

SOIL, WATER AND TISSUE HEAVY METAL OF COMMUNAL SHEEP AND THE POSSIBLE PUBLIC HEALTH IMPLICATIONS AROUND THE POTENTIALLY POLLUTED AREA OF KHUTSONG, SOUTH AFRICA

BY

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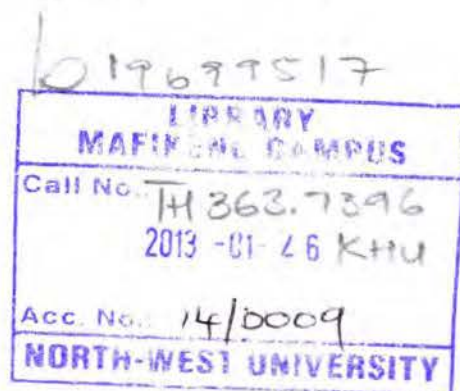
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DECLARATION

I, Khunou Letlhogonolo, hereby declare that the work on which this dissertation is based is original (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree at this or any other university.

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ABSTRACT

The present study was carried out to determine the levels of heavy metals, Arsenic (As), Chromium (Cr), Cadmium (Cd) and Lead (Pb) in samples of water, sediments, and specimens from sheep known to graze and drink from Wonderfontein stream around the Khutsong area in the North West Province of South Africa. Determination of heavy metal levels was carried out using Atomic Absorption Spectrophotometer. Abundance of metals in water samples followed the trend $As > Pb > Cd > Cr$, while that in sediments followed the trend: $Pb > As > Cr > Cd$. Faecal levels were highest for Cr, followed by Cd, As then Pb, while serum levels were highest for As, Cd Cr and then Pb. The metal concentration in liver, kidney and muscle showed the following trends respectively: $As > Cd > Cr > Pb$; $As > Pb > Cd > Pb$ and $As > Cd > Cr > Pb$. The liver, kidney and muscle samples had higher concentration of As compared to other heavy metals. Generally, most samples showed a higher concentration in As. The mean concentrations of heavy metals in ppm were compared with European Commission Regulation, World Health Organisation, Korean Soil Environmental Conservation Act and the Australian New Zealand Food Standards maximum acceptable levels. The metal levels generally tended to be higher than the permissible levels and thus, public health risks. A survey conducted also revealed that the community in Khutsong does not have the knowledge on environmental contamination due to mining effluents and the effects thereof. The varying levels of water and sheep specimen contamination with As, Cd, Cr and Pb revealed in this study imply public health risks. Further biomonitoring, public and animal health studies are therefore indicated in this area.

CHAPTER 1

1.1 INTRODUCTION

The term heavy metal refers to any chemical element that has a relatively high density and is toxic or poisonous at a low concentration. Although these toxic metals are natural components of the environment, human activities, notably industrial and mining processes, have been responsible for increasing their prevalence (Miranda *et al.*, 2005). Sources of heavy metal contamination include a number of old and abandoned mine sites (Moss and Constanzo, 1998). Heavy metals can enter a water supply from industrial and consumer waste or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes and ground water.

Vegetation can be a useful indicator of heavy metal contamination in an environment in that root uptake of metals can integrate scales. In addition, heavy metal accumulation by vegetation can be further magnified within ecosystems via food webs (Pugh *et al.*, 2002). Soil pollution with potentially toxic metals and metalloids represents one of the most prominent environmental hazards from abandoned mine lands, which affects many countries having historic mining industries. As a direct result of the open pit mining operations, soil is destroyed over a considerable area and what is left of it is generally degraded and may continue causing environmental damage long after the mining period (Carvallo and Fernández, 2008).

Toxic metals cannot be degraded or destroyed. These metals enter human and/ or animal bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, zinc, selenium) are essential to maintain the metabolism of the body. However, at higher concentrations, they can lead to poisoning. When toxic metals are accumulated in soils and plants, animals fed with these plants will tend to accumulate toxic metals in their bodies, it is noteworthy that contamination of animal feed by toxic metals cannot be entirely avoided. Given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health (SCAN, 2003).

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical concentration in the environment. Compounds accumulate in living things any time they are up taken and stored faster than they are broken down (metabolism) or excreted.

Heavy metals have been known to cause a variety of effects on humans and animals ranging from biological, pathological (including haematologic and biochemical aberrations), reproductive and even mortality. In production animals, production losses may result when the health of the animal has been compromised.

The main factors affecting the accumulation of potentially toxic metals (PTM) by grazing animals are the presence of the metal, its concentration in herbage at the soil surface and the duration of exposure to the contaminated pasture and soil. In addition, the elapsed time between the contamination of the pasture and grazing, the quality of soil ingested together with herbage, the mechanism of absorption of the metal into blood and the presence or absence of antagonist metals can interact to influence the rate and extent of accumulation of heavy metals in body tissues (Wilkinson *et al.*, 2003).

The disposal of mine wastes often produces more environmental problems than the mining operations themselves. The pollutants may be transferred from tailings and waste rock dumps to nearby soils by acid mine drainage and or atmosphere deposition of windblown dust, depending on climatic and hydrologic conditions which determine locations of potentially contaminated areas. Upon exposure to the surface environment, over burden materials can be weathered leading to soil development in the abandoned mine sites (Carvallo and Fernández, 2008).

The Khutsong area is host to a wide range of gold mining activities. Other gold mining catchments have been associated with varying levels of heavy metal contamination that has posed potential risks to the surrounding communities. In such situations, inhabitants of informal settlements are more at risk (Winde and van der Walt, 2004). The Khutsong area boasts of high gold mining activities and this has exposed the area to high risk of pollution with heavy metals.

The area is presumed to be heavily contaminated with heavy metals to the extent that municipal authorities actually advise residents not to use, for any domestic purposes, water from the Wonderfontein stream that passes through Khutsong. Nevertheless, local residents engage in a lot of fishing activities in an effort to supplement dietary protein for the largely low income community and the surrounding informal settlements. Also, sheep, goats, and cattle graze and drink in and around the stream, thereby, possibly bioaccumulating pollutants. The same livestock are sold and slaughtered within the communities, further exposing residents to the pollutants.

Despite these presumptions of high levels of contamination, no scientific studies have been conducted to determine the range and levels of heavy metal pollution in the area. The presumption of high levels of heavy metal contamination in the Khutsong area warrants urgent investigations into the prevalence and levels of heavy metals in this area. The scarcity of information on aspects of animal health and productivity is contrary to the vital and integral role that livestock species play in the livelihoods of their respective communities, as a source of food, income and security. Livestock also forms a vital link between humans and the food chain, thereby, wielding great possibilities of exposing humans to toxic heavy metals. After conducting this study, the heavy metal content in the water, plants and livestock species in the Khutsong catchment will be established. This may lead to policy changes that will promote animal, human and environmental safety.

CHAPTER 2

2.1 OBJECTIVES

The objectives of this study were to:

- i. Determine the prevalence and concentrations of heavy metals in water and sediments around the Khutsong area;
- ii. Determine the concentrations of heavy metals in sheep specimens (liver, muscle, kidney, faeces and blood) around the Khutsong area; and
- iii. Determine the knowledge of heavy metal pollution on human health.
- iv. Determine the potential health effect on human exposed to these metals.

CHAPTER 3: LITERATURE REVIEW

3.1 EFFECTS OF SOME HEAVY METALS

i. URANIUM (U)

Uranium is a radio-toxic and chemotoxic heavy metal (Stojanovic *et al.*, 2008). Uranium is a naturally occurring element that can be found in low levels within all rocks, soil, and water. Uranium is also the highest-numbered element to be found naturally in significant quantities on earth and is always found combined with other elements (Hammond, 2000). An animal can be exposed to Uranium (or its radioactive daughters such as radon) by inhaling dust in air or by ingesting contaminated water and food. The uptake and accumulation of uranium has been studied in plants native to uranium mine sites but not in cultivated plants which are commonly consumed by humans (Stojanović *et al.*, 2008). It was generally observed that plant species differ in uranium accumulation. Uranium accumulates mainly in the roots and the depth of uranium placement and soil properties influence adsorption by plants (Stojanovic *et al.*, 2008).

