of arsenic is absorbed by plants where it enters food chains, despite arsenic having no known benefits to living organisms (Marques, et al., 2011). If the body burden of arsenic exceeds a threshold, the affected individual could have cancers of the bladder, or lung, cardiovascular and skin disease among others (Ernst, 2012). This called for understanding of ecological migration of arsenic especially in uranium mining and processing where over 70 % of arsenic is released into mine soil waste (IAEA, 1993)

2.2.4. Cobalt

Cobalt is a transition element preferring +II oxidation state in aqueous media to thermodynamically unstable +III. It is found in association with nickel as part of vegetation, water, soil and rocks (Gal, Hursthouse, Tatner, Sreward, & Welton, 2008). In uranium ore, only 30 % of Co is leached with uranium during uranium recovery leaving a large portion in mine soil waste (IAEA, 1993). Due to its high affinity for organic ligands and Mn/Fe hydroxides, Co is mostly immobilised. However, in acid media which prevails in mine soil waste, the Co^{2+} ions are hydrolysed rendering them more bioavailable (Johnson, et al., 2011). Plants absorb the bioavailable Co fraction although the metal having no known physiological function. Lack of biological function means that no deficiency is observed, but at high Co concentrations, stunted growth, folia necrosis and reduced biomass production (Bakkaus, et al., 2005). In contrast, cobalt is an essential micronutrient in prokaryotes and animals as it is incorporated in metalloenxymes and vitamin B12 respectively. While these organic forms of Co are clearly beneficial, inorganic forms have no known purpose but are toxic at high concentrations especially where they take precedence over calcium is calcium activated enzymes (Simonsen, Harbak, & Bennekou, Cobalt metabolosm and toxicity, 2012).

2.2.5. Copper

Copper is an essential nutrient as it is an important component in sulphur containing proteins. Once incorporated in metallothionines, the transition metal's ability to undergo redox reactions, switching between cuprous, Cu (II) and cupric (II) oxidation states, makes the proteins effective enzymes (Stillman, 1995). Cuproenzymes give copper an essential role in ecosystem which it enters by being absorbed from soil pore water by plants in the form of highly mobile cupric cations. Cupric cations are the predominant species of copper in aerobic soils. In organic soils, the divalent copper cations are immobilised by forming complexes with amines and carboxyl groups in organic matter (Fulda, Voegelin, Ehlert, & Kretzschmar, 2013). The cations can also be immobilised by adsorbing to iron and manganese (oxyhydr) oxides' reactive phases.

Sequestering copper presents a challenge because it is mobile under both acidic and highly alkaline conditions, leaving a small window for reduced mobility between neutral to slightly alkaline pH (Fan, et al., Immobilization of copper in contaminated sandy soils using calcium water treatment residue, 2011). In strongly basic solution the Cu^{2+} cations adsorbed by organic matter is remobilised as organic matter is digested. In contrast, Al, Mn and Fe hydroxides which are also good scavengers of Cu disintegrate in acidic conditions also remobilising copper. (Contin, Mondini, Leita, & De Nobili, 2007). To sustain the sequestering of Cu by hydroxides, soil needs a buffer system to absorbed H^+ released during copper absorption. The protons, released at about twice the normality of Cu^{2+} cations lowers soil pH (Fan, et al., Immobilization of copper in contaminated sandy soils using calcium water treatment residue, 2011) which in turn destroys the hydroxides. The mobilisation of copper may cause plants to absorb more of the metal leading to toxic effects like disruption of enzyme activities and photosynthesis which may lead to reduced biomass production or even death

2.2.6. Zinc

Zinc is a peculiar transition element in that it forms only divalent cations, Zn^{2+}. Moreover, it is the only metal with dual functionality where it is involved in both structural and catalytic roles in biological systems (Gutierrez-Carbonell, et al., 2013). Like most metals it enters ecosystem through absorption from rhizosphere by plants roots as Zn^{2+} cations. The cations are formed by acid mobilisation of sequestered zinc in soil where its abundance is rivalled only by that of iron. The high abundance means that lower pH will mobilise more of the metal which cause toxicity because Zn is an essential micro-nutrient (Jovanovic, et al., 2011). The tell-tales of zinc toxicity include disruption of enzyme activities. However, some plant species have the ability to accumulate high concentrations of zinc isolated in their vacuoles. The isolated zinc does not affect physiology of the plants, but pose danger to herbivoures feeding on these plants as they face over exposure to zinc (Gutierrez-Carbonell, et al., 2013).

2.2.7. Lead

Lead is a grey and soft group IV metal characterised by low melting point in relation to other metals. It forms both covalent and ionic bonds because of its borderline electronegativity, exhibiting both IV and II oxidation states. It has high affinity for particles adsorbing on organic particles, hydroxides of Fe, Mn and Al reducing its mobility. Reduced mobility leads to less bioavailable fraction, which in turn culminates in reduced amounts being absorbed by plant roots (Jovanovic, et al., 2011). The little that is absorbed is not mobile even within plants such that more than 90 % of lead detected in plant shoot is from folia input, that is, atmospheric deposition rather than soil origin (Zheljazkov, et al., 2008). Though incorporated into plant structures, lead plays no physiological role even in animals that subsequently feed on plants. The animals have a very low tolerance limit to lead because it causes retarded mental development in young children which is observed as learning difficulties (Needleman, et al., 1979) while in adults it causes cardiovascular diseases.

As a metal, lead is non-biodegradable and persistence in the environment resulting in bioaccumulation through trophic levels. Problems with lead bioaccumulation are exacerbated by low lead excretion in exposed humans (Ahmed & El Razek, 2013). Once in the human body, lead is distributed primarily between soft tissues, blood and bones with bones having the lion’s share of total body burden (Henricsson, Ranebo, Hansson, Raaf, & Holm E, A biokinetic study of polonium-210 in man, 2012). However, the highest concentration is found in blood where the target cells are erythrocytes due their high affinity for the metal. Lead interferes with hem formation in erythrocytes, resulting in free ion accumulation within the cells. The iron ions are liable to redox reaction and produce oxidative species which can be toxic to the erythrocytes (Rendon-Ramirez, et al., 2013)

2.2.8. Cadmium

Cadmium is a highly toxic metal that readily dissolves in water to form Cd^{2+} cations (Xhao, et al., 2013) The cations are absorbed by higher plants from soil pore water and disrupt physiological processes; at concentrations exceeding 5 ppm in dry plant material (Jovanovic, et al., 2011), cadmium induces reduced photosynthesis owing to inhibited chlorophyll formation (Liang, et al., 2013). Some plant can counteract adverse impacts of cadmium resulting in increased cadmium concentration exposure for herbivores feeding on the plants (Xhao, et al., 2013). Cadmium is accumulated in humans because of its long biological half-life of 10 to 30 years (Cheng, et al., 2013), and the elevated concentrations can cause necrosis which disrupts normal organ functions leading to hepatic, pulmonary and renal tubular diseases (Suhartono, Triawanti, Yunanto, Firdaus, & Iskandar, 2013).

2.3. Soil-Plant transfer

Soils serve as indicators of environmental pollution due to metals as they act as reservoirs for these pollutants (Navel & Martins, 2014). The pollutants are taken up by plants from soil which is a universal substrate for plant growth. The primary pathway followed by trace metals as they enter plants is absorption of the aqueous metal ions in soil pore water in the rhizosphere (De Vries, McLaughlin, & Groenenberg, 2011; Jovanovic, et al., 2011). Since the fraction that is assimilated by plants is in water, the portioning of minerals between soil particles and pore water is the one that is biologically important to plants. This has led to the determination of the concentration of trace metals in soil pore water rather than total soil concentration taking centre stage in studies on mobility, accumulation and accessibility of metals to plants (De Vries, McLaughlin, & Groenenberg, 2011).

Soil pore water concentration is not the only factor that affects the behaviour of metal within and between biotic and abiotic environments. De Vries, McLaughlin, & Groenenberg (2011) argue that, before metals can be bioavialable, they have to be geoavailable. The aurthors define geoavailability as the migration of minerals from abiotic solid phases to biosphere under the influence of environmental conditions and processes which can be mechanical, biological or chemical in nature (Molina, Schaider, Donaghey, & Shine, 2013). Some of the environmental processes and conditions influencing partitioning of metals are pH, organic matter content, Al, Mn and Fe (oxyhydr) oxides and cation exchange capacity (Jovanovic, et al., 2011) as well as activity of earthworms which play both a mechanical and biological roles in metal migrations (Leveque, et al., 2013).

As they move through soil, earthworms break down soil into smaller particle which can be attacked easily by soil chemical aiding in binding or loosening metals from soil particles (Leveque, et al., 2013). Generally, soils with high pH, organic matter and clay content and have low metal mobility. The sluggish movement is also observed in plants resulting in metal concentration being high in plant roots compared to shoots. Cu, Mn, Zn, Mo, and Cd are exception as they migrate quickly throughput plant and are found in fruits and leaves (Jovanovic, et al., 2011)

3. Materials and methods

3.1. Site description

The study area is a proposed uranium mining site, Letlhakane project, Figure 5 and this study could provide the currently non-existent background data of trace metal and radionuclide prevalence in the ecosystem. It is located in the east of Botswana, 21°50’S and 27°25’E along the country’s major transport corridors; the main highway called A1 and the only railway line. The routes connect the biggest settlement areas in the country with the capital, Gaborone lying 350 km south and Francistown 90 km north while the copper mining town of Selebi Phikwe is 60 km to east (A-Cap Resources, 2012) Electricity is also readily available with power cables leading from Serule to Gojwane passing close to the site. The mining project will source water from underground reservoirs. All these are expected to give the project a favourable chance of being undertaken.

The area is characterised by average annual rainfall between 350 and 400 mm, most of which falls between October and March and the mean minimum temperatures are 8 °C in the June/July while the hottest moths are December and January averaging 33 °C at the other extreme. The dominating geological formation is arkoses and carbonaceous shale with coal seam lithology belonging to dwyka and ecca series stratigraphic unit of late carboniferous to Jurassic era Karoo systems. (De Wit and Bekker, 1990; Geological Surveys and Mines Department, 1973). This geology is the foundation of vastly occurring luvisol soils. The prominent vegetation is the mopane savannah which is dominated by *Colophospermum mopane,* the preferred host to the monophagous *Imbrasia belina* caterpillar, a larval stage of these emperor moths (Ekosse *et. al.* 2005 and Hrabar *et. al.* 2009) The terrain is flat to gently undulating plains sandstone of the sandveld origin.

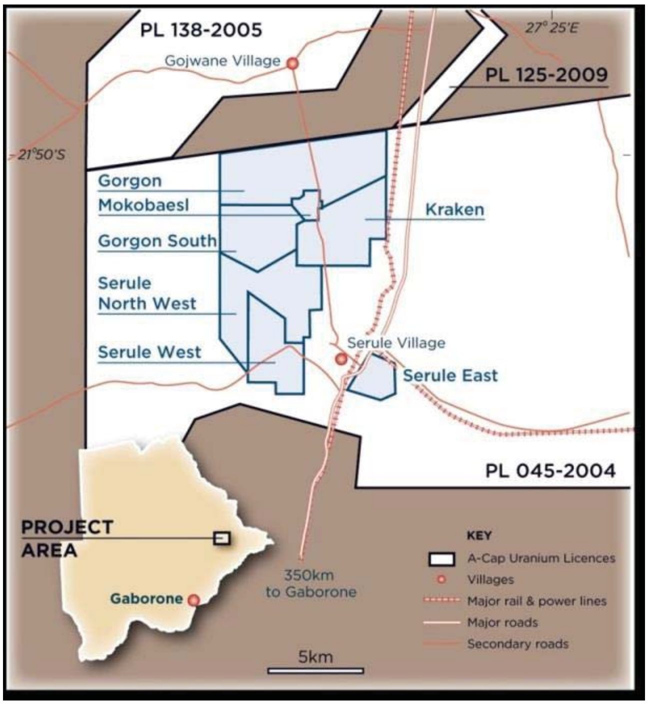

Figure 5: Map of Letlhakane Uranium Mining Project, Serule Botswana. Adopted from A Cap Resources Annual Report, 2011

3.2. Sampling

A random sampling method was applied within the study area. The boundaries of the study area were used to identify the confines of sampling and the exact locations were informed by presence of *Imbrasia belina*. *I. belina* is situated at the top of the studied food chain which made its presence crucial. *I. belina* is bivoltine, i.e. produces two generation per annum; the first and more dominant generation reaches outbreak proportion from October to December (Greyling, van der Bank, Grobler, & Wessels, 2001). The presence of *I. belina*, particularly in the March/April season is dependent on rainfall, sampling was planned when the rainfall was predicted to be above average by Botswana Metrological Services, this proved to be wrong. However, sampling was done in the last two weeks of March 2012 even though the vegetation especially the herbaceous plants were dry but *C. mopane* was green due to its efficient root system, Figure 6.

Figure 6: A pond with the study area characterised by dried out grass and green leafy C. Mopane due to the latter having overreaching root network which in enables it to access water below the former's range

3.2.1. Colospermum mopane leaves

Only trees infested with *Imbrasia belina* were samples because the insect caterpillars have poor mobility hence spend most of their feeding live on a single tree. This feature made the choscе of a nesting host plant by *I. belina* moths very important as it shall have enough leaves to support her hatchlings. The poor mobility means that the only nutrients including metals are supplied by the *C. Mopane* host. Only the leaves of the trees are used for food, hence about 25 leaves were harvested from each plant, stored in polyethylene bags and sent to the Isotope Laboratory at NMBU in Ås, Norway.

Figure 7: Imbrasia belina infested Colospermum mopane trees were identified by crown with lost foliage due to the caterpillars feeding on the leaves

3.2.2. Sampling of *Imbrasia belina* caterpillars

Due to limited rainfall, *Imbrasia belina* caterpillars were not only scarce but even infested trees were not heavily laden with them, Figure 7. While the usual number of caterpillars is eighty, less than thirty were found per tree during sampling and twenty of them were harvested for analysis. This resulted in reduced mass of *Imbrasia belina* being harvested. One sample was collected at each of the five site; the caterpillars were degutted, Figure 8 and put in polyethylene bags and transported to Gaborone where they were partly dried at 30°C in an oven for six hours. Each sample was then sealed in three layers of self-locking polyethylene bags and transported to Norwegian University of Life sciences.

Figure 8: Imbrasia belina caterpillars were plucked from the trees and degutted on site.

3.2.3. Soil

Three shallow pits of 0.3 m by 0.3 m width and length were made with a spade to a depth of 0. 3m, Figure 9. The excavations were made within a meter radius from the tree that leaves were sampled and the depth represented the root zone for *C. mopane*. A hand trowel was used to scrape equal amounts soil from the bottom of each excavation and the three soil samples from excavation were mixed to make a one composite sample. A composite sample was chosen to reduce effects of local variation in chemical composition. The soil samples were packaged like the dried biological samples and also sent to UMB.

Figure 9: A typical soil sampling pit showing exposed roots of Colospermum mopane

3.3. Sample treatment

Upon reaching NMBU, all samples were transferred to paper bags and dried in cupboards at 40 °C over a two week period. At the end of this incubation period, the soil samples were grounded by hand mortar and pestle and particles larger than 0.2 mm were sieved out and discarded. The organics, leaves and caterpillars, were ground using a blender. Each of the ground samples were transferred 100 ml air tight polyethylene bottles to keep them from reabsorbing moisture prior to analysis.

3.4. Analysis

3.4.1. Polonium analysis

For the ^{210}Po determination, ^{209}Po was added to the sample prior to digestion, and used as an internal analytical tracer (yield monitor) in order to correct for losses throughout the analytical procedure. The internal standards are advantageous in that they easily work with prevailing complex sample matrices compensating for chemical losses and counting variability (Lin & Wu, 2009).