The amount of uranium in air is usually very small. However, people who work in factories that process phosphate fertilizers, live near government facilities that make or test nuclear weapons, live or work near a modern battle field where

depleted uranium weapons have been used or live or work near coal fired power plant, facilities that mine or process uranium ore, or enrich uranium for reactor fuel, are highly exposed to uranium (USEPA, 2009). Normal functioning of the kidney, brain, liver, heart and other systems can be affected by uranium exposure, because in addition to being weakly radio-active, uranium is a toxic metal (Craft *et al.*, 2004). Uranium is also a reproductive toxicant (Arfsten *et al.*, 2001) and other hexavalent uranium compounds have been shown to cause birth defects and immune system damage in laboratory animals (Domingo, 2001).

Table 1. Some effects of uranium toxicity in humans and animals (Craft et al., 2004)

Body system	Human studies	Animal studies	<i>In vitro</i>
Renal	Elevated levels of protein excretion, urinary catalase and diuresis	Damage to Proximal convoluted tubules, necrotic cells cast from tubular epithelium, glomerular changes	No studies
Brain/CNS	Decreased performance on neurocognitive tests	Acute cholinergic toxicity; Dose-dependent accumulation in cortex, midbrain, and vermis; Electrophysiological changes in hippocampus	No studies
DNA	Increased reports of cancers	Increased urine mutagenicity and induction of tumours	Binucleated cells with micronuclei, Inhibition of cell cycle kinetics and proliferation; sister chromatid induction, tumorigenic phenotype
Bone/muscle	No studies	Inhibition of periodontal bone formation; and alveolar wound healing	No studies
Reproductive	Uranium miners have more first born female children	Moderate to severe focal tubular atrophy; vacuolization of Leydig cells	No studies
Lungs/respiratory	No adverse health effects reported	Severe nasal congestion and hemorrhage, lung lesions and fibrosis, edema and swelling, lung cancer	No studies
Gastrointestinal	Vomiting, diarrhea, albuminuria	n/a	n/a
Liver	No effects seen at exposure dose	Fatty livers, focal necrosis	No studies
Skin	No exposure assessment data available	Swollen vacuolated epidermal cells, damage to hair follicles and sebaceous glands	No studies
Tissues surrounding embedded DU fragments	Elevated uranium urine concentrations	Elevated uranium urine concentrations, perturbations in biochemical and neuropsychological testing	No studies
Immune system	Chronic fatigue, rash, ear and eye infections, hair and weight loss, cough. May be due to combined chemical exposure rather than DU alone	No studies	No studies
Eyes	No studies	Conjunctivitis, irritation inflammation, edema, ulceration of conjunctival sacs	No studies
Blood	No studies	Decrease in RBC count and hemoglobin concentration	No studies
Cardiovascular	Myocarditis resulting from the uranium ingestion, which ended 6 months after ingestion	No effects	No studies

ii. Cadmium and Lead

Cadmium (Cd) and Lead (Pb) are environmental pollutants toxic to humans and animals (Liu *et al.*, 2007) and are also pervasive environmental pollutants with public health hazard as contaminants of food from animal origin (Swarup *et al.*, 2005). Man-made activities including mining of ores and industrial activities lead to the emission of lead, resulting in environmental pollution and contamination of forages for animal consumption (Swarup *et al.*, 2005). Significant amounts of Cd and Pb can be transferred from contaminated soil to plants and grass (Pugh *et al.*, 2002; Zhu *et al.*, 2007) causing accumulation of these potentially toxic metals in grazing ruminants (Farmer and Farmer, 2000; Wilkinson *et al.*, 2003; Wlostowski *et al.*, 2006), particularly in cattle. Accumulation of Cd and Pb in ruminants causes toxic effects in cattle but also in humans consuming meat contaminated with toxic metals (Cai *et al.*, 2009).

Animals get access to lead from soil, water, feed and fodder and varied degrees of lead poisoning have been reported in animals reared around different polluted areas (Kottferova and Korenekova, 1995). The poisoning is more common in farm ruminants, considered most susceptible to the toxic effects of lead (Swarup *et al.*, 2005). The excess transfer of metals to the food chain is thought to be controlled by a soil-plant barrier. However, this barrier fails when metal concentrations reach critical limits, especially for toxic metals such as Cd and Pb (Cataldo and Wildung, 1978).

The basis of cadmium toxicity is its negative influence on enzymatic systems of cells, resulting in other metal ions (mainly Zn, Cu and Ca) in metalloenzymes and its very strong affinity to biological structures containing -SH (sulphydryl) groups such as protein enzymes and nucleic acids. Many effects of cadmium action result from interaction with necessary micro and macro elements, especially Ca, Zn, Cu, Fe and Se. These interactions can take place at different stages of the absorption, distribution and excretion of the bioelements and cadmium as well as at the stage of biological functions of essential elements (Brzóška and Moniuszko-Jakoniuk, 2001).

A survey of heavy metals and pesticides in bovine liver, kidney and muscle (meat) was conducted in Guizhou, China from 2001 to 2005. Cadmium and Pb concentrations in bovine kidneys were higher than the Food Safety Standards in 94% and 32% of the samples respectively (Cao *et al.*, 2006).

iii. Mercury

Mercury (Hg) is a heavy metal which occurs in several forms, and can produce toxic effects in high enough doses. Toxic effects include damage to the brain, kidneys, and lungs (Davidson *et al.*, 2004). The consumption of fish is by far the most significant source of ingestion-related mercury exposure in humans, although plants and livestock also contain mercury due to bioaccumulation of mercury from soil, water, and atmosphere. Biomagnifications through the ingestion of other mercury containing organisms may also be an important source of mercury.

In humans, exposure to mercury can also occur from breathing contaminated air (ATSDR, 1999), from eating foods containing mercury residues from processing, from exposure to mercury vapour in mercury amalgam dental restorations (Levy, 1995) and from improper use or disposal of mercury containing objects, for example, after spills of elemental mercury or improper disposal of fluorescent light bulbs (Goldman *et al.*, 2001). Coal plants emit approximately half of atmospheric mercury, with natural sources such as volcanoes responsible for the remainder. An estimated two thirds of mercury comes from stationary combustion, mostly of coal. Other important human-generated sources include gold production, ferrous metal production, cement production, waste disposal, crematoria, caustic soda production, pig iron and steel production, mercury production (mostly for batteries) and biomass burning (Pacyna *et al.*, 2006).

Mercury is such a highly reactive toxic agent whose specific mechanism of damage is difficult to identify (Clarkson *et al.*, 2006). It damages the central nervous system, endocrine system, kidneys, and other organs and adversely affects the mouth, gums and teeth. Exposure over long periods of time or heavy exposure to mercury vapour can result in brain damage and ultimately death. Mercury and its

compounds are particularly toxic to developing fetuses and the young, and may result in birth defects following exposure during pregnancy (Hendry *et al.*, 1993).

Animal data indicate that less than 0.01% of ingested mercury is absorbed through the intact gastrointestinal tract; though it may not be true for individuals suffering from ileus. Though not studied quantitatively, the physical properties of liquid elemental mercury limit its absorption through intact skin, and in light of its very low absorption rate from the gastrointestinal tract, skin absorption would not be high (ATSDR, 1999). In humans, approximately 80 % of inhaled mercury vapour is absorbed via the respiratory tract where it enters the circulatory system and is distributed throughout the body. Chronic exposure by inhalation, even at low concentrations in the range 0.7 to 42µg/m³ has been shown in case control studies to cause effects such as tremors, impaired cognitive skills, and sleep disturbance in workers (Ngim *et al.*, 1992).

iv. Copper

Copper is a reddish metal that occurs naturally in rock, soil, water, sediment and at low levels in air. Its average concentration in the earth's crust is about 50 ppm. Copper also occurs naturally in all plants and animals; it is an essential element for all known living organisms (ATSDR, 2004). Copper is a trace element essential to the function of specific proteins and enzymes. The increasing industrial activities and the use of Copper sulphate as a fungicide in agricultural practices as well as in the control of algae and pathogens in fish culture ponds have increased the copper concentration in aquatic systems. Furthermore, occasional accidents have aggravated this situation by suddenly introducing substantial amounts of copper into aquatic environments, which may be accompanied by changes in water pH, depending on the type of industrial effluent in question (Carvalho and Fernandes, 2008).

The toxicity of copper to fish has been well documented. In addition to its acute lethality, a wide range of toxicological responses of several organs to this metal

has been reported in a number of fish species (Wood, 2001 ; Dautremepuits *et al.*,2004). Copper alters the function of the gills and liver (Grosell *et al.*, 2002 ;Dautremepuits *et al.*, 2004) by causing severe histological changes in these organs .Copper uptake in freshwater fish occurs mainly by the gills, followed by the skin and intestines.

The liver is the major organ in which copper homeostasis occurs. Copper is accumulated in the liver to be excreted via the bile even though gills and kidneys also participate in its excretion. (Grosell *et al.*, 1998; Mazon and Fernandes, 1999). Copper toxicity depends on chemical and physical characteristics of water. Temperature, PH, hardness and alkalinity are the main factors influencing copper bioavailability in aquatic environments (Tao *et al.*, 2001). Both on mammalian cell systems, both deficiency and excess of copper induce toxic effects in-vivo and in-vitro.

Copper is essential for plant and animal nutrition and is an essential micronutrient. It is involved in several metabolic processes. It is a component of many proteins, and also plays a vital role in many enzyme systems. Copper deficiency symptoms in animals include low plasma copper and ceruloplasmin levels, anaemia, demyelination, and skeletal defects (McDonald *et al.*, 1995). It has been previously reported that copper deficiency is also associated with decreased cytochrome-c-oxidase and super oxide dismutase in the heart and liver of rats (Rossi *et al.*, 1998). Copper is therefore essential for good health, however, exposure to higher doses can be harmful (ATSDR, 2004).

v. Zinc

Zinc is one of the most common elements in the earth's crust. It is also an environmental pollutant and omnipresent in the environment (Weltje, 1998). Zinc is found in the air, soil and water and is present in all foods. Zinc enters the air, water, and soil as a result of both natural processes and human activities. Most Zinc enters the environment as a result of mining, the purifying of zinc, lead and cadmium ores, steel production and coal burning of waste (ATSDR, 2005). Millions of tons of zinc metal are used commercially, principally to galvanise iron and to manufacture brass (Barceloux, 1999). It is also used widely in preservative treatment, fungicidal action and medicine, etc. (Barceloux, 1999).

Zinc or zinc salts may enter the body by inhalation, through the skin or by ingestion and induce irritation of the respiratory or digestive system, and dental deterioration and ulceration of the skin, and zinc fumes cause fever, chills nausea and vomiting, and muscular aches and weakness (Barceloux, 1999). Therefore, zinc is a definite environmental hazard. It is well known that zinc is an essential trace element and has important biological functions that control many cell processes including DNA synthesis, normal growth, brain development, behavioural response, reproduction, fetal development, bone formation, and wound healing (Barceloux, 1999; Calesnick and Cla, 1988).

Zinc deficiency results in growth retardation, testicular atrophy, skin changes, and suppressed appetite. Because zinc is a nutrient, many people assume that if a little is good, more will be better. Some people even believe that zinc will cure various ailments, including growth failure, cancer, infection, skin diseases, and wounds since these manifestations can result from the Zn-deficiency (Shah *et al.*, 1988; Yadrick *et al.*, 1989; Batra *et al.*, 1998). Misunderstanding the availability of zinc supplements, lack of knowledge about zinc toxicity and the ease with which many preparations of zinc salts can be obtained over the counter in drug stores and in health food stores, have led to zinc supplements being widely used by the public as self-medication at unknown dosages (Sandstead, 1995).

Some cases of intoxication following ingestion of elemental zinc in an attempt to promote wound healing or control anger were reported (Broun *et al.*, 1990; Forman *et al.*, 1990; Lewis and Kokan, 1998).

Zinc is an essential element needed by the body and is commonly found in nutritional supplements. However, taking too much zinc into the body can affect health (Fosmire *et al.*, 1990; ATSDR, 2005). The free zinc ion in solution is highly toxic to plants, invertebrates and even vertebrate fish (Eisler, 1993). Concentrations of Zinc as low as 2 ppm can adversely affect the amount of oxygen that fish can carry in their blood (Heath, 1995). Levels of zinc in excess of 500 ppm in soil interfere with the ability of plants to absorb other essential metals, such as iron and manganese (Emsley *et al.*, 2001). Studies on rats have shown that excessive dietary zinc in these animals induces deficiencies of copper and iron, producing poor growth and anemia (Llobet *et al.*, 1988; Calesnick and Cla, 1988).

These findings indicate that excessive intake of zinc supplements is also a potential risk to humans. Although some toxic effects in human subjects, rodents and sheep have been reported (Ryun *et al.*, 2002; Chen, 1992), because research on the possible toxic effects of zinc in man and experimental animals has lagged behind the progress made in studying its other characteristics, there has been sporadic reports on the toxicity of zinc and relatively little information is available from systemic observation of zinc toxic effects (Cassel, 1978).

Cadmium and zinc are elements having similar geochemical and environmental properties (Tingqiang *et al.*, 2009). Cadmium is commonly found in zinc ores which are the principal commercial sources of cadmium. Both metals are classified commonly with mercury (Hg) in group ii B of post- transition elements of the periodic table. In this way, one of the metals can influence the uptake and action of the other, depending on their levels (in experimental studies it has been demonstrated that even low-level cadmium intake can inhibit zinc absorption (Coppen –Jaeger and Wilhelm, 1989).

Disturbances in zinc function and metabolism, independently of cause, may have serious consequences for health. This element plays an important role in growth, development and functioning of all living cells. It is involved as a co-factor in a number of metalloenzymes (over 200) and regulatory proteins, including enzymes of both DNA and RNA biosynthesis and repair, the principal mechanism of the activity of enzymes participating in replication, transcription and translation process. By influencing the activity of many enzymes, zinc regulates overall metabolism of the organism (Brzóška and Moniuszko-Jakoniuk, 2001).

iv. Nickel

Nickel, together with other pollutants are released into the environment as a result of combustion of fossil fuels, crude oil, and coal. Nickel in the atmosphere can be combined with other pollutants, producing various nickel compounds which have varying animal toxicities (Novelli *et al.*, 1997). Nickel is a natural element of the earth's crust, therefore, small amounts are found in food, water, soil, and air (ATSDR, 1997). Nickel and its compounds are widely used in commerce and are also widely distributed throughout the environment. Natural source of atmospheric nickel is derived from volcanic emissions and the weathering of rocks and soils, and natural source of aqueous nickel include biological cycles and solubilisation of nickel compounds from soils (Kasprzak *et al.*, 2003).

It is well known that nickel exposure is associated closely with skin allergies, lung fibrosis and cancer risk (Kasprzak *et al.*, 2003). Animal and human studies have revealed an increased risk of lung and nasal cancer, pulmonary fibrosis, renal edema and immune effects following exposure to nickel refinery dust, nickel subsulfide, or soluble nickel compounds (nickel carbonyl) (USEPA, 1986; ATSDR, 1997).

Nickel is considered an industrial health hazard, since exposure to highly nickel-polluted environments, such as those associated with nickel refining, electroplating, and welding, has the potential of producing a variety of pathologic or adverse effects (Kasprzak *et al.*, 2003). In addition, nickel has been regarded as the predominant occupational allergen in women and the eighth most common in men

(Wall and Gebauer, 1991). Nickel can generate reactive oxygen species to cause DNA damage and inhibit DNA repair (Kasprzak *et al.*, 2003; Kodipura *et al.*, 2004; Wozniak and Blasiak, 2004). It has also been found to be able to affect the heterochromatin and inhibit histone acetylation (Costa *et al.*, 2001).