2.4.1.1. Digestion for Po-determination

A gram of soil sample was digested in a Teflon tube with 10 ml of 65 % HNO_3 using Ultra Clave high performance microwave reactor model by Milestone, Shelton, United States of America shown in Figure 10 providing controlled temperature and pressure. Po – 209 tracer corresponding to 0.3 Bq was used as internal standard. The digestion was aided by immersing the reaction vessels in 5 ml and 10 ml of H_2SO_4 and H_2O_2 respectively dissolved in 300 ml water and exposing them to microwave with an automated protocol. The automated protocol started at 29 °C and 50 bar which rose to 50 °C and 54 bar in two minutes and held at these conditions for eight minutes, after which they were gradually upped to 110 °C and 75 bar over a period of ten minutes. The physical conditions were held for a further ten minutes prior to being subjected to half an hour of raise to reach 258 °C and 145 bar where they remained for 20 minutes. At the end of sample digestion protocol, the reaction vessels were cooled and depressurised over an hour to reach 70 °C and half a bar. Finally the samples were quantitatively transferred to beakers by washing the Teflon tubes consecutive times with 5 ml 65 % HNO_3 portions. The reaction mixture was evaporated to dryness over a sand bath set at 65 °C.

Figure 10: Ultra Clave High Performance Microwave Reactor by Milestone Incorporated, Shelton, USA

2.4.1.2. Oxidation

The dry contents of the beakers were oxidised with 5 ml hydrogen peroxide and heated in a sand bath at 200 °C for ten minutes. Another ten minutes were spent heating the samples at 200 °C after being acidified with 0.8 ml of 12 M hydrochloric acid. The acidified samples were diluted with 15 ml of MilliQ water and heated for 15 minutes in the same sand bath after which a gram of $NH_2OH*HCl$ was added and allowed to react for ten minutes at the same temperature in order to supress interfering metals like Fe and Mn. The beakers were covered with watch glass during each incubation stage to curb Po losses due to its volatilisation at high temperatures.

2.4.1.3. Deposition

The solution was filtered into deposition glass column fitted with a polished nickel disc such that only one side of the discs was in contact with the solution. The residue was rinsed thrice with 3ml 1mg/ml NaCl in 0.1 M HCl to ensure qualitative transfer of polonium solution to the column. The column were sealed with Al foil and incubated at 90 °C to reduce polonium volatilisation during the four hour deposition periodFigure 11. At the end of incubation period, the columns were allowed to cool to room temperature, solution discarded and discs removed with great care take not to touch the side on which Po was deposited. The discs were rinsed with milli Q water and dried in an oven at 60 °C.

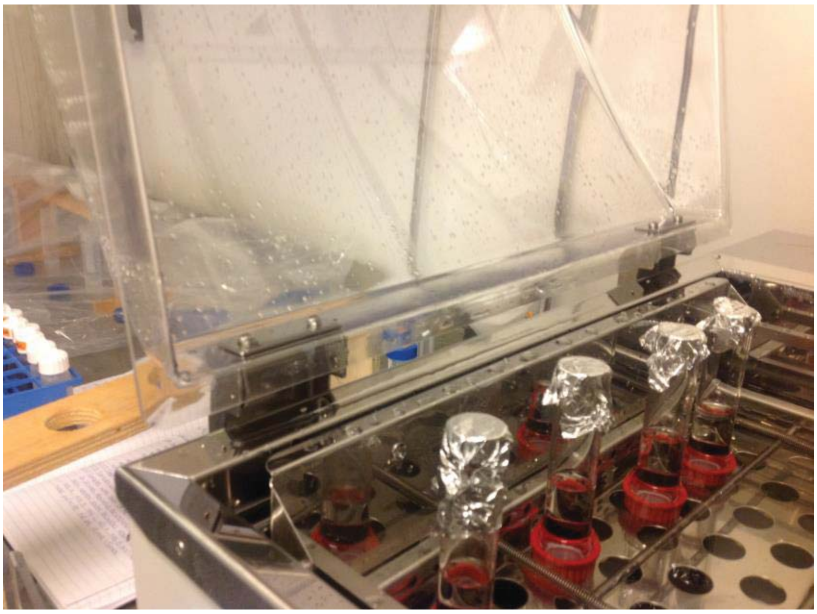

Figure 11: Set-up for depositing ^{210}Po onto nickel discs in a water bath at 90°C

2.4.1.4. Counting of alpha spectrum

Counting of alpha spectrum of Po deposited on nickel discs was performed on Canberra Alpha Spectrometer equipped with 7401 and 7401 VR detectors housed in a vacuum stainless steel chamber which reduces background reading below 1count/hour for energy above 3MeV. Signal was stabilised by a microprocessor and rendered on to the display by Gene2000 software, a product of Canberra Inc. Australia that control the whole spectrometer.

The software quantified ^{210}Po at its characteristic 5.34 MeV peak against the 1Bq ^{209}Po with a peak of 4.88 internal standard at 4.88. Example of a spectrum is given in Figure 12

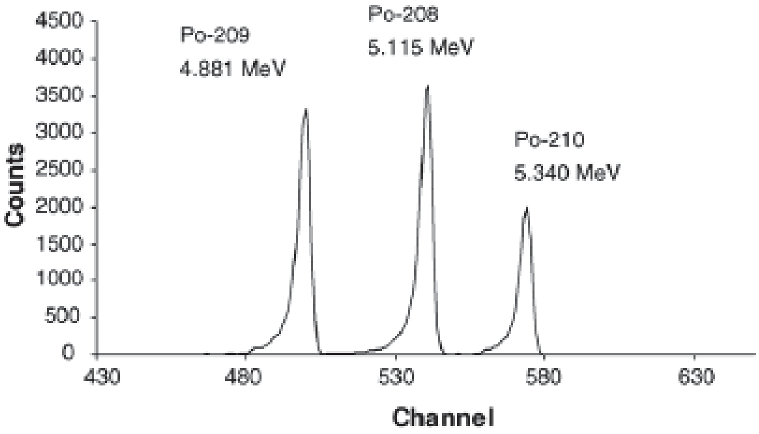

Figure 12: Graphic representation of alpha spectrum depicting energies of different polonium isotopes (Vesterbacka & Ikaheinmonen, 2005)

Figure 13:VR 740 Alpha spectrometer by Canberra, Sidney, Australia

Analysis of polonium in organic samples; *Colospermum mopane* leaves and *Imbrasia belina* caterpillars.

The organic samples were treated like soil samples except that the sample size was doubled to 2 g while ^{209}Po internal standard was reduced to 0.1 Bq in anticipation of reduced concentration of polonium in *C. mopane* leaves and *I. belina* caterpillars. The last modification was to increase nitric acid used for digestion to 15ml and this volume was added gradually to avoid over-boiling due to the exothermic reaction of organic material with acid.

A duplicate of each sample, three standard samples, three analytical blanks and three reagent blanks were used to control quality of analytical process

3.4.2. Trace metals quantification

3.4.2.1. Digestion

The same quantities were weighed out as in ^{210}Po analysis and a solution of In, Tl, Te and Rh in 2 % HNO_3 were added as internal standards. Five analytical blanks, four soil reference material and three plant (bush twigs and leaves; spinach; pine needles) reference materials were digested in parallel with triplicate of each soil and *C. mopane* samples. There was no reference material for *I. belina* hence the samples were only ran against analytical blanks. The contents of all reaction vessels were quantitatively transferred to 50 ml plastic tubes and diluted to the mark with Milli-Q water before being taken for identification and quantification of trace elements.

3.4.2.2. Identification and quantification

All trace elements were identified and quantified buy the use of triple quadrupole inductively coupled plasma-mass spectrometer from Agilent, USA as shown in Figure 14 by Karl Andreas Jensen at UMB, Aas.

Figure 14: Triple quadrupole inductively coupled plasma-mass spectrometer model 8000 equipped with automatic sampler from Agilent, USA

3.5. Standard reference materials

For purposes of quality control, standard reference materials from reputable organisations were used to correct losses of analytes during sample handling. The standard reference materials came in the form of media similar to that of samples doped with known amounts of analytes. The reference materials are analysed parallel to analytical and blank samples where the result are thought as being obtained in similar matrix. Results from reference materials are used to calculate percentage yield from which accuracy of the analytical method can be inferred. Precision of the results is obtained from spread around the mean of a triplicate run for each sample. Finally the blank samples quantify contamination from the analyst and, or laboratory apparatus.

Three standard reference materials were for plant samples;

- NCS DC 73348 comprising twigs and leaves of different plant species. The sample was prepared in 2008
- NCS ZC 730313 contained spinach leaves packaged in 2010
- 1575 was a standard reference material made up of pine needles packaged by US Department of Commerce’s National Institute of Standards and Technology

Although there were no reference materials for *Imbrasia belina*, it was improvised and plant reference materials used in quantification of trace metal concentration in *I. belina*. On the other hand, the following three soil reference materials were used

- GBW 07401 from China National Analysis Center for Iron and Steel
- NCS DC 73324
- 2709a San Joaquin from US Department of Commerce's National Institute of Standards and Technology

Reference concentrations of the trace elements are shown in attachments I to VIII

3.6. Transfer factors (TF) and concentration ratios (CR)

The study is to determine means by which concentration of trace elements and radionuclides could be predicted throughout the food chain if concentration in the preceding trophic level is known. The predictions are usually made from simplistic modelling where linearity is assumed and the system is regarded as being in equilibrium. Therefore, transfer factor can be presented as Equation 1

Equation 1: Transfer factor formula

$$Transfer\ Factor = \frac{Concentration\ in\ C.mopane}{Concentration\ in\ soil}$$

While Concentration factor is the ratio of trace element concentration in animal species and concentration in food source, expressed as

Equation 2: Concentration factor

$$Concnetration\ factor = \frac{Concentration\ in\ I.belina}{Concentration\ in\ C.mopane}$$

3.7. Statistics

Statistical analysis was done with MiniTab version 16. Descriptive statistics expressed the mean for each analytes and central tendency was evaluated by standard deviation. The variation of analytes with each other was analysed with principal component analysis, PCA correlation between any chosen analytes was expressed by correlation data.

4. Results and discussion

4.1. Detection limits, precision, accuracy

Polonium was quantified by a single run of alpha spectrometry over two days or until uncertainty fell below 10%. Quantification of ^{210}Po was calculated against ^{208}Po internal standard which presented a clearly resolved peak relative the analyte. However, the rest of the analytes were quantified in triplicates and precision expressed as standard deviation while accuracy was based on percentage yields calculated against standard reference materials listed in appendices 1-8. The reference materials were materials with similar sample matrices to the samples doped with known quantities of analytes. The analytes' % recovery was calculated from standards prepared using reference materials and these recoveries used to quantify analytes in samples.

All blank samples recorded concentrations below detection limits of both alpha spectrometry and ICP - MS systems used in this study. This was a testament that there was no cross contamination of the samples. ^{209}Po internal standard used in alpha spectroscopy was measured above 95 % recovery of the initially amount added which implied that only small quantity polonium was lost during sample analysis hence it can be concluded that the analytical method employed to quantify ^{210}Po was accurate. By the same token, reference samples doped with trace metals exhibited % recoveries in access of 90 % also implying accuracy of the ICP – MS in quantifying trace metals. Precision was measures by % standard error which was always below 10 %.

4.2. Soil properties

Some of soil properties that influence migration of metal ions from soil to plant are listed in Table 1 below. Soil pH ranged from slightly acidic 6.15 to basic 8.15 for analytical samples which presented a wider range in comparison to 6.2 to 7.1 found for the same study area (Dikinya, 2013) while the control sample recorded slightly more acidic 5.51. Acidic control sample could be due to some richness in organic matter and or a result of increased precipitation due to the sample having been collected over 100km away from proposed uranium mine. The proposed mining site had slightly acidic to basic soils which could be attributed to low annual precipitation common in arid to semi-arid areas (Tserenpil, et al., 2013).

Ion migration is also influenced by abundance and quality of Fe and Mn compounds in soil hence these also need to be quantified (Sen, Mahajan, & Khilar, 2002). Total specific concentration of Fe in dry soil ranged from 12 ± 0.0 to 23.5 ± 0.7 g/kg with reference sample at 16.5 ± 0.7 which is close to mean, 17.9 ± 4.4 g/kg implying no difference between analytical and reference site. Mn was below Fe by two degrees of magnitude with a mean of 0.25 ± 0.3g/kg and the reference site had 0.46.3 ± 1.5 g/kg. Therefore, ANOVA analysis at 95% confidence and $p < 0.001$, mean Mn for analytical samples is statistically lower than Mn at reference site while Fe showed no significant differences. The high ratio of Fe to Mn makes Fe the dominant source of soil ameliorants because high Fe concentration does not pose toxicological risk to plants (Contin, Mondini, Leita, & De Nobili, 2007).

Table 1: Properties of soil from proposed uranium mining site in north-east Botswana

Sample No.	**Fe (g/kg)**	**Mn (g/kg)**	**pH**
1	12000 ± 0.0	240 ± 0.0	8.13
2	23500 ± 7.1	255.0 ± 7.1	6.15
3*	24000	280.0.	6.44
4	12500 ± 7.1	205.0 ± 7.1	7.89
5	16000 ± 0.0	270.0 ± 0.00	6.17
6	16005 ± 7.1	255.0 ± 0.71	5.51

NB: * Sample 3 duplicate had an error because internal standard for ICP-MS was not detected hence the run was discarded resulting in no standard deviation.

The two metals exhibited good correlations with pH: Mn in soil (R^2 = -0.67) and Fe in soil (R^2 = -0.80). The negative correlation observed here contradicts the theory that at high proton, (H^+) concentration observed at low pH, less reactive metals like Fe and Mn are displaced from their compounds by protons and are lost through leaching (Sen, Mahajan, & Khilar, 2002). This inverse correlation is shown inFigure 15. The decline in Mn and Fe concentration in soil with increase in pH could be explained in terms of heterogeneity of soil chemistry, for example the sites with high pH could have high oxidizing potential which would lead to dissolution of Mn and Fe oxyhydroxides (Sen, Mahajan, & Khilar, 2002).

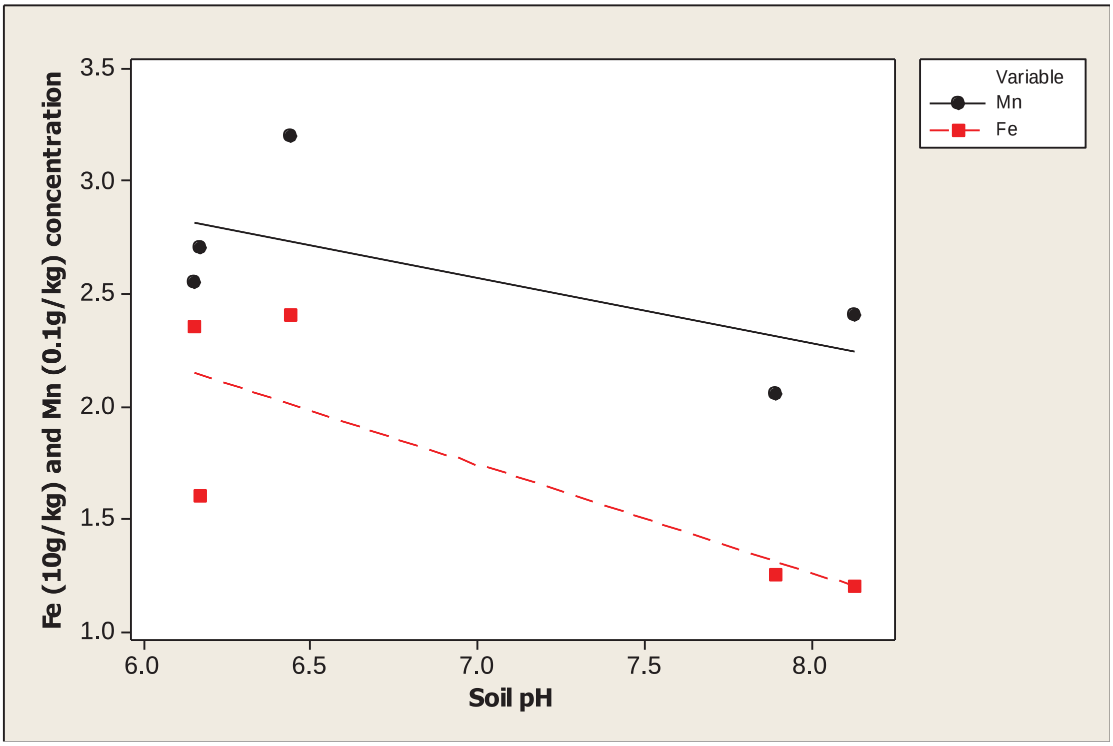

Figure 15: Correlation between Fe and M-n specific concentrations to soil pH at the proposed uranium mining site in north-east Botswana

4.3. Concentration of metals and radionuclides in soil

The concentration of radionuclides and trace metals are given in Table 2. The mean specific concentrations of trace metals was of the order Cr > Zn > Ni > Cu >Pb >Th > As ≈ Co, > Cd. A similar order in heavy metal concentrations was observed for Cd, Cu, Pb and Zn in Korean soils adjacent to Zn/Pb mine (Jung & Thornston, 1996) suggesting this to be the order of natural abundances of these metals in soil. Table 2 also shows the range of radionuclide and heavy metals concentrations, and the wide ranges point towards highly heterogeneous chemical composition of soil even within minute concentrations (Sheppard S. C., Sheppard, Ilin, Tait, & Sanipelli, 2008).

Table 2: Abundances of metals and radionuclides in soil from proposed uranium mining site in north-east Botswana

Variable	Mean	StDev	Minimum	Maximum
Cr	63.9	15.8	41.0	77.0
Mn	250.0	29.4	205.0	280.0
Co	9.2	2.3	6.2	12.5
Ni	35.3	8.1	23.0	45.0
Cu	29.8	9.0	17.5	40.5
Zn	41.9	14.9	27.5	61.5
As	9.4	2.2	7.4	13.0
Cd	0.05	0.011	0.022	0.069
U	7.6	9.6	1.2	24.5
Th	13.5	5.1	7.2	20.0
Pb	18.5	6.3	9.7	25.0
Po	345	514	45	1259
Fe	17000	5111	12000	23500
S	147.0	47.4	110.0	230.0

Principal component analysis was employed to explain the relationships between abundances of radionuclide and heavy metals in soil, Figure 16 below. In the figure, 64 % of the variation was defined by the first component while a combination of the first and second components account for 86 % of the variability. This data compression allowed for an easier interpretation of the data, Th, Pb, Cr, Cu, Zn, Ni, Co are all clustered around Fe exhibiting strong correlations with R^2 values 0.64, 0.65, 0.71, 0869, 0.93, 0.70 and 0.64 respectively against iron,***Table 3***. These correlations suggest that metal ions were trapped in an iron containing compound, probably iron oxyhydroxides (Ehlken & Kirchner, 2002; Sen, Mahajan, & Khilar, 2002).

No correlation was observed between Fe and the trace elements As and Cd, which were weakly correlated to Mn with R^2 of 0.27 and 0.50 respectively. This correlation implied that Cd migration in soil was governed by Mn oxides while As seemed to move about more independently. Most trace metals were correlated with Fe while the expectation was that they should correlate with Mn because Mn oxides have forty times more binding capacity for metals ion than Fe oxides (Contin, Mondini, Leita, & De Nobili, 2007). This discrepancy could imply that Mn exists predominately as abiotic oxides which have reduced binding capacity for metals while Fe is in the form of amorphous oxides that efficiently scavenge metal ions (Battlefield, Saldotova, Lee, Spiro, & Tebo, 2013).

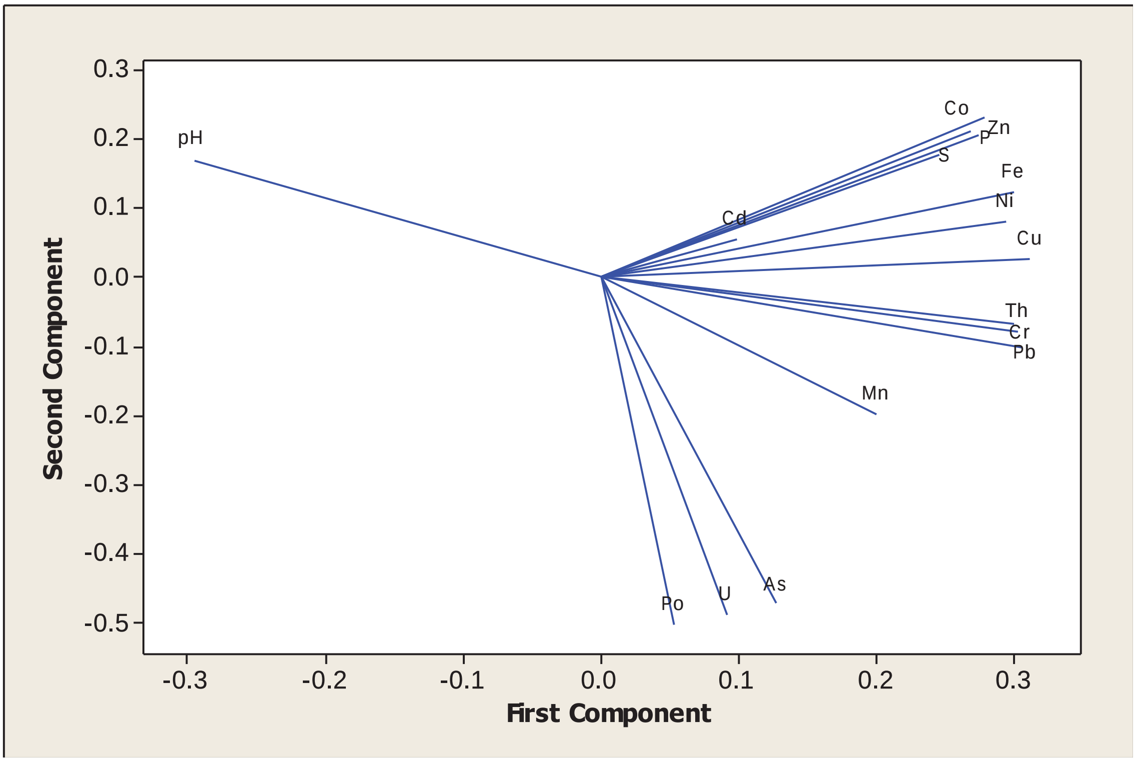

Figure 16: Loading plot for trace metal composition at proposed uranium mining site soil in north-east Botswana

The 9.40 ± 2.15 mg/kg abundance of As in the investigated soils compares favorably with range (1 to 40 mg/kg) being characteristic of uncontaminated sandy soils (Mandal & Suzuki, 2002). The soil at the proposed uranium mine in north-east Botswana falls in the category of sandy soils (Dikinya, 2013). Sandy soils are usually low in organic matter making them dominant in minerals and leaving As sequestrations to be a function of Mn and Fe oxides/hydroxides. However, since good correlation, $R^2 = 0.68$ was found between As and Mn while non was observed between As and Fe, it is inferred that As adsorbed on the large surface area of Mn (IV) oxides (Hennebel, De Gusseme , Boon, & Verstraete, 2009). The relationship between As and Mn is also visible from Figure 16, where the two analytes are principally influenced by the second component.

Table 3: Correlation coefficient between chemical components in soil from proposed uranium mining site in north-east Botswana

	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	U	Th	Pb	Po	P	S	pH
Mn	0.65														
Co	0.63	0.17													
Ni	0.65	0.32	0.95												
Cu	0.66	0.51	0.88	0.97											
Zn	0.45	0.39	0.87	0.88	0.91										
As	-0.05	0.38	0.08	0.35	0.47	0.33									
Cd	0.12	0.70	0.09	0.19	0.36	0.54	0.37								
U	0.26	0.39	-0.04	0.21	0.29	-0.09	0.73	-0.12							
Th	0.70	0.52	0.81	0.90	0.94	0.76	0.50	0.19	0.48						
Pb	0.65	0.54	0.78	0.93	0.97	0.80	0.62	0.30	0.50	0.97					
Po	0.14	0.29	-0.13	0.13	0.10	-0.16	0.74	-0.15	0.98	0.38	0.42				
P	0.53	0.40	0.87	0.83	0.86	0.92	0.19	0.39	-0.06	0.82	0.76	-0.17			
S	0.16	0.07	0.78	0.79	0.79	0.83	0.47	0.19	0.10	0.76	0.75	0.05	0.85		
pH	-0.95	-0.69	-0.44	-0.46	-0.53	-0.26	0.06	-0.04	-0.37	-0.64	-0.54	-0.24	-0.43	-0.05	
Fe	0.78	0.64	0.85	0.85	0.90	0.88	0.16	0.48	0.03	0.84	0.81	-0.10	0.92	0.64	-0.66

Table 4: Principal components influencing trace metals and ^{210}Po in soil from proposed uranium mining site in north-east Botswana

Variable	PC1	PC2	PC3
Cr	0.30	-0.07	0.05
Mn	0.20	-0.20	0.49
Co	0.27	0.21	-0.22
Ni	0.29	0.08	-0.18
Cu	0.31	0.03	-0.04
Zn	0.28	0.23	0.09
As	0.13	-0.47	0.07
Cd	0.10	0.06	0.70
U	0.10	-0.49	-0.16
Th	0.33	-0.08	-0.14
Pb	0 31	-0.10	-0.08
Po	0.05	-0.50	-0.17
Fe	0.30	0.12	0.14
P	0.28	0.21	0.01
S	0.25	0.18	-0.28
pH	-0.30	0.17	-0.06

Sulphur in this study is equally influenced by both the first and third components, in principal component analysis as shown in Table 4. It then follows that trace metals whose variability is principally influenced by the first component - Co, Ni, Cu, Zn, Th and Pb – and those metals that are explained by the third component –Mn and Cd – are strongly correlated with S in soil. These relationships imply that the trace metals are bonded to S and that S influences their distribution in soil as all these metals have R^2 ranging from 0.78 to 0.96 between each other. Since Zn is the most abundant of the metals and is strongly correlated with S, the other metals are probably incorporated as sulphides in ZnS (Ernst, 2012). The occurrence of metals as sulphides could be attributed to existence of the primary ores of Cu, Ni, Pb and Zn as sulphur compounds.

Actinides, Th and U have 13.5 ± 5.1 and 7.6 ± 9.6 mg/kg which are within universal averages of 5 to 20 and 1 to 10 mg/kg respectively (Elderstein, Fuger, Katz, & Morss). The highest concentration of uranium, 24.5 mg/kg was found in sample 5 which was 20 times more enriched than the lowest concentration, 1.25 mg/kg in sample 1. The large spread expressed here as standard deviation can be attributed to the inherent spatial variability of soil chemistry even within minute distances (IAEA, 2006) due to geological deposition during rock and soil formation (El Aassy, et al., 2012). Th/U ratio ranged from 2.62-5.72 with a mean of 3.87±127 when the U enriched sample 5 characterised by Th/U 0.67 was considered an outlier. The Th/U is skewed towards Th with the experimental values overlapping with theoretical ones, 2.75-3.68 (Labhart & Rybach, 1971) because U is more mobile than Th due to the latter's higher solid-liquid partition ratio rendering it less soluble (Mihucz, et al., 2008). Th/U relationship is captured in Figure 17. Th showed strong correlation to Fe (R^2 = 0.85) and moderate correlation to Mn, (R^2 = 0.55) while U showed only weak correlation to Mn (R^2 = 0.40) and no correlation to Fe suggesting that the mobility for U could be attributed to weak sequestration of U by Fe and Mn oxides.

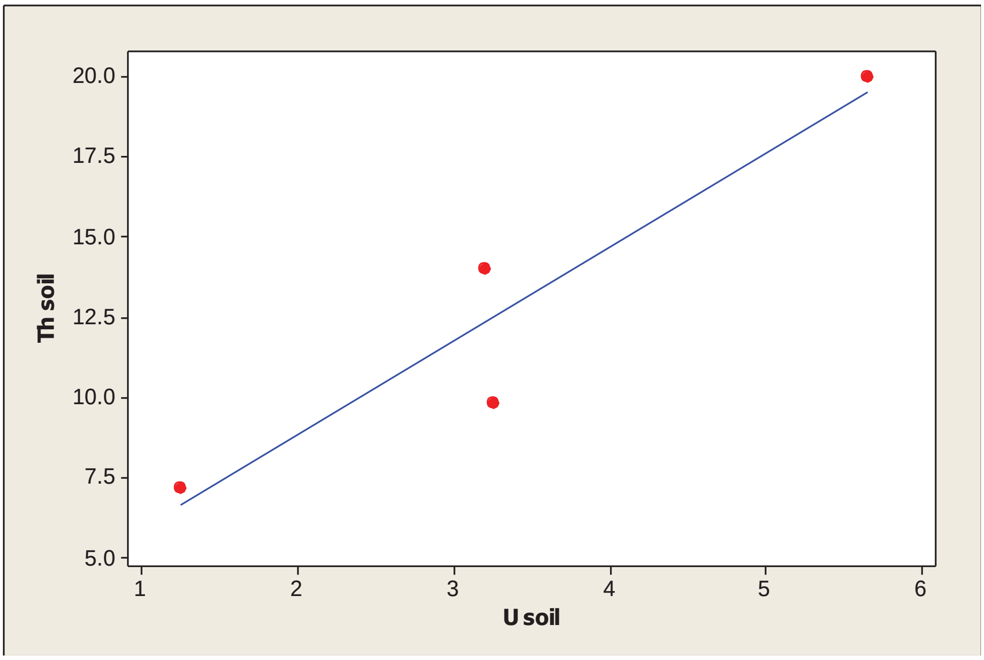

Figure 17: Variation of U with Th in soil from proposed uranium mining site in north-east Botswana

The ^{210}Po activity concentrations in the same soil samples is also shown in Table 2 and ranged from 91 to 1259 Bq/kg (dry weight). All of them are within the world range of 20 – 240 Bq/kg (Parfenov, 1974) except for sample 5. The arithmetic mean of the activity concentration was 355 ± 507 Bq/kg which like uranium shows a wide spread of values around the mean owing to variability of soil chemistry. This argument is further reinforced by sample 5 which has activity concentration with one degree of magnitude higher than the remaining samples, reflecting a hot spot commonly found in radiological analysis of lithogenic materials exemplified by 100 µG/h uranium hotspots observed in an area characterised by 03 to 06 µG/h dose rates (Lind, et al., 2013).

The measured ^{210}Po activity concentration is within the range, 0.5 – 1.25Bq/g, found in literature for areas that boarder phosphate mines in Syria but having experienced minimal anthropogenic activities (Aoun, El Samrani, Lartiges, Kazpad, & Saad, 2010). The activity concentration recorded here can thus be regarded as reference ^{210}Po activity concentration in soil for undisturbed soils bordering areas with known elevated naturally occurring radioactive materials such as phosphate or uranium.