In addition, nickel exposure has immunotoxicity on rats (Harkin *et al.*, 2003). Nickel exposure also results in reproductive toxicity by reducing seminal vesicles' weight and size, sperm motility and count in mice (Pandey *et al.*, 1999; Pandey and Singh, 2001). Therefore, the nickel exposure has multiple biological toxicities on animal differentiation and development. Though it is highly toxic, nickel is an essential element in animals, where its deprivation is associated with depressed growth, reduced reproductive rates, and alterations of serum lipids and glucose (Barceloux, 1999).

vii. Chromium

Chromium (Cr) is an essential trace element metal for living organisms and it is believed to work as a cofactor with insulin (Anderson, 1997). However, its high toxicity, mutagenicity and carcinogenicity render it hazardous at very low concentration. Chromium pollution often derives from the effluents of many industries; especially chrome plating and chrome tanning industries, and lead to surface and ground water pollution (Yayintas *et al.*, 2007; Saxena *et al.*, 2009). Waste water pollution in industrial areas is one of the most important environmental problems. Heavy metal pollution, especially chromium species in waste water sources from tannery affects lives (Yayintas *et al.*, 2007).

Chromium is an essential oligo-element for human metabolism. It is present in nature in different oxidative forms, of which trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) chromium are the most common. The daily chromium requirement, which is constituted exclusively of Cr^{3+} , ranges from 5 to 115 $\mu\text{g}/\text{day}$. Chromium gastrointestinal absorption is very low and only 1–25% of ingested chromium is absorbed. The combination of Cr^{3+} with certain amino acids produces organic complexes such as polynicotinate and nicotinate, which have a much higher rate of absorption than inorganic chromium. Chromium toxicity is closely related to its valency. Cr is 100 times more toxic than Cr^{3+} . Hexavalent chromium, used

industrially as an insoluble salt, has a well-documented toxic effect that is dose-dependent (Lança *et al.*, 2002).

vii. Cobalt

Cobalt is a naturally occurring element found in rocks, soil, water, plants, and animals. It is used to produce alloys used in the manufacture of aircraft engines, magnets, grinding and cutting tools, and artificial hip and knee joints. Cobalt compounds are used to colour glass, ceramics and paints. Cobalt is essential for animals since it is a part of Vitamin B12. However, exposure to high levels of cobalt can result in heart effects and dermatitis. Liver and kidney effects in animals and memory deficits in humans have also been observed following exposure to high levels of cobalt (ATSDR, 2004).

Direct injection of cobalt under the muscles or skin of hamsters resulted in tumors at the site of injection, while none were noted following prolonged exposure by inhalation (Calabrese and Kenyon, 1991). Although cobalt is an essential nutrient, excessive oral doses result in a variety of adverse responses. In higher concentrations, cobalt is toxic to humans and to terrestrial and aquatic animals and plants (Nagpal 2004). The best characterised toxic responses are increases in red blood cell counts (polycythemia), cardiomyopathy and effects on male reproductive systems. However, cobalt can be used safely in monitored medication to treat non-iron anaemia (Barceloux, 1999). Exposure to high levels of cobalt may also cause asthma, pneumonia and wheezing (ATSDR, 2004).

3.2 EFFECTS OF HEAVY METAL TOXICITY ON REPRODUCTION AND HAEMATOLOGY

Changes in Zn distribution have also been noted in pregnant animals dosed with Cd and in their fetuses. Low dietary Zn cannot only increase Cd accumulation in various internal organs of experimental animals, especially in the liver and kidney, but also alter its distribution in cytosolic proteins of these target organs (Brzóška and Moniuszko-Jakoniuk, 2001). Cd has been shown to produce a variety of adverse reproductive effects in humans and experimental animals. Even low-level exposure to this metal leads to its accumulation in placenta, placental abnormalities, decrease in birth weight, foetal growth retardation and malformations (Kantola *et al.*, 2000; Moniuszko-Jakoniuk, 2001).

Zn is required for many aspects of foetal growth and plays important roles in both prenatal and postnatal development. Exposure to Cd during pregnancy is associated with alterations in maternal and foetal disposition of Zn. Maternal Zn retention is thought to be one of the causes of foetal Zn deprivation and impaired foetal growth. Decreased Zn concentrations in foetal tissue, accompanied by reduction in the activities of Zn metalloenzymes in both maternal and foetal tissues, may also be responsible in part for the adverse reproductive outcomes commonly associated with exposure to cadmium during pregnancy. A relationship between reduced brain level of Zn and dysfunction of the central nervous system in adulthood of offspring of female rats exposed to Cd during gestation. Conversely, a protective effect of Zn against Cd induced embryonic and foetal toxicity and teratogenicity has been described in experimental animals (Kantola *et al.*, 2000; Moniuszko-Jakoniuk, 2001).

A positive correlation between birth weight and Zn status of newborn infants and a negative correlation between their Zn status and Cd concentrations in maternal blood and placenta were observed in women exposed to Cd via smoking. In smoking women, blood Cd, placental Cd and placental Zn levels were negatively related to birth weight. Moreover, decreased Zn levels in cord vein red blood cells

were significantly related to decreased birth weight in non-smokers. (Brzóška and Moniuszko-Jakoniuk, 2001). The concentration of Zn in the red blood cells from cord vein was also positively related to birth weight. Available data suggest that interactions between Cd and Zn occur in the placenta even at "normal" levels of exposure to Cd and over a very short period of time (Brzóška and Moniuszko-Jakoniuk, 2001). A significant positive correlation was noted between placental Cd and Zn, but only in multiparous women but there are also studies in which no negative effect of Cd on Zn status was noted (Osman *et al.*, 2000). The available literature suggests that multiparous women, particularly cigarette smokers, may be the most vulnerable to embryo fetal actions of Cd due to decreased Zn concentrations in serum, placenta and cord blood (Osman *et al.*, 2000).

3.3 EFFECTS OF HEAVY METAL TOXICITY ON PRODUCTION

Heavy metals normally occurring in nature are not harmful to the environment, because they play an essential role in tissue metabolism and growth of plants and animals. However, severe metal imbalances are toxic and marginal imbalances contribute to deformities and impede health (Birungi *et al.*, 2007).

The lead level in milk from animals exposed to environmental pollutant has serious public health concern. A linear dose related excretion of lead from plasma into milk was found in rats and mice after intravenous injection and the lead concentration in milk was approximately 100 times higher than that in plasma 24 h after administration demonstrating a very efficient transport of lead into milk. This is substantiated by the findings that rat neonates exposed to lead via the placenta and milk, had more than 6 times greater blood and brain lead concentrations than neonates exposed only via placenta. Oral feeding of lead acetate at the dose rate of 500 mg/day to limited number of lactating cows has been reported to significantly increase the milk lead excretion (Swarup *et al.*, 2005).

The normal functions of fish are susceptible to adverse changes in water quality. Occurrence of aquatic pollutants (such as heavy metals) has been correlated to alterations in the fish immune system and the incidence of infectious diseases. Even very low sub lethal doses of certain heavy metals can have profound effects

on the structure and / or functions of the immune system that could be almost as harmful as direct toxic doses. The heavy metal Chromium is often found in the effluents of many industries, especially Chrome plating and Chrome tanning industries which are a major source of pollution of surface and ground water. Pollution of water with heavy metals may adversely affect the immune system of fish leading to decreased production, increased susceptibility to diseases and mortality (Saxena *et al.*, 2009).

3.4 EFFECTS OF HEAVY METAL TOXICITY ON HISTOPATHOLOGY

3.4.1 Hepatotoxicity

Liver is a target organ following acute Cd intoxication. High doses of Cd administered to experimental animals lead to morphological and functional changes in this organ. It has been shown that Zn administered prior to Cd protects against Cd-induced liver toxicity, including lipid peroxidation and cell damage, even using otherwise lethal doses of Cd (Brzóška and Moniuszko-Jakoniuk, 2001).