The ^{210}Po activity concentration and uranium specific concentration in soil were found to be closely correlated as depicted by the scatter plot shown in Figure 18 below. Correlation depicted in Figure 18 means that for a particular soil sample, activity concentration of polonium can be predicted from specific concentration of uranium by formula

Activity concentration (^{210}Po) < 0.054(Uranium concentration) – 0.078

characterised by a correlation coefficient of 97% at $p < 0.001$. This statistical analysis support the theory that ^{210}Po is a progeny of ^{238}U which constitutes 99 % of natural uranium (Choppin, Liljenszin, & Rydberg, 2002), and hence ^{210}Po and uranium should be closely correlated. Furthermore, the correlation indicates supported ^{210}Po which can be expected for soils deeper than 0.2 m in direct contrast to the unsupported ^{210}Po on the surface dominated by atmospheric fall out following radon decay through ^{210}Pb (Pham, Povinec, Nies, & Betti, 2013).

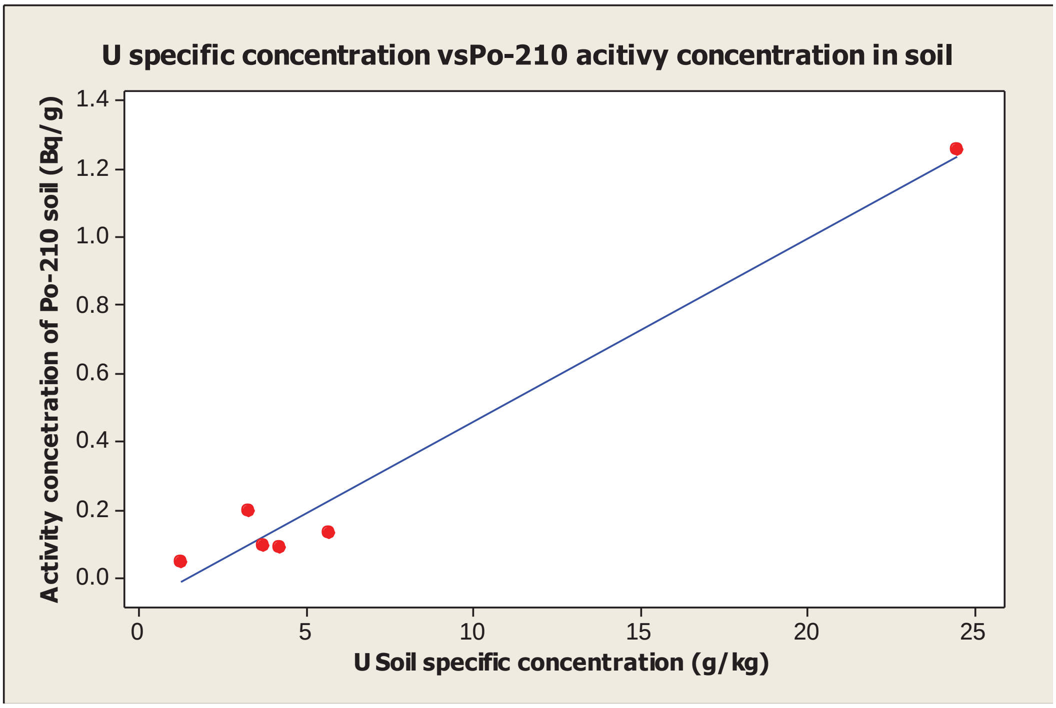

Figure 18: Variation of ^{210}Po activity with U specific concentration in soil from proposed uranium mining site in north-east Botswana

4.4. Concentration of trace metals and radionuclides in *Colospermum mopane* leaves

Table 5 summarises the concentration of heavy metals indicating mean and range of trace metals and ^{210}Po. Generally, concentrations of trace metals in the *Colospermum mopane* leaves follow the same trends, Cr > Zn > Ni > Cu >Pb >Th > As ≈ Co, > Cd as in soils from which the *C. mopane* trees grew implying that most of the minerals were absorbed from the soil substrate. This similarity in relative mineral content between substrate soil and plants mean that total concentration of metals in soil has a large bearing on the metal quantities appropriated by plants grown on such soils. Furthermore, the detection of trace metals in *C. mopane* leaves is testimony to the easy with which the metals translocate from roots to above ground vegetative parts.

Table 5: Abundances of trace metals and radionuclides in Colospermum mopane leaves from proposed uranium mining site in north-east Botswana

Variable	Mean	StDev	Minimum	Maximum
Cr plant	0.56	0.26	0.32	0.98
Mn plant	20.58	7.35	11.56	31.33
Co plant	0.061	0.025	0.031	0.098
Ni plant	0.91	0.34	0.52	1.47
Cu plant	4.41	0.54	3.80	5.09
Zn plant	21.60	5.91	14.67	30.00
As plant	0.064	0.019	0.043	0.096
Cd plant	0.0075	0.0036	0.0041	0.012
U plant	0.016	0.018	0.0034	0.048
Th plant	0.015	0.0071	0.0080	0.025
Pb plant	0.062	0.012	0.051	0.082
Po plant	70.8	32.3	47.3	127.3
Fe plant	46.71	13.95	31.56	61.11
S plant	1191	254	778	1400

Cr concentration ranged from 0.31 to 0.98 mg/kg in wet Colospermum mopane leaves and showed a strong correlation of (R2 = 0.80) to Cr concentration in dry soil, Table 7. The highest concentration, 0.97 ± 0.05 mg/kg found in sample 2 which had the lowest pH at 6.17 was well above the mean, 0.56 ± 0.26 mg/kg suggesting that more Cr was mobilised from soil and absorbed by plants through the roots. Since Cr and Fe in soil are closely correlated, it is expected that Cr was trapped in Fe (oxy) hydroxides which would have positive adsorption sites in acidic condition and mobilise Cr from solid into solution in soil pores. The Cr in soil pores is easily appropriated by plants hence the high concentration transferred to C. mopane leaves. Figure 19 shows that total Cr concentration in Colospermum mopane leaves increased in a linear fashion with amounts of Cr in substrate soil suggesting that root uptake is the principal entry point for Cr into C. mopane. Since Cr was quantified in C. mopane leaves, it can be concluded that Cr was easily translocated in the plant,

Table 7.

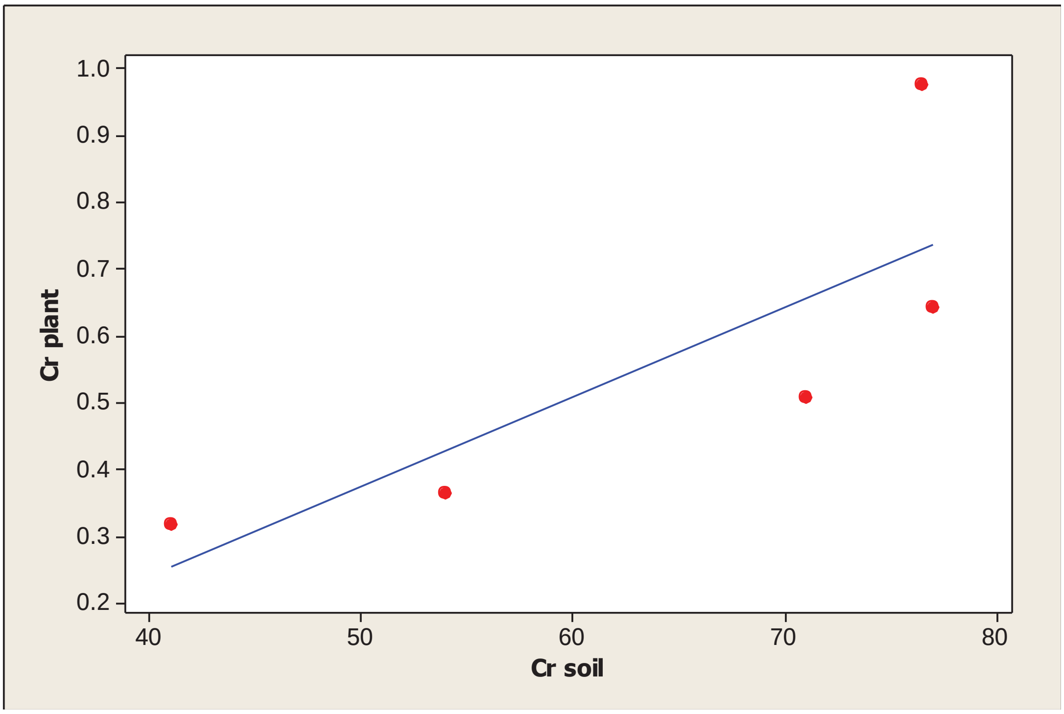

Figure 19: Variation of Cr in C. mopane leaves with Cr in soil substrate at proposed uranium mining site in north-east Botswana

Co, Zn, Th and Pb exhibited the same behaviour and this is captured in Figure 20, all the variations listed above here are primarily influenced by the first component.

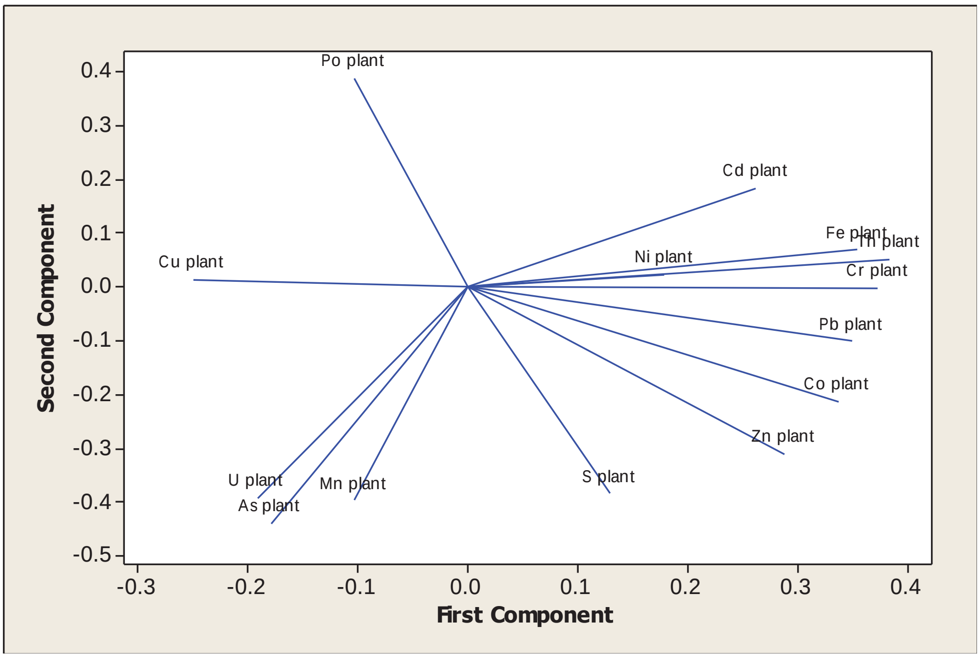

Figure 20: Loading plot depicting abundances of heavy metals in C. mopane leaves in relation to soil Mn, S, pH and Fe

Even though the Cu concentration is principally explained by the first component like Pb, Th, Cr, and Ni,Table 6 showed ordinates for Cu, Pb, Th, Cr and Ni along first component (PC1) were -0.25, 0.35, 0.38, 0.37 and 0.18. Out of all these trace metal, Cu ordinate is the only one bore a negative charge which implied the effect of the first component on Cu is opposite to the remaining trace metals. Figure 20 showed this relationship graphically as only copper laid to the left of PC1. This simply means that copper increases as the rest of the trace metals decreased

Table 6: Principal component matric for trace metals in C. mopane leaves from a proposed uranium mining site in north-east Botswana

Variable	PC1	PC2	PC3
Cr plant	0.373	-0.003	0.168
Mn plant	-0.104	-0.398	0.288
Co plant	0.337	-0.213	0.184
Ni plant	0.179	0.024	0.053
Cu plant	-0.250	0.013	0.451
Zn plant	0.288	-0.311	0.198
As plant	-0.179	-0.439	0.172
Cd plant	0.262	0.184	0.422
U plant	-0.191	-0.392	0.282
Th plant	0.384	0.050	0.028
Pb plant	0.349	-0.102	-0.113
Po plant	-0.103	0.389	0.417
Fe plant	0.355	0.068	0.119
S plant	0.129	-0.385	-0.348

At near neutral pH, Cr concentration in *C. mopane* leaves increased exponentially with Cr concentration in substrate soil as Figure 21: Variation of Cr in C. mopane leaves with Cr in substrate soil from proposed uranium mining site in north-east Botswana, excluding sample 2

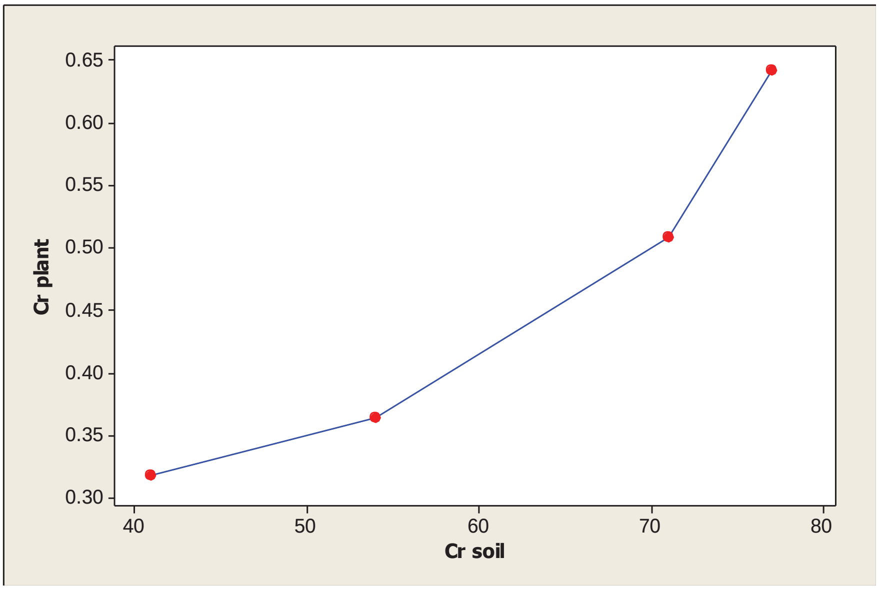

Figure 21: Variation of Cr in C. mopane leaves with Cr in substrate soil from proposed uranium mining site in north-east Botswana, excluding sample 2

However, plotting original data shows a distortion from the figure 13 due to influence of acidity on the bioavailability of Cr, Figure 22: Variation of Cr in soil with Cr in C. mopane leaves from proposed uranium mining site in north-east Botswana.