Cd is a cumulative element with biological half-life of about 20 years. The main accumulation sites for this metal in humans are the kidney and liver, responsible for half of the whole body retention. Cd accumulation in the organism is accompanied by changes in levels of some essential elements, including Zn. The Cd-induced changes in Zn homeostasis result in an increased retention of Zn in the liver and/or kidneys which decreases its availability for other tissues (for example bone) and many biochemical processes. A highly positive correlation between Cd and Zn concentrations in liver and kidneys has been noted (Oishi *et al.*, 2000). The Cd-induced retention of Zn in the liver and/or kidney is due to Cd accumulation and in these organs (Brzóška and Moniuszko-Jakoniuk, 2001).

3.4.2 Nephrotoxicity

Cadmium (Cd) is a toxic metal. It has no essential biological function and is extremely toxic to humans. Cadmium is used widely in many industries and is an important source of environmental pollution. When cadmium is absorbed into the body, it usually accumulates in the kidney for long periods of time. When it reaches a critical threshold, it leads to serious kidney failure. The results of blood cadmium levels are used to diagnose toxicity. Whole blood cadmium levels have been used to evaluate occupational exposure (Foihirun *et al.*, 2006).

The kidneys are major sites of antagonistic interactions between essential elements (including Zn) and Cd, and a target organ for Cd toxicity. Long-term, even low-level exposure to this metal leads to kidney damage characterised by tubular dysfunction (Zn deficiency may enhance renal Cd toxicity in animals. Degenerative changes in the proximal convoluted tubules of kidneys such as cytoplasmic vacuolation, mitochondrial swelling and coagulative necrosis were noted in rats fed Zn deficient (0 mg Zn/kg) but not Zn adequate (30 mg Zn/kg) diet containing 100 mg Cd/kg. (Brzóska and Moniuszko-Jakoniuk, 2001).

3.4.3 Action in bone

Long-term Cd exposure leads among other effects, to bone lesions (osteoporosis, osteomalacia) in humans and experimental animals. It is thought that bone damage is a result of both direct (influence on bone cells) and indirect (by influence on kidneys and gastrointestinal tract) actions of Cd. This can have important consequences for bone calcification, since this Zn dependent enzyme is involved in the formation of bone mineral matrix. In the ribs of Cd exposed subjects, an increase in Cd concentration was noted to correlate significantly with a decrease in Ca/Zn Ratio in ribs of Cd-exposed subjects. Moreover, the Ca/ Zn ratio was related to the degree of osteomalacia in these persons.

There is limited data on the influence of dietary Zn status on Cd action in bone tissue. Because of the important role of Zn in bone metabolism, its deficiency can lead to disturbances in bone growth and mineralisation and make the bone more susceptible to Cd. On the other hand, zinc supplementation can protect from Cd-induced bone loss). Cd-induced (100 mg Cd/kg of diet for up to 5 months) diminished bone growth and cortical thinning of femur were most evident in rats raised on a Zn-deficient diet (0 mg Zn/kg) in comparison with Zn-sufficient (30 mg Zn/kg) diets (Brzóska and Moniuszko-Jakoniuk, 2001). Persons exposed environmentally to excessive Cd levels may be at increased risk of bone diseases in later life. Recently, it has been hypothesized that Cd may promote skeletal demineralisation, which may lead to enhanced bone fragility and increased risk of fractures, at much lower levels of exposure than previously thought (Staessen *et al.*, 1999).

3.5 EFFECTS OF HEAVY METAL TOXICITY IN PLANTS

Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr concentration, Cr is toxic to most higher plants (Davies *et al.*, 2009). The first interaction Cr has with a plant is during its uptake process. Cr is a toxic, non-essential element to plants; hence, they do not possess specific mechanisms for its uptake. Therefore, the uptake of this heavy metal is through carriers used for the uptake of essential metals for plant metabolism. Since seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal (Peralta *et al.*, 2001). Plant growth and development are essential processes of life and propagation of the species.

They are continuous and mainly depend on external resources present in soil and air. Growth is chiefly expressed as a function of genotype and environment, which consists of external growth factors and internal growth factors. Presence of Cr in the external environment leads to changes in the growth and development pattern of the plant. It has long been established that Co like a number of other elements, is relatively toxic to plants when given in supernormal doses. Plants can

accumulate small amounts of Co from the soil, especially in the parts of the plant that are more routinely consumed, such as the fruit, grain, and seeds (ATSDR, 2004). The distribution of Co in plants is entirely species-dependent and uptake is controlled by different mechanisms in different species. Soil water status has a major influence on the amount of Co available for plant uptake. In poorly drained soils, the amount of extractable Co is greater than in areas which are well drained resulting in significantly increased plant uptake.

CHAPTER 4

MATERIALS AND METHODS

4.1 Study area

The study was carried out in Khutsong, near Carletonville, North West Province, South Africa. This is a settlement through which the Wonderfontein stream flows. The Wonderfontein stream is itself catchment to drainage and waste water from various gold mining activities in the area.

4.2 Ethical considerations

4.2.1 BLOOD

Blood was collected directly from the sheep jugular vein after restraining, ensuring that animals were neither hurt nor injured. Gloves were worn to prevent the transmission of zoonotic diseases. Sterile Vacuitainer needles were used to maintain sterility and the blood samples were transferred into red and purple stoppered tubes and stored in cooler boxes.

4.2.2 FAECES

Faeces samples were collected directly from the sheep rectum with the use of lubricated gloves. The animals were handled in a humane manner in order not to stress or hurt them. The samples were then placed in an air-tight plastic container.

4.3 Sample collection

Water and sediment were collected from around the Khutsong area, which is home to a huge low income community. Various samples were also collected from sheep grazing and watering from the stream.

The following samples were collected:

4.3.1 WATER

Water samples were collected in 100 ml polyethylene plastic bottles with screw caps from the Wonderfontein stream.

4.3.2 SEDIMENTS

Soil sediments were collected about 30cm into the stream at a depth of 10 cm and stored in plastic containers.

4.3.3 TISSUE SAMPLES (MUSCLE, KIDNEY AND LIVER)

Sheep liver, muscle and kidney tissues (from Foscville abattoir) were collected on a once off basis from animals grazing and watering from the Wonderfontein stream and stored in iced cooler boxes and kept away from direct sunlight or warm environments prior to transportation to the laboratory for further analysis.

4.3.4 BLOOD

Blood was collected from a jugular vein of 10 each of randomly selected male and female sheep of adult ages grazing and drinking from wonderfontein stream. Vacutainer needles were used, and the blood samples were collected into serum tubes (red stopper tubes) and stored in cooler boxes. These samples were used for the determination of heavy metal levels in the serum.

4.3.5 FAECES

Faeces samples were collected directly from the rectum of animals known to graze and drink from wonderfontein using lubricated gloves. The samples were then placed on aluminium plates for drying.

4.4 Sample preparation

4.4.1. WATER

Each sample was filtered through a 0.45 micron microspore membrane filter in order to avoid clogging of the burner capillary, then the samples were diluted with 5%(v/w) La solution and HCl.

4.4.2. SEDIMENTS

The samples were put on aluminum plates and left to air dry for about a week. They were then refined through a 2mm screen grinder. About 5 g each of soil

sediments were mixed with 10ml of distilled water and shaken for 30 minutes. The solution was filtered through Whatman filter paper no.42 into a suitable container. The extracts were used for analysis.

4.4.3. TISSUE SAMPLES (MUSCLE, KIDNEY AND LIVER)

A 5-g tissue sample was placed in an acid cleansed crucible and dried in a drying oven at 106°C for 16hrs. After drying, the crucible containing the samples were placed in a dessicator for 6 hrs to cool and then weighed to determine the dry weight, which was recorded. Samples were then ashed in a muffle furnace at 800°C for 16hrs. After ashing, samples were allowed to cool in a dessicator for 6 hrs and then weighed to determine the ash weight. 1 ml of 32% concentrated Nitric acid(HNO_3) was added to the ashed samples in the crucibles and evaporated on a hot plate at a low temperature of 60°C. The crucibles were then re-ashed in the muffle furnace for 2 hours at 600°C, they were then removed and cooled. 10mL of 5 N HCl (5 N =415 ml concentrated HCl + 500ml distilled water) was added to each crucible and was also evaporated at a very low heat of 60°C. The solution was then transferred to 100-mL volumetric flask and diluted to volume with distilled water using a glass funnel. The solution was left overnight so as to let the sediment settle. The following day, the supernatant was taken without disturbing the sediment and transferred and stored in McCartney bottles for heavy metal analysis.