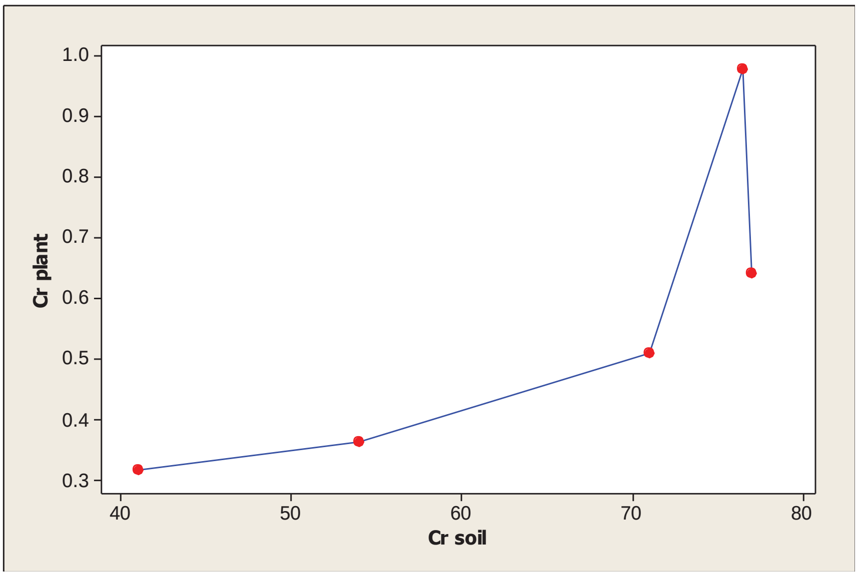

Figure 22: Variation of Cr in soil with Cr in C. mopane leaves from proposed uranium mining site in north-east Botswana

Table 7: Correlation table of heavy metals in C. mopane leaves from proposed uranium mining site in north-east Botswana

	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	U	Th	Pb	Po	P
Mn	-0.11												
Co	0.91	0.19											
Ni	0.38	-0.49	0.42										
Cu	-0.50	0.27	-0.40	0.10									
Zn	0.80	0.40	0.97	0.29	-0.33								
As	-0.42	0.83	-0.03	-0.15	0.52	0.18							
Cd	0.82	-0.12	0.61	0.20	-0.11	0.48	-0.49						
U	-0.39	0.83	-0.01	-0.12	0.62	0.19	0.10	-0.38					
Th	0.97	-0.32	0.84	0.47	-0.61	0.70	-0.57	0.74	-0.55				
Pb	0.87	-0.02	0.82	0.10	-0.83	0.76	-0.38	0.51	-0.41	0.87			
Po	-0.11	-0.23	-0.38	-0.09	0.50	-0.47	-0.32	0.46	-0.19	-0.17	-0.46		
P	-0.58	0.44	-0.46	-0.15	0.97	-0.35	0.60	-0.16	0.69	-0.72	-0.82	0.54	
S	0.18	0.15	0.46	0.34	-0.45	0.53	0.33	-0.40	0.23	0.24	0.43	-0.96	-0.49

The U/^{210}Po correlation coefficient in C. mopane leaves, $R^2 = 0.19$ in is lower than 0.98 as observed in corresponding soil samples ***Table 3***. The weakened linear relationship of the two metals in plants leaves indicates another ^{210}Po source to plants besides soil uptake via their root system. Studies have identified atmospheric deposition as the dominant source of for ^{210}Po to above ground vegetative parts (Persson & Holm, 2011; Brown, et al., 2011; Lin & Wu, 2009). From Table 5, ^{210}Po activity concentration ranged from 47.3 to 127.3 Bq/kg averaging 70.8 ± 32.3.5 Bq/kg (wet weight). The standard deviation here is lower than that recorded for ^{210}Po activity concentration in soil because unlike soil which is characterised by variable chemistry, most ^{210}Po found on plant leaves is a result of atmospheric deposition which is uniform for local sites (Persson & Holm, 2011). This is emphasised by the analytical samples which have 55 ± 10 Bq/kg when sample 5 is excluded indicating that for region where ^{210}Po soil activity is within a degree of magnitude, the effect of ^{210}Po uptake through the root system is negligible. However, sample 5 contains a degree of magnitude ^{210}Po activity higher than the rest of the samples and has elevated ^{210}Po levels in C. mopane leaves even though atmospheric deposition is expected to be uniform implying a marked input from the soil system through the root system. Uptake through root system is enhanced for metals with elevated concentration in substrate as it increases the metal's chances of being appropriated when different ions compete for absorption

Staying with the theory that atmospheric deposition of radionuclides varies between regions, sample 6, collected more than 100km from the analytical samples to serve as control, recorded 6.67 Bq/kg of ^{210}Po activity concentration which an order of magnitude below analytical samples' average as found by Perrson and Holm in 2011. This is beside the fact that the soil on which it grew had 90.75 Bq/kg ^{210}Po which is within range for analytical samples suggesting that sample 6 could be a hot spot in a low activity area hence radon gas emanation from the soil is reduced leading to low atmospheric deposition of ^{210}Po on above ground vegetative parts. (Watson, 1985). The significant difference in ^{210}Po activity in sample 6 and average for analytical samples lend more weight to the dominance of atmospheric deposition in plants' ^{210}Po activity owing to polonium's low solid-liquid partition ratio (Mihucz, et al., 2008) which limits ^{210}Po migration through the root system.

4.5. Soil to plant transfer factors for heavy metals and radionuclides

Table 8 lists means, standard deviations and ranges of soil to plant transfer factors for metals at proposed uranium mining site in north-east Botswana. The transfer factors were calculated as a quotient of metal concentration in *Colospermum mopane* leaves (wet weight) and metal concentrations in substrate soil (dry weight). All the ranges of transfer factors were within a degree of magnitude; 0.00675 - 0.128, 0.00494 – 0.00780, 0.0193 – 0.0376, 0.0938 – 0.230, 0.407 – 0.720, 0.0840 – 0.270, 0.000754 - 0.00140, 0.00225 - 0.00609, 0.101 - 1.480 and 0.00225 - 0.00609 for Cr, Co, Ni, Cu, Zn, Cd, Th and Pb respectively. All the transfer factors were below unity because these metals exist in nature as solid materials in rocks and soils, metals ions are then released from the host material in response to change in ambient chemistry (Devkota & Schmidt, 2000). The chemical changes mobilise metal ions according to their solid to liquid partition ratio which means some ions will always remain in soil form; hence remain unavailable to plant life. Less mobile metals like Pb have strong affinity for soil particles leading to reduced transfer factors, 00369 than the more mobile Zn which has a transfer factor that is two degrees of magnitude higher at 0.535 (Zhaung, Zou, & Shu, 2009; Selvaraj & Agoramoorthy, 2006)

Table 8: Mean, standard deviation and range of soil to plant transfer factors for metals at proposed uranium mining site in north-east Botswana

Variable	Mean	St.Dev	Minimum	Maximum
Cr	0.00856	0.00244	0.00675	0.0128
Co	0.00633	0.00111	0.00494	0.00780
Ni	0.0259	0.00698	0.0193	0.0376
Cu	0.161	0.0550	0.0938	0.230
Zn	0.535	0.115	0.407	0.720
As	0.0172	0.0230	0.0065	0.0584
Cd	0.180	0.0882	0.0840	0.270
U	0.00430	0.00598	0.00063	0.0150
Th	0.00112	0.000312	0.000754	0.00140
Pb	0.00369	0.00146	0.00225	0.00609
Po	0.571	0.542	0.101	1.480

No correlations were found between Fe soil concentration against Pb, Co, Cd and Ni transfer factors, meaning that these metals are not trapped in amorphous Fe (oxy) hydroxides known to scavenge metal ions and render them non-bioavailable. These findings were consistent with existing studies where treatment of soil with Fe oxide had no impact on wheat uptake of Pb and Cd (Chen, Lee, & Liu, 2000). However, Pb is known to have very strong affinity for Fe oxides which means that Pb could be irreversibly bonded to organic carbon (Stille, et al., 2011). The irreversible bonding of Pb makes it immobile and hence unavailable for plant uptake (Chen, Lee, & Liu, 2000) leading to observed conservative soil to plant transfer factors, $2.25 * 10^{-3}$ to $6.09 * 10^{-3}$ as depicted in Table 1

As transfer factor was a paltry 0.0172 ± 0.0230 implying that As is largely retained in soil rather than being availed to plant uptake through root absorption from soil pore water. From correlation data, As transfer factor has a strong correlation to soil pH and Mn concentration, $R^2 = -0.60$ and 0.69 respectively. The opposing polarity of the correlation coefficient is in agreement with literature where by Mn exists in soil primarily as oxide, the oxides have negatively charged reactive sites at high pH (Meng, Zheng, Zhang, & He, 2009). The negatively charged reactive sites then bind As electrostatically, rendering As non-bioavailable to plants hence an increase in pH means reduced As transfer factors (Hennebel, De Gusseme , Boon, & Verstraete, 2009)

Co had one of the lowest transfer factors at $(6.33 \pm 1.11)10^{-3}$ because it exhibits limited mobility in soil due to its high affinity for organic ligands as well as oxides of Mn and Fe (Blanco Rodriguez, Vera Tome, & Lazano, 2002) (Johnson, et al., 2011). In this study, no correlation was found between Co and Mn but Fe/Co correlation was very strong, $R^2 = 0.82$ while Co/pH had a weak $R^2 = -0.54$ which implied that at weakly acidic to basic soils Co is bound to reactive sites of gelatinous Fe oxides hence immobilised resulting in low transfer factors.

The highest transfer factor was recorded for Zn, 0.535 ± 0.115, Table 8 which was at least a degree above the rest of the metals except Cu and Cd. Zn and Cu have relatively high concentrations in soil as shown in Table 2: Abundances of metals and radionuclides in soil from proposed uranium mining site in north-east Botswana and follow similar migration patterns in soil. Both Zn and Cu out-compete other metals at the rhizosphere due the two's numeric advantage in terms of ions available at absorption site resulting in Zn and Cu having higher transfer factors (Ehlken & Kirchner, 2002).

Table 9: Correlation matrix for heavy metals transfer factors and soil Fe, Mn, S and pH

	Cr	Th	Pb	Po	S	pH	Fe	Mn	Co	Ni	Cu	Zn	As
Th	0.44												
Pb	-0.12	0.52											
Po	-0.01	0.76	0.91										
S	-0.55	0.30	0.52	0.46									
pH	-0.51	0.07	0.83	0.58	0.71								
Fe	0.81	0.35	-0.55	-0.30	-0.47	-0.83							
Mn	0.27	0.33	-0.46	-0.05	-0.36	-0.78	0.64						
Co	0.74	0.12	-0.49	-0.48	-0.29	-0.54	0.82	0.11					
Ni	-0.36	0.21	-0.32	-0.07	0.50	-0.22	0.20	0.52	0.01				
Cu	-0.74	-0.16	0.69	0.49	0.53	0.88	-0.98	-0.59	-0.86	-0.19			
Zn	-0.44	-0.64	0.20	-0.22	0.22	0.63	-0.69	-0.93	-0.18	-0.46	0.58		
As	-0.42	-0.56	0.03	-0.35	0.41	0.53	-0.50	-0.86	0.08	-0.14	0.37	0.91	
Cd	0.40	-0.62	-0.65	-0.75	-0.89	-0.59	0.35	0.04	0.42	-0.51	-0.48	0.16	0.05

At 0.161 ± 0.055, Cu transfer factor is second only to Zn transfer factor among all metals studied here. This represents the complexities associated with sequestering Cu because Cu dissolves in acidic as well as basic soils alike (Fan, et al., Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils, 2012). Mn and Fe concentration in soil were weakly, $R^2 = -0.59$ and strongly, $R^2 = -0.98$ to Cu transfer factor proving the theory that as the two known good scavengers of Cu in soil decrease, Cu transfer factor went up (Contin, Mondini, Leita, & De Nobili, 2007). However, a strong correlation, $R^2 = 0.88$ was observe for pH/Cu transfer factor which implies that Mn and Fe oxide were not the only chemicals binding Cu but organic ligands were also present. The ligands are digested as the pH becomes more basic and mobilise Cu leading to an increase in Cu transfer factor (Contin, Mondini, Leita, & De Nobili, 2007).

Cd has a comparable transfer factor, 0.180 ± 0.088 to Zn and Cu even though it has trace concentration because it is more mobile than many other metals in soil rendering it bioavailable (Xhao, et al., 2013). The high mobility of Cd is attributed to lack of correlation between Cd and neither Mn nor Fe suggesting that Cd is not sequestered. Moreover, Cd has similar chemical properties to Zn (Devkota & Schmidt, 2000) hence follows similar migration patterns further increasing its uptake by plant roots. Pb had a low transfer factor, $(3.69 \pm 1.46)10^{-3}$ while showing weak correlations, $R^2 = -0.55$ and -0.46 to Fe and Mn pointing to a reduced impact of these Mn and Fe on Pb sequestration. However, Pb/soil pH were strongly correlated, $R^2 = 0.83$ implying that Pb was bonded to organic ligands which immobilised Pb leading to low transfer factors. Pb transfer factor increased with pH because organic matter disintegrates at high pH (Chen, Lee, & Liu, 2000; Stille, et al., 2011; Jovanovic, et al., 2011)

4.6. Concentration of metals and radionuclides in *Imbrasia belina* larvae

Arithmetic mean, standard deviation and maximum and minimum concentrations of all the analytes are presented in Table 10 and Appendix III. From the tables, Zn, Fe and Mn had the highest concentrations at 167.3 ± 40.7, 107.5 ± 73.4 and 27.5 ± 3.15 mg/kg respectively keeping with the latter two's high concentrations from soil through *C. mopane* leaves to *I. belina* larvae. However, Zn received preferential bioaccumulation from producers, to primary consumers in the studied food chain because it has both catalytic and structural functions in biological systems (Gutierrez-Carbonell, et al., 2013). Mn exhibited the least variability with a percentage standard error of about 10%. Reduced variability was probably accomplished by

I. belina rigorously regulating intake and depuration of Mn to maintain stable tissue concentration in a bid to keep delicate balance between Mn essentiality and toxicity (Martinez-Finley, Gavin, Aschnar, & Gunter, 2013). This trend was also observed, though to a lesser extent for the rest of the metals except Cd and ^{210}Po because metals are expected to be more concentrated in higher trophic levels than in lower ones (Laskowski & Maryanski, 1993). Most metals here are also cofactors – Cr, Zn, Co, Mn, Cu, Ni - in enzyme activity hence the metal tend to concentrate in species having high levels of protein of which *I. belina* is one.

Table 10: Abundances of heavy metal and ^{210}Po in Imbrasia belina from proposed uranium mining site in north-east Botswana

Variable	Mean	StDev	Minimum	Maximum
Cr	2.883	1.600	0.917	4.333
Mn	27.53	3.15	25.00	33.00
Co	0.0673	0.0418	0.0257	0.1333
Ni	1.292	0.803	0.410	2.433
Cu	8.047	0.516	7.467	8.533
Zn	167.3	40.7	146.7	240.0
As	0.809	0.974	0.223	2.533
Cd	0.005893	0.001825	0.003200	0.007933
U	0.03947	0.01220	0.02633	0.05167
Th	0.0433	0.0388	0.0137	0.1067
Pb	0.1438	0.0698	0.0690	0.2533
Po	55.0	36.0	13.0	103.0
Fe	107.5	73.4	42.0	223.3
S	5.562	0.470	5.070	6.230

Metals with no known physiological function in organisms, As, ^{210}Po, Pb, Th, and U exhibited linear relationships between concentrations in *C. mopane* leaves and *I. belina* larvae with $R^2 = 0.99, 0.70, 0.87, 0.68$ and -0.76 respectively as could be expected from literature (Blanco Rodriques, Vera Tome, & Lozano, 2002). The linearity plots are shown in , From the this data, uranium is the only one with a negative correlation, implying that while all the metal concentration in larvae increase with metal concentration in leaves, the reverse is true for uranium.