4.4.4. BLOOD

The blood samples were left to stand at room temperature of 4°C for 24 hours to allow clotting, and care was taken to avoid haemolysis. They were then centrifuged at 2600 rpm for 10 minutes. After centrifuging, serum was then transferred to clean bottles using a pipette. To precipitate the protein in the serum, 0.7 ml of serum in duplicate was added to 6.65ml of stock trichloroacetic acid in clean test tubes which were covered, mixed individually on an electric stirrer and left to stand at room temperature for 5 minutes. 5 ml of the supernatant fluid from each sample was taken off with the pipette and transferred to clean tubes without disturbing the centrifuged material at the bottom for heavy metal analysis.

4.4.5. FAECES

1-g duplicate air dried faeces samples were weighed in dried, acid cleansed crucibles. Both the weight of the crucible with the fresh sample and the weight of an empty crucible were recorded. The crucible containing the sample was placed in a drying oven and dried at 106⁰ C for 16 hours. After drying, the crucibles were removed and placed in a desiccator for 6 hours to cool and then weighed. The difference between the crucible with the dried sample and the empty crucible were also recorded again to determine the dry weight of the faeces sample. The samples were then placed in a muffle furnace at 800⁰ C for 16 hours to be ashed.

After ashing, the samples were again placed in a desiccator for 6 hours to cool then the crucibles were weighed to determine the ash weight of the sample. 1 ml of 32% concentrated Nitric acid(HNO₃) was added to the ashed samples in the crucibles and evaporated on a hot plate at a low temperature of 60⁰C. The crucibles were then re-ashed in the muffle furnace for 2 hours at 600⁰C, they were then removed and cooled and 10ml of 5 N of Hydrochloric acid (5 N =415 ml concentrated HCl + 500ml distilled water) was added to each crucible and was evaporated on a very low heat of 60⁰C until approximately 3ml was left in the crucible. The solution was then transferred to 100 ml volumetric flask and filled to volume with distilled water using a glass funnel. The solution was left overnight so as to let the sediment settle. The following day, the supernatant was taken without disturbing the sediment, transferred and stored in Mc Cartney bottles for heavy metal analysis.

4.6 Preparation of Standards

Standard solutions of heavy metals (1000 mg/L) were procured from Merck. Solutions of varying concentrations were prepared for all the metals by diluting the standards.

4.6.1 PREPARATION OF WORKING As STANDARD

As solutions of 5µg/l , 10µg/l ,20µg/l,30µg/l and 50µg/l were prepared by dilution of the standard solution 1000mg/l(MERK).

4.6.2 PREPARATION OF WORKING Cd STANDARD

Cd solutions of 0.5µg/l, 1µg/l, 2µg/l, 3µg/l and 5µg/l were prepared by dilution of the standard solution 1000mg/l(MERK).

4.6.3 PREPARATION OF WORKING Cr STANDARD

Cr solutions of 0.5µg/l, 1µg/l, 2µg/l, 3µg/l and 5µg/l were prepared by dilution of the standard solution 1000mg/l(MERK).

4.6.4 PREPARATION OF WORKING Pb STANDARD.

Pb solutions of 5µg/l , 10µg/l ,20µg/l,30µg/l and 50µg/l were prepared by dilution of the standard solution 1000mg/l(MERK).

4.7 HEAVY METAL SAMPLE ANALYSIS

4.7.1 WATER

Elements in water sample were analysed using the Perkin Elmer AAnalyst 700 (Atomic Absorption Spectrophotometer).

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the 'ground state'. If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the 'excited state'. Since this state is unstable, the atom will immediately return to the 'ground state' releasing light energy. The sample is subjected to a high-energy thermal environment in order to produce excited state atoms. The environment can be provided by a flame or more recently, plasma. However, since the excited state is unstable, the atoms spontaneously return to the ground state and emit light energy which corresponds to the concentration of the element in the solution. The emission spectrum of an element consists of a collection of emission wavelengths called emission lines. Thus, for Pb =283.3nm, for Cr= 357.9nm, for Cd =228.8nm and for As =193.7nm wavelengths. The concentration of each element is measured by the AAS machine which directly converts the light energy emitted from each element into ppm at the corresponding wavelengths in nm (nanometres).

4.7.2 SEDIMENTS

The elements As, Cr, Cd, Pb in sediment samples were analysed using the Perkin Elmer AAnalyst 700 machine (Atomic Absorption Spectrophotometer) as for water samples (4.7.1).

4.7.3 TISSUE SAMPLES (MUSCLE, KIDNEY AND LIVER)

The elements As, Cr, Cd, Pb in tissue samples (muscle, kidney and liver) were analysed using the Perkin Elmer AAnalyst 700 machine (Atomomic Absorption Spectrophotometer) as for water samples (4.7.1).

4.7.4 BLOOD

The elements As, Cr, Cd, Pb in blood samples were analysed using the machine Perkin Elmer AAnalyst 700 (Atomomic Absorption Spectrophotometer) as for water samples (4.7.1)

4.7.5 FAECES

The elements As, Cr, Cd, Pb in faeces samples sample were analysed using the Perkin Elmer AAnalyst 700 machine (Atomomic Absorption Spectrophotometer) as for water samples(4.7.1).

4.8 Preparation of laboratory equipment and reagents

All laboratory equipment used for sample digestion and analysis were soaked in 32% HCl overnight. They were rinsed with distilled water 3 times and dried in a hot plate for 16 hours at 106°C. A dessicator was used for 6 hours to cool crucibles.

4.9 Experimental design and statistical analysis

4.9.1 EXPERIMENTAL DESIGN

Ten adult sheep were randomly selected from a communal herd known to graze and drink from the Wonderfontein stream in Khutsong. The stream is itself a catchment to drainage and waste water from various gold mining activities in the area. Samples of organs (kidney, liver and muscle), serum, sediments, faeces and

water were collected and analysed and the results were compared to the normal values according to the World Health Organisation (WHO, 2005). A survey was also conducted to determine if the community around the area was aware of the environmental contamination due to mining activities.

4.9.2 STATISTICAL ANALYSIS

All the data obtained were analysed using the Statistical Package for the Social Sciences (SPSS) (version 10.0) following general linear model. The results were expressed as means and pooled SE of mean (SEM). The means were then compared using Independent t tests. Probability of $P < 0.01$ and $P < 0.05$ was described as highly significant (at 1% level) and significant (at 5% level) respectively.

CHAPTER 5

5.1 RESULTS AND DISCUSSIONS

Ten composite samples, each of stream water and sediments were collected from Wonderfontein stream. Liver, kidney, muscle, blood and faeces were collected from sheep known to graze and drink from the stream. The mean recoveries of metal concentrations of heavy metals are shown in Table 2 below.

Table 2: mean concentrations (ppm) of As, Cr, Cd and Pb.

Sample	As	Cr	Cd	Pb
Muscle	3.66±00.2 ^a	0.76±0.00 ^c	1.33±0.006 ^c	0.002±0.00003 ^a
Kidney	16.30±00.11 ^b	0.53±0.02 ^{ab}	1.65±0.0032 ^c	2.02±0.0001 ^b
Liver	10.43±03.22 ^c	0.52±0.003 ^{ab}	0.53±60336 ^b	2.213±0.0001 ^b
Fecal	17.88±02.04 ^b	50.22±30.32 ^d	36.55±09.77 ^d	6.22±0.005 ^c
Serum	5.66±00.55 ^a	0.23±0.0005 ^a	0.55±0.00005 ^a	0.000012±0.00 ^a
Soil-upstream	350.00±20.32	66.66±07.66	53.32±13.22	752.22±20.32
Soil- midstream	325.33±26.55	75.20±06.33	40.23±09.32	665.32±23.22
Soil-lower stream	396.25±33.60	89.36±10.21	55.52±14.87	1021±50.20
Water next to sewage	510.25±16.32 ^c	73.25±19.32 ^c	90.10±11.22 ^b	121.25±11.87 ^c
Water upstream	256.12±23.65 ^a	50.33±10.21 ^a	60.22±13.44 ^a	60.22±07.89 ^a
Water midstream	300.35±50.22 ^b	65.51±7.35 ^{ab}	95.80±14.11 ^b	70.36±0866 ^b
Water lowstream	359.00±23.32 ^b	80.99±0866 ^c	101.00±0563 ^c	64.23±0950 ^a

^{a, b, c} letters with same superscript in a column are significantly ($P < 0.05$) different.

The mean recoveries in stream water samples revealed the following trend in metal concentration $As > Pb > Cd > Cr$. The concentration of metals in water next to the sewage was generally due to the direct disposal of waste water into the stream. Wonderfontein water samples had a mean As, Pb, Cd and Cr levels of 356, 79, 87, and 67 ppm that were many times higher than the permissible levels for drinking water (EU, 2005) as shown in table 3 for all the metals analysed. This is suggestive of a high risk of toxicity to the local Khutsong human population and livestock. The mean sediment recoveries indicated the following trend in metal concentration $Pb > As > Cr > Cd$ (Table 2).

The soil heavy metal levels also tended to be higher than the water levels especially for Pb. This observation was not surprising since it is reported that sediments serve as a sink for various anthropogenic pollutants (Davies and Aboweni, 2009). The Korean Soil Environmental Conservation Act prohibits the use of soils containing over 12 ppm Cd for agricultural purposes (Lee *et al.*, 2001). The maximum permissible Cd level for irrigation purposes is 0.05 ppm (Umali, 1999) as shown in Table 4. In this study, the level of Cd is more than 1000 times higher, indicating high risk of toxicity in the environment. The estimated natural concentration of soil Pb ranges from 5-25ppm (Ona *et al.*, 2006). The higher mean sediment Pb concentration of 1021 ppm found in this study falls far above the recommended range of 5-25ppm. However, a soil concentration level of 1.37 ppm Pb was reported in the same river catchment area at Koekemoerspruit, probably due to different locality contamination to metal levels (Dzoma *et al.*, 2010). According to Lee *et al.*, (2001), the Korean Soil Environmental Act requires that soils containing over 400ppm of Pb need not be used for agricultural purposes. The maximum permissible Pb level for irrigation is 0.5ppm (Umali, 1999). Heavy metals in plants are a result of their absorption from the soil into roots and other plant parts. Plants uptake of heavy metals varies with soil pH, plant species, type of metal and season among others (Lee *et al.*, 2001; Abrahams and Steigmajer, 2003). Animal tissue and fluid heavy metal analysed are shown in Table 2. The faecal heavy metal levels tended to be higher compared to serum, liver, kidney and muscle for As, Cr, Cd and Pb. Faeces are used to indicate the animal mineral's status because most of the dietary minerals are excreted in faeces (Mc dowell, 1985). The higher levels in

faeces therefore directly indicate that the sheep were consuming lots of heavy metals in question from the pasture i.e the occurrence of trace metals in plants and animal specimens indicates the upward mobility of heavy metal pollutants through the food chain. Faecal samples indicated the following tend of metal recoveries: Cr>Cd>As>Pb and serum: As>Cd>Cr>Pb. The levels of As in serum and faeces in this study were above 0.284 and 0.643ppm in clinically-affected cattle respectively (Rana *et al.*, 2008c) of 5.66 and 17.88 ppm (Table 2). This clearly indicated some degree of As risk toxicity in livestock in this area of the study. Heavy metal levels in animal specimens are influenced by environmental levels of the metals (Ona *et al.*, 2006). The usual sources of environmental As pollution include mining activities, smelters, fossil fuel power plants and agricultural applications like pesticides, insecticides ,cattle and sheep dips (Juhasz *et al.*,2007). Mining activities in Khutsong area could have been responsible for the pollution.

The metal concentration in liver, kidney and muscle samples showed the following trends respectively As >Cd >Cr >Pb ; As> Pb>Cd>Cr and As>Cd>Cr>Pb (Table 2). The liver, kidney and muscle samples had higher concentrations of As compared to other heavy metals. Also, the kidney and liver combined had significantly more ($P<0.05$) As levels compared to the As levels in the muscle tissue (Table 2) although the kidney As levels were significantly more ($P<0.05$) than the liver tissue. The kidney tissue as an excretory organ had the ability to concentrate lots of dietary minerals as they get excreted from the body (Ternouth, 1990). Almost all metabolic activities take place in the liver by microsomal enzymes. Therefore, the liver cells concentrate heavy metals as well as other digestive products as they are anabolised into big food molecules or incorporated with other endogenous metabolites for excretion in the bile (Ruth, 1978) and thus higher levels of As in this study. As levels in this study were several times higher in liver and kidney samples compared to the recommended levels by Australian New Zealand Food Standards (ANFS, 2011) as shown in Table 3. This indicates a high degree of As toxicity to the livestock and Khutsong human community who consume the sheep meat and other products. Also, the same study reveals a degree of Pb toxicity, much higher than that recommended by ANFS (2011) in liver and kidney tissues. However, Cd liver and kidney Cd levels were below the

recommended level of 1,25 and 2.5 ppm respectively (Table 3) (ANFS, 2011). In the muscle tissue, Pb levels (0.002ppm) were significantly lower than the ANFS (2011) of 0.1 ppm, indicating some degree of Pb safety in the Khutsong environment. However, Cd had much higher level of 1.33 ppm compared to 0.05 ppm recommended by ANFS (2011) and Miranda *et al.*, (2005), in sheep muscle tissue of 0.02 ppm. Literature is not available on the normal levels of As and Cr in sheep muscle tissue. Exposure of livestock to high levels of toxic metals leads to adverse effects such as reproductive impairment, physiological abnormalities, behavioural modification or even death (Sarker *et al.*, 2008). Arsenic is one of the most toxic and carcinogenic metals derived from the natural environment and is relatively one of the common elements that occur in air, soil and living tissues (WHO, 2001; Rana *et al.*, 2008c). Exposure to As could be through water, plants and soil (Rahman *et al.*, 2008). According to Rana *et al.*, (2008c) clinical signs of cattle suffering from As toxicity include depression, weight loss, weakness, dehydration, anaemia, anorexia, bloody diarrhea, ruminal stasis, lethargy, dermatosis and reddish urine, dry dull rough, epilated hair coat and anoestrus.

With respect to lead (Pb), there are reports of Pb toxicity to animals, mainly sheep through soil and vegetation although, it is stated that the soil-plant transfer of Pb is low at only 1-10% (Abrahams and Steigmajer 2003; Ona *et al.*, 2006). Abrahams and Steigmajer (2003) further noted that the daily intake of metals by sheep reflects the degree of soil metal enrichment and is elevated during winter/spring, coinciding with the higher rates of soil ingestion and the generally higher pasture herbage metal concentration as was the case in this study done in the dry season (winter). Varying degrees of Pb poisoning have been reported in animals reared around different polluted areas (Kottferero and Korenekova , 1995; Ancora *et al.*,2008). Animals studied provide evidence that Cd has developmental effects, such as low fetal weight, skeletal malformations, interference with fetal metabolism and impaired neurological development. Cadmium can be acquired through inhalation and oral exposure. In animals, chronic inhalation or oral exposure to Cd results in effects on the kidney, liver, lung, bone, immune system, blood and nervous system (Calabrese and Kenyon, 1991).

The fact that, in this study none of the randomly selected sheep showed signs of ill health, apparently supports the assertion of low environmental pollution in the sampled area. However, a comprehensive health check is required in order to determine the heavy metals on animal and human health in this region.

Table 3: Comparison of the Mean recoveries of heavy metals analysed from the mean water samples collected at the Wonderfontein stream (with WHO recommended levels (European Commission Regulation - EU (2005).)

SAMPLE	As	EU	THRESHOLD ABOVE EU	Cr	EU	THRESHOLD ABOVE EU	Cd	EU	THRESHOLD ABOVE EU	Pb	EU	THRESHOLD ABOVE EU
Water	356.43	0.05	7180	67.52	0.1	809.9	86.78	0.005	20200	79.015	0.001	64230

Mean concentrations (ppm) of As, Cr, Cd and Pb -European Commission Regulation - EU (2005).