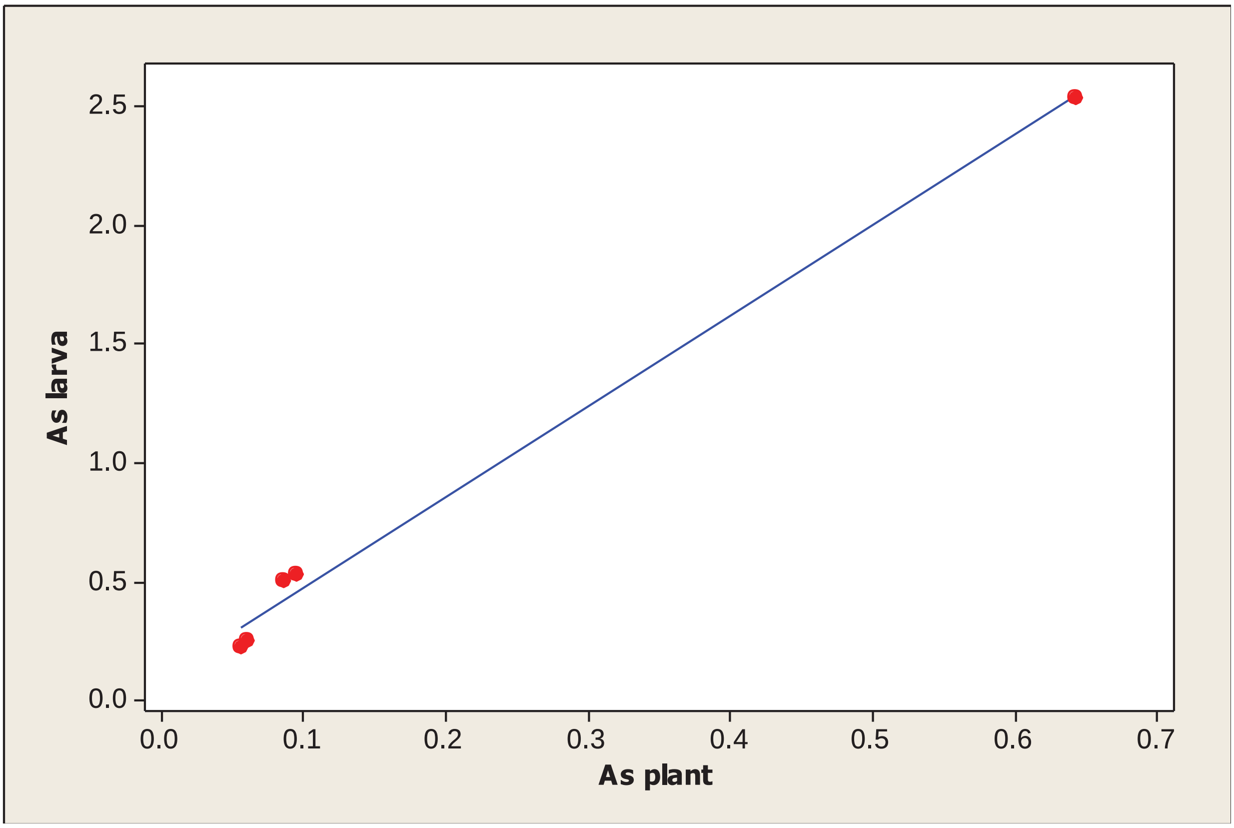

Figure 23: Linearity plot for As concentration in I. belina larva and C. mopane leaves at the proposed uranium mining site in north-east Botswana

Uranium concentration in *I. belina* caterpillars ranges from 2.50 * 10^{-2} to 5.17 * 10^{-2} g/kg and averages 3.71 * 10^{-2} ± 1.24 * 10^{-2} g/kg. The only source of U to the caterpillars is their diet consisting solely of *C. mopane* leaves since the caterpillars are monophagous and hatch from eggs attached to the underside of the leaves (Madibela, Seitiso, Thema, & Letso, 2007). From this direct dependence, a linear relationship is expected between plant and caterpillar concentrations of uranium and was obtained, Figure 24. However, the correlation had a negative sign, Table 11which implied that U concentration in *I. belina* was reduced with an increase with U concentration in *C. mopane* leaves. This decrease in U concentration in larvae could be attributed to *I. belina* probably being capable of restricting U absorption because U has no known physiological benefits to the caterpillars.

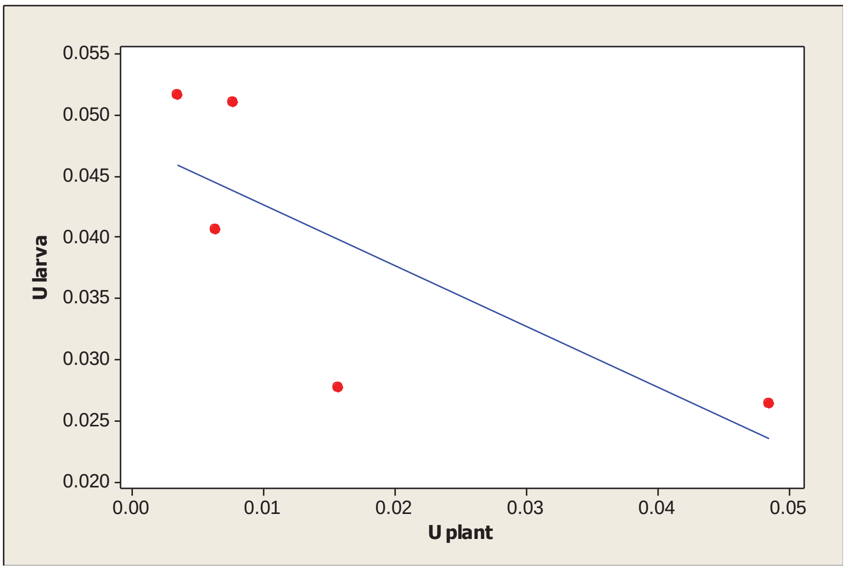

Figure 24: Linear plot for U concentration in I. belina larvae and C. mopane leaves at the proposed uranium mining site in north-east Botswana

In the caterpillars, it is shown that both As, Zn and Mn are principally explained by the second component but As increased with the component while Zn and Mn increase as the second component decreases, *Figure 25*. Therefore the As/Mn and As/Zn have inverse relationships expressed as R^2 = -0.20 and -0.36 while Mn/Zn has R^2 = 0.95 from The strong correlation between Fe and Th, Pb, Ni, Cr and Co is retained throughout the food chain

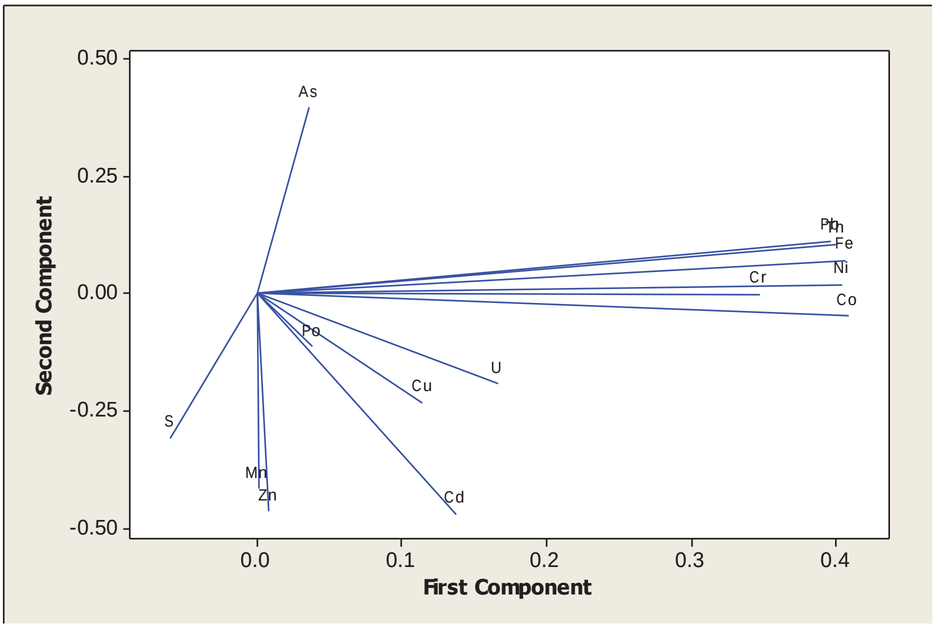

Figure 25: Loading plot for heavy metal and ^{210}Po in I. belina larvae from proposed uranium mining site in north-east Botswana

Table 11: Correlation matrix for heavy metal and ^{210}Po concentrations in I. belina from proposed uranium mining site in north-east Botswana

	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	U	Th	Pb	Po	Fe
Mn	0.30												
Co	0.84	0.06											
Ni	0.92	0.07	0.97										
Cu	-0.03	0.26	0.31	0.17									
Zn	0.27	0.95	0.10	0.07	0.15								
As	0.41	-0.20	0.01	0.22	-0.53	-0.36							
Cd	0.13	0.52	0.42	0.25	0.56	0.65	-0.84						
U	0.09	-0.16	0.46	0.31	0.04	0.12	-0.64	0.65					
Th	0.78	-0.18	0.95	0.95	0.28	-0.20	0.19	0.17	0.31				
Pb	0.80	-0.22	0.95	0.95	0.10	-0.18	0.19	0.16	0.44	0.98			
Po	-0.15	0.17	0.10	0.00	0.94	-0.02	-0.30	0.30	-0.28	0.15	-0.07		
Fe	0.84	-0.08	0.97	0.98	0.25	-0.09	0.20	0.21	0.32	0.99	0.98	0.10	
S	0.21	0.67	-0.09	-0.05	-0.44	0.82	-0.12	0.32	0.20	-0.34	-0.22	-0.57	-0.24

4.6. Concentration factor

Table 12: Concentration factors for heavy metal and ^{210}Po from C, mopane to I. belina at proposed uranium mining site in north-east Botswana

```
Variable        Mean      StDev    Minimum    Maximum
Cr CF           5.51       3.47       1.80      11.16
Mn CF          1.532      0.777      0.851      2.856
Co CF          1.067      0.312      0.553      1.367
Ni CF          1.474      0.913      0.450      2.808
Cu CF          1.848      0.264      1.541      2.237
Zn CF           8.18       2.59       5.00      10.91
As CF          4.697      0.915      3.938      5.857
Cd CF          0.886      0.369      0.514      1.366
U CF            6.19       5.80       0.54      15.30
Th CF           2.96       2.35       1.10       6.51
Pb CF          2.246      0.749      1.362      3.081
Fe CF          2.277      1.285      0.922      3.655
Po CF          0.781      0.416      0.197      1.293
S CF        0.004836   0.001029   0.004042   0.006517
```

^{210}Po activity concentration in *I. belina* ranged from 26.3 to 51.7 Bq/g and averages 39.5 ± 12.2 Bq/kg. Generally, the activity in the caterpillars was higher than that measured in the leaves the caterpillars feed on in agreement with the expectation that high protein species accumulate more polonium due to polonium's high affinity for high molecular weight proteins (Henricsson, Ranebo, Hansson, Raaf, & Holm, A biokinetic study of 209-Po in man, 2012). *I. belina* caterpillars contain over half of their body weight as protein (Pharithi, Suping, & Yeboah, 2004). As a chalcogens, polonium substitutes selenium in some proteins (Lee & Wang, 2013) because of their similar chemistry as members of the same group in the periodic table. However, there is no evidence that ^{210}Po follows sulphur as expected of chalcogens because the data shows no correlation between the two element.

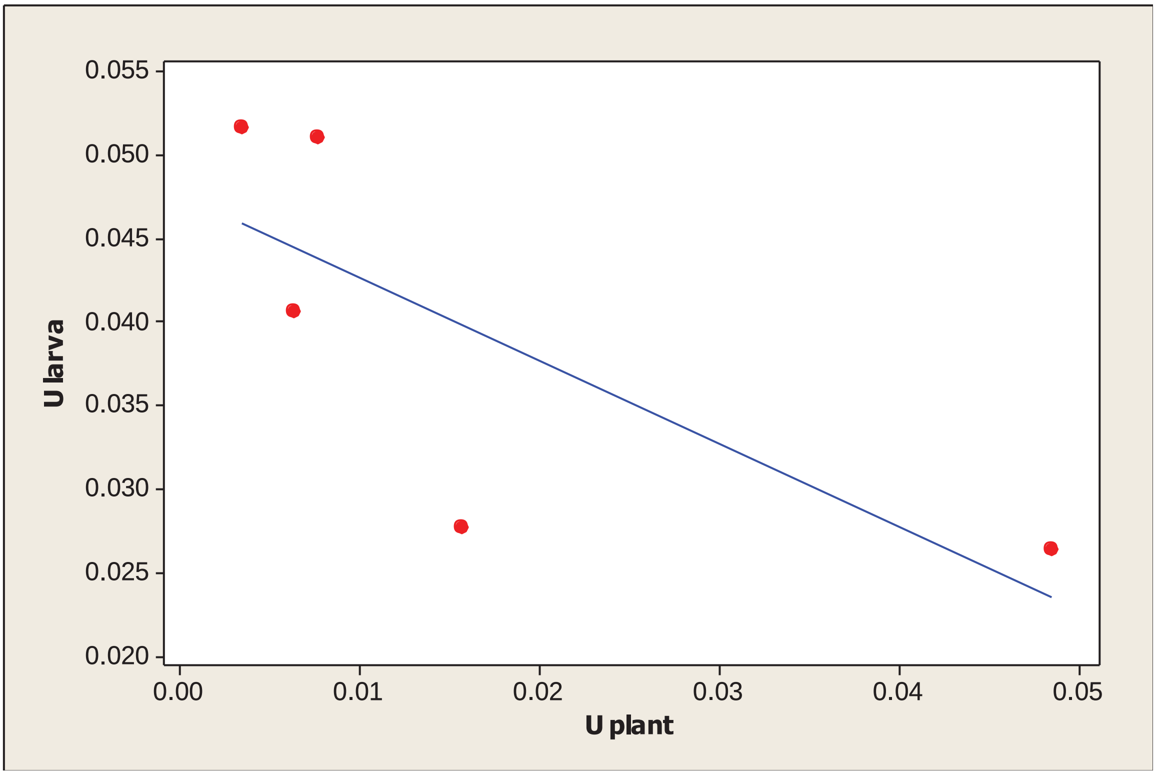

Figure 26: Relationship between concentration of uranium in C. mopane and I belina at the proposed uranium mining site in north-east Botswana

5. Conclusions

Trace metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni Pb, Th, U and Zn as well as radionuclide, ^{210}Po were quantified in soil – tree – herbivorous insect larva food chain. Soil is the sink for trace metal contamination hence, the trace metal were quantified in soil. The specific concentration of the trace element were either lower or at the same level with global averages for trace metal abundances which put them well below the set thresholds. Based on these low specific concentrations, the proposed uranium mining site in the north-east of Botswana can be regarded as being pristine.

Since the land is sparsely populated and the principal land uses are harvesting of *Imbrasia belina* larvae and subsistence arable and pastoral farming, it was imperative that the transfer of the trace metals to plants were investigated. Emphasis was put *Imbrasia belina* larvae which are .dominant protein source for communities inhabiting the proposed mining site and its surroundings. However, *I. belina* is monophagous, feeding exclusively on the leaves of *Colospermum mopane* and the *C. mopane* trees acted as a conduits trace metal migration from soil to the larvae. Relative concentrations of trace metals to each other in *C. belina* leaves reflected similar pattern to that observed in soil suggesting that soil concentration has a profound impact on leaves concentration.

Fe soil concentration and soil pH were identified as some of the soil properties that affected the transfer factor of trace metals from soil to plants. The transfer factor was always below unity implying a low solid/liquid partition ratio of the trace metals which encourages retention of trace metal in soil. Once in the plants, trace metals are consumed by *I. belina* caterpillars which tend to accumulate the as caterpillars had higher specific concentrations of all trace metals than *C. mopane* leaves. There the ratio of trace metal concentration in larvae to that in leaves, called concentration factor was above unity. Therefore, the trace metals move easily from *C. mopane leaves* to *I. belina* caterpillars than from soil to *C, mopane* leaves.

^{210}Po concentration in soil tended to follow its predecessor in the 4n + 2 natural decay series headed by ^{238}U. The close correlation of U specific concentration to ^{210}Po activity concentration observed in samples was weakened probably due to the potential atmospheric depositions in on the *C. mopane* leaves. Atmospheric deposition introduced some unsupported ^{210}Po. The transfer factors appeared higher than expected due probably to atmospheric deposition because ^{210}Po is known to have affinity for partition which makes ^{210}Po unavailable for root uptake.

Almost all ^{210}Po from *C. mopane* leaves was passed on to the *I. belina* larvae because the concentration in the two trophic levels was almost equal resulting in average concentration factor of just one. This in contrast to interaction of this food chain with the trace metals because the transfer factor increased is such a trace metal was physiologically needed more than others.

Appendices

Appendix I: Colospermum mopane leaves wet/dry weight conversion factor

Sample number	Wet weight (g)	Dry weight (g)	Conversion factor
1A	70.19	50.25	1.39
1B	65.99	46.80	1.41
2A	45.03	31.71	1.42
2B	70.16	45.05	1.55
3A	68.65	48.68	1.41
3B	85.09	53.18	1.6
4A	63.95	44.10	1.45
4B	70.00	48.57	1.56
5A	58.15	37.34	1.6
5B	56.31	38.05	1.48
6A	75.88	52.33	1.45
6B	60.45	39	1.55

Appendix II: pH of soils samples

Sample number	Run 1	Run 2	Run 3
1	8.12	8.14	8.13
2	6.14	6.16	6.14
3	6.41	6.45	6.43
4	7.88	7.88	7.89
5	6.14	6.20	6.17
6	5.52	5.50	5.53

Appendix III: Concentration of heavy metals in Imbrasia belina from proposed uranium mine in north-east Botswana

	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Th	U	P	S
Larva 1	1.40	25	49	0.038	0.63	7.4	150	0.230	0.0060	0.120	0.012	0.052	7.0	5.7
Larva 1A	1.40	25	51	0.037	0.65	7.6	150	0.220	0.0055	0.110	0.017	0.052	6.9	5.8
Larva 1B	1.40	25	49	0.040	0.67	7.6	160	0.220	0.0059	0.110	0.013	0.051	6.8	5.8
Larva 2	4.30	26	24	0.140	2.40	8.6	150	0.510	0.0066	0.280	0.110	0.053	6.6	5.2
Larva 2A	4.30	26	20	0.130	2.40	8.3	150	0.500	0.0071	0.230	0.110	0.049	6.6	5.1
Larva 2B	4.40	26	20	0.130	2.50	8.6	150	0.510	0.0078	0.250	0.100	0.051	6.5	5.2
Larva 3	3.80	33	97	0.074	1.40	8.2	220	0.250	0.0080	0.110	0.050	0.042	6.4	6.2
Larva 3A	3.60	33	95	0.070	1.40	8.2	250	0.250	0.0081	0.130	0.019	0.039	6.4	6.2
Larva 3B	3.70	33	96	0.078	1.40	8.2	250	0.260	0.0077	0.120	0.021	0.041	6.6	6.3
Larva 4	3.90	26	120	0.063	1.50	7.4	140	2.500	0.0029	0.160	0.049	0.027	5.5	5.5
Larva 4A	4.20	27	140	0.070	1.60	7.5	140	2.600	0.0034	0.170	0.050	0.025	5.7	5.6
Larva 4B	4.10	27	120	0.062	1.60	7.5	160	2.500	0.0033	0.160	0.058	0.027	5.7	5.6
Larva 5	1.20	29	50	0.030	0.45	9.0	150	0.570	0.0066	0.079	0.017	0.030	4.3	5.6

Larva 5A	0.70	26	36	0.024	0.39	8.3	130	0.500	0.0050	0.062	0.012	0.023	3.8	4.8
Larva 5B	0.85	26	40	0.023	0.39	8.3	160	0.510	0.0045	0.066	0.012	0.030	3.8	4.8
Larva 6	2.30	48	80	0.065	0.50	9.0	110	0.050	0.0027	0.084	0.019	0.026	4.8	5.3
Larva 6A	2.10	45	70	0.066	0.45	9.0	120	0.041	0.0024	0.080	0.015	0.024	4.6	5.4
Larva 6B	1.90	46	74	0.068	0.40	9.3	110	0.039	0.0026	0.084	0.025	0.025	4.6	5.3

Appendix IV: Descriptive statistics for heavy metals in soil

Descriptive Statistics: Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, Th, U, P, S

```
Variable  C1       N      Mean     StDev   Minimum   Maximum
Cr        Larva 1  3    1.4000  0.000000    1.4000    1.4000
          Larva 2  3    4.3333    0.0577    4.3000    4.4000
          Larva 3  3    3.7000    0.1000    3.6000    3.8000
          Larva 4  3    4.0667    0.1528    3.9000    4.2000
          Larva 5  3     0.917     0.257     0.700     1.200
          Larva 6  3     2.100     0.200     1.900     2.300

Mn        Larva 1  3    25.000  0.000000    25.000    25.000
          Larva 2  3    26.000  0.000000    26.000    26.000
          Larva 3  3    33.000  0.000000    33.000    33.000
          Larva 4  3    26.667     0.577    26.000    27.000
          Larva 5  3     27.00      1.73     26.00     29.00
          Larva 6  3    46.333     1.528    45.000    48.000

Fe        Larva 1  3    49.667     1.155    49.000    51.000
          Larva 2  3     21.33      2.31     20.00     24.00
          Larva 3  3    96.000     1.000    95.000    97.000
          Larva 4  3    126.67     11.55    120.00    140.00
          Larva 5  3     42.00      7.21     36.00     50.00
          Larva 6  3     74.67      5.03     70.00     80.00

Co        Larva 1  3  0.038333  0.001528  0.037000  0.040000
          Larva 2  3   0.13333   0.00577   0.13000   0.14000
          Larva 3  3   0.07400   0.00400   0.07000   0.07800
          Larva 4  3   0.06500   0.00436   0.06200   0.07000
          Larva 5  3   0.02567   0.00379   0.02300   0.03000
          Larva 6  3  0.066333  0.001528  0.065000  0.068000

Ni        Larva 1  3    0.6500    0.0200    0.6300    0.6700
          Larva 2  3    2.4333    0.0577    2.4000    2.5000
          Larva 3  3    1.4000  0.000000    1.4000    1.4000
          Larva 4  3    1.5667    0.0577    1.5000    1.6000
          Larva 5  3    0.4100    0.0346    0.3900    0.4500
          Larva 6  3    0.4500    0.0500    0.4000    0.5000

Cu        Larva 1  3    7.5333    0.1155    7.4000    7.6000
          Larva 2  3     8.500     0.173     8.300     8.600
          Larva 3  3    8.2000  0.000000    8.2000    8.2000
          Larva 4  3    7.4667    0.0577    7.4000    7.5000
          Larva 5  3     8.533     0.404     8.300     9.000
```

```
          Larva 6  3     9.100     0.173     9.000     9.300

Zn        Larva 1  3    153.33      5.77    150.00    160.00
          Larva 2  3    150.00  0.000000    150.00    150.00
          Larva 3  3     240.0      17.3     220.0     250.0
          Larva 4  3    146.67     11.55    140.00    160.00
          Larva 5  3    146.67     15.28    130.00    160.00
          Larva 6  3    113.33      5.77    110.00    120.00

As        Larva 1  3   0.22333   0.00577   0.22000   0.23000
          Larva 2  3   0.50667   0.00577   0.50000   0.51000
          Larva 3  3   0.25333   0.00577   0.25000   0.26000
          Larva 4  3    2.5333    0.0577    2.5000    2.6000
          Larva 5  3    0.5267    0.0379    0.5000    0.5700
          Larva 6  3   0.04333   0.00586   0.03900   0.05000

Cd        Larva 1  3  0.005800  0.000265  0.005500  0.006000
          Larva 2  3  0.007167  0.000603  0.006600  0.007800
          Larva 3  3  0.007933  0.000208  0.007700  0.008100
          Larva 4  3  0.003200  0.000265  0.002900  0.003400
          Larva 5  3  0.005367  0.001097  0.004500  0.006600
          Larva 6  3  0.002567  0.000153  0.002400  0.002700

Pb        Larva 1  3   0.11333   0.00577   0.11000   0.12000
          Larva 2  3    0.2533    0.0252    0.2300    0.2800
          Larva 3  3   0.12000   0.01000   0.11000   0.13000
          Larva 4  3   0.16333   0.00577   0.16000   0.17000
          Larva 5  3   0.06900   0.00889   0.06200   0.07900
          Larva 6  3   0.08267   0.00231   0.08000   0.08400

Th        Larva 1  3   0.01400   0.00265   0.01200   0.01700
          Larva 2  3   0.10667   0.00577   0.10000   0.11000
          Larva 3  3    0.0300    0.0173    0.0190    0.0500
          Larva 4  3   0.05233   0.00493   0.04900   0.05800
          Larva 5  3   0.01367   0.00289   0.01200   0.01700
          Larva 6  3   0.01967   0.00503   0.01500   0.02500

U         Larva 1  3  0.051667  0.000577  0.051000  0.052000
          Larva 2  3   0.05100   0.00200   0.04900   0.05300
          Larva 3  3  0.040667  0.001528  0.039000  0.042000
          Larva 4  3  0.026333  0.001155  0.025000  0.027000
          Larva 5  3   0.02767   0.00404   0.02300   0.03000
          Larva 6  3  0.025000  0.001000  0.024000  0.026000

P         Larva 1  3    6.9000    0.1000    6.8000    7.0000
```

```
          Larva 2  3    6.5667    0.0577    6.5000    6.6000
          Larva 3  3    6.4667    0.1155    6.4000    6.6000
          Larva 4  3    5.6333    0.1155    5.5000    5.7000
          Larva 5  3     3.967     0.289     3.800     4.300
          Larva 6  3    4.6667    0.1155    4.6000    4.8000

S         Larva 1  3    5.7667    0.0577    5.7000    5.8000
          Larva 2  3    5.1667    0.0577    5.1000    5.2000
          Larva 3  3    6.2333    0.0577    6.2000    6.3000
          Larva 4  3    5.5667    0.0577    5.5000    5.6000
          Larva 5  3     5.067     0.462     4.800     5.600
          Larva 6  3    5.3333    0.0577    5.3000    5.4000
```

Bibliography

A-Cap Resources. (2012). *A-Cap Resources annual report.* Sydney: A-Cap Resources.

Ahmed, A., & El Razek, A. (2013). The mobility and speciation of lead and cadmium in Bahr El Baqar region, Egypt. *Journal of Environmental Chemical Engineering*.

Akai, J., & Anawar, H. M. (2013). Mineralogical approach in elucidation of contamination mechanism for toxic trace elements in the environment: Special reference to arsenic contamination in groundwater. *Physics and Chemistry of the Earth* , 2-12.

Akyil, S., Gurboga, G., Aslani, M. A., & Aytas, S. (2008). Verical distribution of 226Ra and 210Po inagricultural soils in Buyuk Menderes Basin, Turkey. *Journal of Hazardous Materials*, 328-334.

Aoun, M., El Samrani, A. G., Lartiges, B. S., Kazpad, V., & Saad, Z. (2010). Releases of phosphate fertiliser industry in the surrounding environment: Investigation on heavy metals and polonium-210 in soil. *Journal of Environmental Radioactivity*, 1387-1397.

Au, C., Benedetto, A., & Aschner, M. (2008). Manganese transport in eukaryotes: The role of DMT1. *Neurotoxicology*, 569-576.

Bakkaus, E., Gouget, B., Gallien, J., Khodja, H., Carrot, F., Morel, J. L., & Collins, R. (2005). Concentration and distribution of cobalt in higher plants: The use of micro-PIXE spectroscopy. *Nuclear Instruments and Methods in Physics Research*, 350-356.

Battlefield, C. N., Saldotova, A. V., Lee, S. W., Spiro, T. B., & Tebo, T. M. (2013). Mn(II,III) oxidation and Manganese (IV) oxide mineralisation byan expressed bacterial multicopper oxidase. *National Academy of Sciences of United States of America*, (pp. 11731-11735).

Blanco Rodriguez, P., Vera Tome, F., & Lazano, J. C. (2002). About the assumption of linearity in soil-to-plant transfer factors for uranium and thorium isotopes and. *The Science of the Total Environment*, 167-175.

Blanco Rodriques, P., Vera Tome, F., & Lozano, J. C. (2002). About the assumption of linearity in soil-to-plant transfer factors for uranium and thorium isotopes and 226Ra. *The Science of the Total Environment*, 167-175.

Brown, J. E., Gjelsvik, R., Roos , P., Kalas, J. A., Outola, I., & Holm, E. (2011). Levels and transfer of 210Pb and 210Po in Nordic terrestrial ecosystems. *Journal of Environmental Radioactivity*, 430-437.

Chen, Z. S., Lee, G. L., & Liu, J. C. (2000). The effects of chemical remediation treatments on the extractability and speciation of cadmium and lead in contaminated soils. *Chemosphere*, 235-242.

Cheng, Q., Zhang, R., Li, W., Niu, Y., Liu, X., Hou, Y., & Zhao, L. (2013). The protective effect of grapeseed procyanidin extract against cadmium-induced rena loxidative damage in mice. *Environmental Toxicology and Pharmacology*, 759-798.

Choppin, G., Liljenszin, J., & Rydberg, J. (2002). *Radiochemistry and nuclear chemistry.* Woburn: Butterworth-Heinneman.

Contin, M., Mondini, C., Leita, L., & De Nobili, M. (2007). Enhanced soil toxic metal fixation in iron (hydr)oxides by redox cycles. *Geoderma*, 164-175.

De Vries, W., McLaughlin, M. J., & Groenenberg, J. E. (2011). Transfer functions for solidesolution partitioning of cadmium for Australian soils. *Emvironmental Pollution*, 3583-3594.

Devkota, B., & Schmidt, G. H. (2000). Accumulation of heavy metals in food plants and grasshoppers from the Tiegetos Mountains, Greece. *Agriculture and Ecosystems*, 85-91.

Dikinya, O. (2013). Land sustainability assessment of the proposed uranium mining area in north-east Botswana. In S. A. Shahid, F. K. Taha, & M. A. Abdelfattah, *Development in soil classifications, land use planning and policy implications: Innovative thinking of soil inventory for land use planning and management of land resources* (pp. 617-631). Dordrecht: Springer Science and Business Media.

Ehlken, S., & Kirchner, G. (2002). Environmental processes affecting plant root uptake of radioactive trace elements and variability of transfer factor data. A review. *Journal of Environmental Radioactivity*, 97-112.

El Aassy, I. E., Nada, A. A., El Galy, M. H., El Feky, M. C., El Maksoud, T. M., Talaat, S. M., & Ibrahim, E. M. (2012). Behaviour of environmental impacts of radionuclides during the hydrometallurgy of calcareous and argillaceous rocks, southwestern Sinai, Egypt. *Applied Radiation Isotopes*, 1024-1033.

Elderstein, N. M., Fuger, J., Katz, J. J., & Morss, L. R. (n.d.). Summary and comparison of actinide and transactinide elements . In *Actinides.*

Ernst, W. G. (2012). Overview of naturally occurring eath materials and human health concerns. *Journa of Asian Earth Sciences*, 108-126.

Fan, J., He, Z., Ma, L. Q., Nogueira, A. R., Wang , Y., Liang, Z., & Stoffela, P. J. (2012). Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils. *Journal of Hazardous Materials*, 375-382.

Fan, J., He, Z., Ma, L. Q., Yang, Y., Yang, X., & Stoffela, P. J. (2011). Immobilization of copper in contaminated sandy soils using calcium water treatment residue. *Journal of Hazardous Materials*, 710-718.

Feng, X. H., Zhai, L. M., Tan, W. F., Liu, F., & He, J. Z. (2007). Adsorption and redox reactions of heavy metals on synthesised Mn oxide minerals. *Environmental Pollution*, 366-373.

Figgins, P. E. (1961). *The radiochemistry of polonium.* Virginia: U.S Department of Commerce.

Fulda, B., Voegelin, A., Ehlert, K., & Kretzschmar, R. (2013). Redox transformation, solid phase speciation and solution dynamics of copper during soil reduction and reoxidation as affected by sulfate availability. *Geochimica et Cosmochimica Acta*, 385-402.

Gal, J., Hursthouse, A., Tatner, P., Sreward, F., & Welton, R. (2008). Cobalt and secondary poising in terresterial food chain: Data review and research gaps to support risk assessment. *Environmental International*, 821-838.

Greyling, M., van der Bank, F. H., Grobler, J. P., & Wessels, D. C. (2001). Allozyme variation in two populations of the Mopane worm, Imbrasia belina (Saturniidae), and the effect of developmental stage and staggered generations. *South African Journal of Animal Science*, 15-24.

Gutierrez-Carbonell, E., Lattanzio, G., Sagardoy, R., Rodriguez-Celma, J., Ruis, J. J., Matros, A., . . . Lopez-Millan, A. (2013). Changes induced by zinc toxicity in the 2-DE protein profile of sugar beet roots. *Journal of Preteomics*, 149-161.

Hansmann, W., & Koppel, V. (2000). Lead-isotopes as tracers of pollutants in soils. *Chemical Geology*, 123-144.

Hartley , W., Edwards, R., & Lepp, N. W. (2004). Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluted by short term and long term leaching tests. *Environmental Pollution*, 495-504.

Hennebel, T., De Gusseme , B., Boon, N., & Verstraete, W. (2009). Biogenic metals in advance water treatment. *Trends in Biotechnology*, 90-98.

Henricsson, C. F., Ranebo, Y., Hansson, M., Raaf, C. l., & Holm E. (2012). A biokinetic study of polonium-210 in man. *Science of Total Environment*, 384-389.

Henricsson, C. F., Ranebo, Y., Hansson, M., Raaf, C. L., & Holm, E. (2012). A biokinetic study of 209Po in man. *Science of Total Environment*, 384-389.

Henricsson, C. F., Ranebo, Y., Hansson, M., Raaf, C. L., & Holm, E. (2012). A biokinetic study of 209-Po in man. *Science of Total Environment*, 684-389.

IAEA. (1993). *Uranium extraction technologies.* Viena: International Atomic Energy Agency.

IAEA. (2006). *Environmental and source monitoring for purposes of radiation protection.* Vienna: International Atomic Energy Agency.

Impellitteri, C. A. (2005). Effects of Ph and phosphate on metal distribution with emphasis on AS specication and mobilisation in soils from lead smelting site. *Science of Total Environment*, 175-190.

International Atomic Energy Agency. (2003). *Technical reports series number 419: Extent of environmental contaminations by naturally occurring radioactive materials (NORM) and technical options for remediation.* Viena: International Atomic Energy Agency.

Johnson, B. E., Santschi, P. H., Addleman, R. S., Douglas, M., Davidson, J. D., Fryxall, G. E., & Schwantes, J. M. (2011). Collection of fission and activation products elements from fresh and marine waters; A comparison of traditional and novel sorbents. *Applied Radiation and Isotopes*, 205-216.

Jovanovic, S. P., Ilic, M. D., Markovic, M. C., Mitic, V. D., Mandic, N. S., & Stojovanovic, G. S. (2011). Wildfire impact on copper, zinc, lead and cadmium distribution in soil and relationwith abundance in related plants of Lamiceae family from Vidlic Mountain (Serbia). *Chemosphere*, 1584-1591.

Jung, M. C., & Thornston, I. (1996). Heavy metal contamination of soils and plants in the vicinity of lead-zinc mine, Korea. *Applied Geochemistry*, 53-59.

Kyle, J. H., Breuer, P. L., Bunney, K. G., Pleysier, R., & May, P. M. (2011). Review of trace toxic elements (Pb, Cd, HG, AS, Sb, Bi, Se, Te) and their deportment in gold processing. Part I: Mineralogy, aqueoues chemistry and toxicity. *Hydrometallurgy*, 91-100.

Labhart, T. P., & Rybach, L. (1971). Abundance and distribution of uranium and thorium syenite of Piz Guiv (Aar-Massif, Switzerland). *Chemical Geology*, 237-251.

Laskowski, R., & Maryanski, M. (1993). Heavy metals in epigeic fauna: Trophic level and physiological hypotheses. *Environmental Contamination and Toxicology*, 232-240.

Lee, H., & Wang, J. (2013). Annual dose of Taiwanese from the ingestion of 210Po in oysters. *Applied Radiation and Isotopes*, 9-11.

Leveque, T., Capowiez, Y., Schreck, E., Mazzia, C., Auffan, M., Foucault, Y., . . . Dumat, C. (2013). Assessing ecotoxicity and uptake of metals and metalloids in relation to two different earthworm species (Eiseina hortensis and Lumbricus terresteris). *Environmental Pollution*, 232-241.

Lewis, J., Stokes, P., Brereton, N., Baxter, M., & Macarthur, R. (2012). Stability of arsenic speciation in fish muscle samples, under different storage and sample preparation conditions. *Microchemical Journal*, 56-59.

Liang, J., Shohag, M. J., Yang, X., Tian, S., Zhang, Y., Feng, Y., & He, Z. (2013). Role of sulfur assimilation pathway in cadmium hyperaccumulation. *Ecotoxicology and Enviromental Safety by Sedum alfredii Hance*.

Lin, Z., & Wu, Z. (2009). Analysis of polonium-210 in food products and bioassay samples by isotope-dilution alpha spectromentry. *Applied Radiatiom and Isotopes*, 907-912.

Lind, O. C., Stegnar, P., Tolongutov, B., Rosseland, B. O., Stromman, G., Uralbekov, B., . . . Salbu, B. (2013). Environmental impact assessment of radionuclide and metal contamination at the former U site in Kadji, Kyrgyzstan. *Journal of Environmental Radioactivity*, 37-49.

Madibela, O. R., Seitiso, T. K., Thema, T. F., & Letso, M. (2007). Effect of traditional processing methods on chemical composition and in vitro true dry matter digestibility of the Mophane worm (Imbrasia belina). *Journal of Arid Environments*, 492-500.

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta*, 201-235.

Mandernack, K. W., Post, F., & Tebo, B. M. (1995). Manganese mineral formation by bacterial spores of marine Bacillus, strain SG-1;Evidence for direct oxidation of Mn (II) to Mn (IV). *Geochemica et Cosmochemica Acta*, 4393-4408.

Marques, A., Lourenco, H. M., Nunez, M. L., Roseiro, C., Santos, C., Barranco, A., . . . Cencic, A. (2011). New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in in meat and seafood. *Food Research International*, 510-522.

Martinez-Finley, E. J., Gavin, C. E., Aschnar, M., & Gunter, T. E. (2013). Manganese neurotoxicityandtheroleofreactiveoxygenspecies. *Free Radical Biology and Medicine*, 65-75.

Matthews, K. M., Kim, C., & Martin, P. (2007). Determination of 210 Po in environmental material: A review of analytical methodology. *Applied Radiation and Isotopes*, 267-279.

Meng, Y., Zheng, Y., Zhang, L., & He, J. (2009). Biogenic Mn oxides for adsorption of Cd from aquatic enviroment. *Environmental Pollution*, 2577-2583.

Mihucz, V. G., Varga, Z., Tatar, E., Vurag, I., van Grieken, R., Koleszar, Z., & Zaray, G. (2008). Redistribution of uranium and thorium by soil/plant interaction in recculticated mining area. *Microchemical Journal*, 44-49.

Molina, R. M., Schaider, L. A., Donaghey, T. C., & Shine, J. P. (2013). Mineralogy affects geoavailability, bioaccessibility and bioavailability of zinc. *Environmental Pollution*, 217-224.

Mora, J. C., Robles, B., Corbacho, J. A., Gasco, C., & Gazquez, M. J. (2011). Modelling of behaviour of polonium-210 in high temperature processes. *Journa of Environmental Radioactivity*, 520-526.

Murray, K. J., & Tebo, B. M. (2007). Cr (III) is indirectly oxidised by Mn (II)-oxidising bacterium Bacillus sp strain SG-1. *Environmental Sciince and Technology*, 528-533.

Navel, A., & Martins, J. M. (2014). Effect of long term organic amendments and vegetation of vineyard soils on the microscale distribution and biogeochemistry of copper. *Science of Total Environment*, 681-689.

Needleman, H. L., Gunnoe, C., Leviton, A., Reed, R., Peresie, H., Maher, C., & Barret, P. (1979). Deficit in psycologic and classroom performance of children with elevated lead dentine. *Journal of Medicine*, 689-695.

O'Brien, R. S., & Cooper, M. B. (1998). Technically enhances normally occurring radioactive materials (NORM);Pathway analysis and radiological impact. *Applied Rdioactive Isotopes*, 227-239.

OECD Nuclear Energy Agency; IAEA;. (2011). *Uranium 2011:*. Geneva: OECD Nuclear Energy Agency.

Parfenov, Y. D. (1974). Polonium-210 in environment and human organism. *Atomic Energy Revision*, 75-143.

Persson, B. R., & Holm, E. (2011). Polonium-210 and lead-210 in terresterial environment; a historical preview. *Journal of Environmental Radioactivity*, 420-429.

Pham, M. K., Povinec, P. P., Nies, H., & Betti, M. (2013). Dry and wet deposition of 7Be, 210Pb and 137Cs in Mexico air during 1998-2010: Seasonal variations of deposition. *Journal of Environmental Radioactivity*, 45-57.

Pharithi, M. T., Suping, S. M., & Yeboah, S. O. (2004). Variation of fatty acid composition in the oil from larval stages of emperor mothcaterpillar, Imbrasia belina. *Bulletin of Chemical Society of Ethopia*, 67-72.

Rendon-Ramirez, A., Maldonado-Vega, M., Quintanar-Escorza, M., Hernadez, G., Arevalo-Rivas, B., Zentella-Dehesa, A., & Calderon-Salinas, J. (2013). Effect of vitamin E and C supplementation on oxidative damage and total antioxidant capacity in lead-exposed workers. *Environmental Toxicology and Pharmacology*.

Ronald, K. L. (1998). NORM sources and their origin. *Applied Radiation Isotopes*, 149-168.

Rothwell, G. (2010). International light water nuclear fuel fabrication supply: Are fabrication services assureg? *Energy Economics*, 538-544.

Saha, P., Raychaudhuri, S. S., Chakraborty, A., & Sudarshan, M. (2010). PIXE analysis of trace elements in relation to chlorophyll concentration in Plantago ovata Forsk. *Applied Radiation and Isotope*, 444-449.

Sartandel, S. J., Jha, S. K., Bara, S. V., Tripathi, R. M., & Puranik, V. D. (2009). Spatial distribution of uranium and thorium in the surface soil around proposed mining site at Lumbapur and its vertical profile in the Nagarjuna Sagar dam. *Journal of Environmentl Radioactivity*, 831-834.

Selvaraj, K., & Agoramoorthy, G. (2006). Taiwans's heavy matal pollution threatens terrestrial biota. *Enviromental Pollution*, 327-334.

Sen, T. K., Mahajan, S. P., & Khilar, A. C. (2002). Adsorption of Ni2+ and Cu2+ on iron oxides and and kaolin and its importance on Ni2+ transport in porous media. *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 91-102.

Shayeganrad, G. (2013). On the remote monitoring of gaseous uranium hexafluoride in the lower atmosphere using lidar. *Opticsand Lasers in Engineering*, 1192-1198.

Sheppard, S. C., Sheppard, M. I., Gallerand, M., & Sanipelli, B. (2005). Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity*, 55-83.

Sheppard, S. C., Sheppard, M. L., Ilin, M., Tait, J., & Sanipelli, B. (2008). Primordial radionuclides in Canadian background sites: Secular equilibrium and isotopic differences. *Journal of Environmental Radioactivity*, 933-946.

Simonsen, L. O., Harbak, H., & Bennekou, P. (2012). Cobalt metabolosm and toxicity. *Science of Total Environment*, 210-215.

Simonsen, L. O., Harbak, H., & Bennekou, P. (2012). Cobalt metabolosm and toxicity - A brief update. *Science of Total Environment*, 210-215.

Stille, P., Pourcelot, L., Granet, M., Pierret, M. -C., Gueguen, F., Perrone, T., . . . Chabaux, F. (2011). Deposition and migration of atmospheric Pb in soil from deforested silicate catchment today and in the past (Strengbach case): Evidence from Po-210 activities and Pb isotope ratios. *Chemical Geology*, 140-153.

Stillman, M. J. (1995). Metallothioniens. *Coordination Chemistry Reviews*, 461-511.

Suhartono, E., Triawanti, Yunanto, A., Firdaus, R. T., & Iskandar. (2013). Chronic Cadmium Hepatooxidative in Rats: Treatment with Haruan Fish (Channa striata) Extract. *APCBEE Procedia*, 441-445.

Thompson, A., Chadwick, O. A., Rancourt, D. G., & Chorover, J. (2006). Iron-oxide crystallinity increases during soil redox oscillations. *Geochemica et Cosmochemica Acta*, 1710–1727.

Tripathi, R. M., Sahoo, S. K., Jha, V. N., Khan, A. H., & Puranik, P. D. (2008). Assessment of environmental radioactivity at uranium mining, processing and tailings management facility at Jaduguda, India. *Applied Radiation and Isotopes*, 1666– 1670.

Tserenpil, S., Maslov, O. D., Norov, N., Liu, Q. C., Fillipov, M. F., Theng, B. K., & Belov, A. G. (2013). Chemical and mineralogical composition of the Mongolian rural soils and their uranium sorption behavior. *Journal of Environmental Radioactivity*, 105-112.

Vesterbacka, P., & Ikaheinmonen, T. K. (2005). Optimisation of 210Po on a silver disc. *Analytica Chemica Acta*, 252-261.

Voigt, D. E., Brantley, S. L., & Rennet, R. J. (1996). Chemical fixation of arsenic in contaminated soils. *Applied Geochemisto*, 633-643.

Walraven, N., van Gaans, P. F., van der Veer, G., van Os, B. J., Klaver, G. T., Vriend, S. P., . . . Davies , G. R. (2013). Lithologically inherited variation in Pb isotope ratios in sedintary rocks in The Netherlands. *Applies Geochemistry*, 228-241.

Waska, H., Kim, S., Kim, G., Kang, M. R., & Kim, G. B. (2008). Distribution patterns of chalcogens (S, Se, Te and 210Po) in variuos tissues of squidm Todarodes pacificus. *Science of Total Environment*, 218-224.

Watson, A. P. (1985). Polonium-210 and lead-210 in food and tobacco products: Parameters and norma; exposure and dose. *Nuclear Safety*, 179-191.

Wei, C., & Murray, J. W. (1994). The behaviour of scavenged isotopes in marine anoxic environments; 210Pb and 210Po in water column of the Black Sea. *Geochemica et Cosmochemica Acta*, 1795-1811.

Xhao, X., Chen, L., Muhammad, M. I., Wang, Q., Wang, S., Hou, P., & Ding, Y. (2013). Effect of Nitric Oxide on Alleviating Cadmium Toxicity in Rice (Oryza sativa L.). *Jaournal of Integrative Agriculture*, 1540-1550.

Yassi, A., Kjellstrom, T., De Kok, T., & Guidotti, T. L. (2001). *Basic environmental health.* New York: Oxford University Press.

Zhaung, P., Zou, H., & Shu, W. (2009). Biotransfer of metals along along a soil-plant-insect-chicken food chain: Field study. *Journal of Environmental Sciences*, 849-853.

Zheljazkov, V. D., Pickett, K. M., Caldwellc, C. D., Pincock, J. A., Roberts, J. C., & Pincock, L. (2008). Cultivar and sowing date effects on seed yield and oil composition of coriander in Atlantic Canada. *industrial crops and products*, 88-94.

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no