Table 4: Comparison of the Mean recoveries of heavy metals analysed from the sediments collected at Wonderfontein (with those of the Korean Soil Environmental Conservation Act (KSEC) (Lee *et al.*, 2001).)

SAMPLE	As	KSEC	THRESHOLD ABOVE KSEC	Cr	KSEC	THRESHOLD ABOVE KSEC	Cd	KSEC	THRESHOLD ABOVE KSEC	Pb	KSEC	THRESHOLD ABOVE KSEC
Sediments	396.3	N/A	N/A	89.3 6	N/A	N/A	55.5 2	0.05	1110	1021	5.0	204.2

Mean concentrations (ppm) of As, Cr, Cd and Pb (Lee *et al.*, 2001)

Table 5: Comparism of the Mean recoveries of heavy metals analysed from the organ samples (muscle, kidney and liver) collected at Wonderfontein (with those of Australian New Zealand Food Standards Code, 2011, (ANFS).)

SAMPLE	As	ANFS	THRESHOLD ABOVE ANFS	Cr	ANFS	THRESHOLD ABOVE ANFS	Cd	ANFS	THRESHOLD ABOVE ANFS	Pb	ANFS	THRESHOLD ABOVE ANFS
Muscle	N/A	N/A		N/A	N/A	N/A	1.33	0.05	2.66	0.002	0.1	0.02
Liver	10.43	0.2	52.15	0.52	N/A	N/A	0.526	1.25	0.421	2.213	0.5	4.426
Kidney	16.3	0.2	81.5	N/A	N/A	N/A	1.65	2.5	0.66	2.02	0.5	4.04

Mean concentrations (ppm) of As, Cr, Cd and Pb (ANFS, 2011)

5.2 RESULTS OF THE SURVEY

The survey conducted included different randomly chosen locations in Khutsong. Communities within the radius of 20 km from the wonderfontein stream were considered. These communities included: Zulu Section, Batswaneng, Emaxhoseni, Khutsong South, Khutsong Plot, Hostel, Ext 1,2,3,5 and 14. The aim of the survey was to determine the community's knowledge of heavy metal pollution on human health. A total number of 60 people of different ages were asked about 17 questions each.

These questions were later categorised according to different activities. In order to analyse the data obtained, all questionnaires were categorised into age and activities, namely: livestock activities, human activities, knowledge of the environmental pollution and knowledge of the effects of pollution. The respondents ages were as follows: < 15 years of age (yoa), 16-25 (yoa), 26-45 (yoa), 46-65(yoa) and > 66 (yoa). The major finding of the survey was that the community around the area does not have the knowledge on the environmental contamination due to mining activities and their effects.

Table 6: The percentages of people interviewed, obtained in livestock activities during the survey.

AGE	WATERING	GRAZING		
		DAM	RIPARAN GRASS(along the banks of wonderfontein)	GRAZING CAMPS
<15 yoa Yoa= years of age	100%	83%	17%	-
Total no. of respondents	12	12	12	12
16-25 yoa	100%	83%	-	12%
Total no. of respondents	17	17	17	17
26-45 yoa	100%	78%	11%	11%
Total no. of respondents	18	18	18	18
46-65 yoa	100%	81%	-	18%
Total no. of respondents	11	11	11	11
>66 yoa	100%	50%	50%	-
Total no. of respondents	02	02	02	02

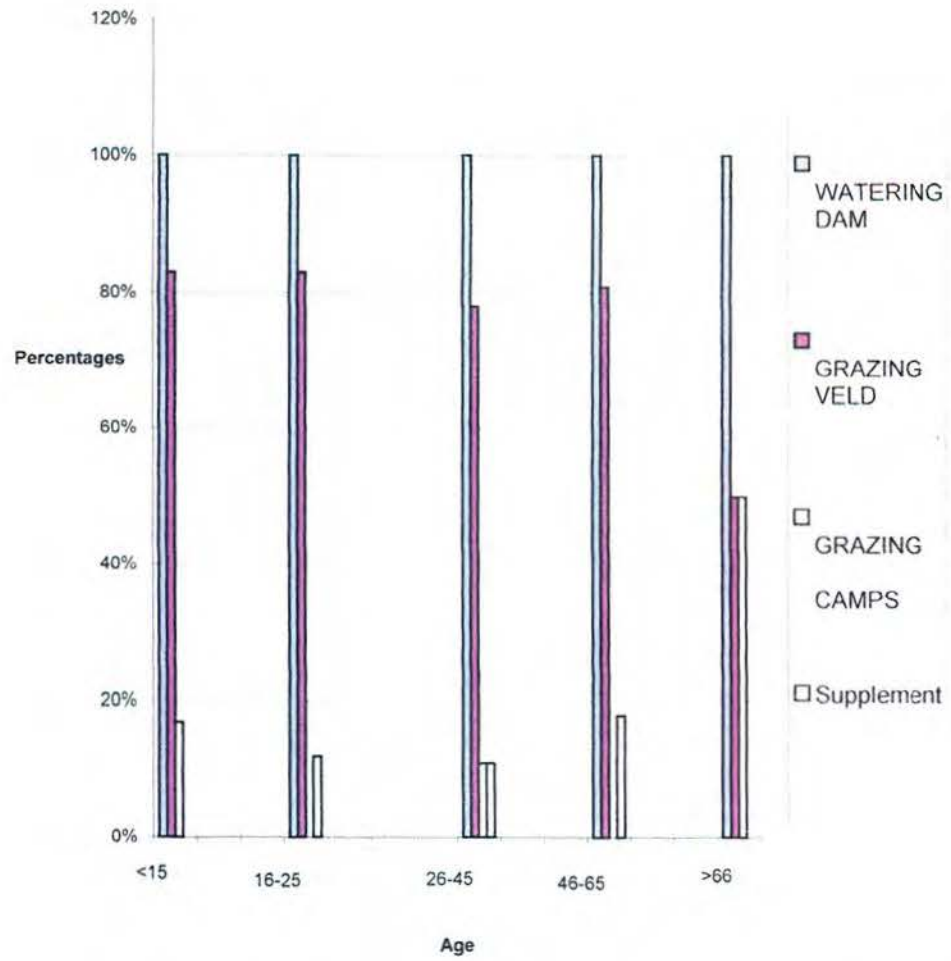


Figure 1: The percentages of people interviewed, obtained in livestock activities during the survey.

Table 6 and Figure 1, represent the percentages obtained in livestock activities (watering and grazing). In terms of watering, all 60 respondents indicated that there is no other source of drinking water for livestock apart from the dam, so it was 100% in watering at the dam in all age groups. Out of 60 people interviewed, 83 % of 12 respondents (<15 years of age) revealed livestock is grazing at the veld, 17% said livestock graze at the grazing camps and none of them mentioned supplements. 17 respondents of between 16-25 years of age, and their response in terms of grazing at the veld it was the same as the <15 yoa, but they disagreed that there are no grazing camps. 12% said livestock are given feed supplements. With the 26-45 yoa category, 18 respondents had a total different story. 78 % said livestock graze at the veld, 11 % said yes there are grazing camps and 11% also said livestock is given feed supplement. 11 respondents of between 46-66 yoa said livestock graze at the veld ,only 18% mentioned feed supplement and none grazing camps (>66 yoa) . The results of the people interviewed showed that 50 % said livestock graze at the veld and 50% grazing camp, and they said livestock is not supplemented.

Table 7: The percentages of people interviewed, obtained in human activities during the survey

AGE	FISHING	SWIMMING	GARDEN	OTHER***
<15 yoa	83%	100%	75%	100%
16-25 yoa	53%	59%	29%	88%
26-45 yoa	56%	83%	56%	83%
46-65 yoa	55%	45%	18%	82%
>66 yoa	50%	100%	-	50%

***LAUNDRY, WASHING OF TENTS, CARS AND RELIGIOUS ACTIVITIES

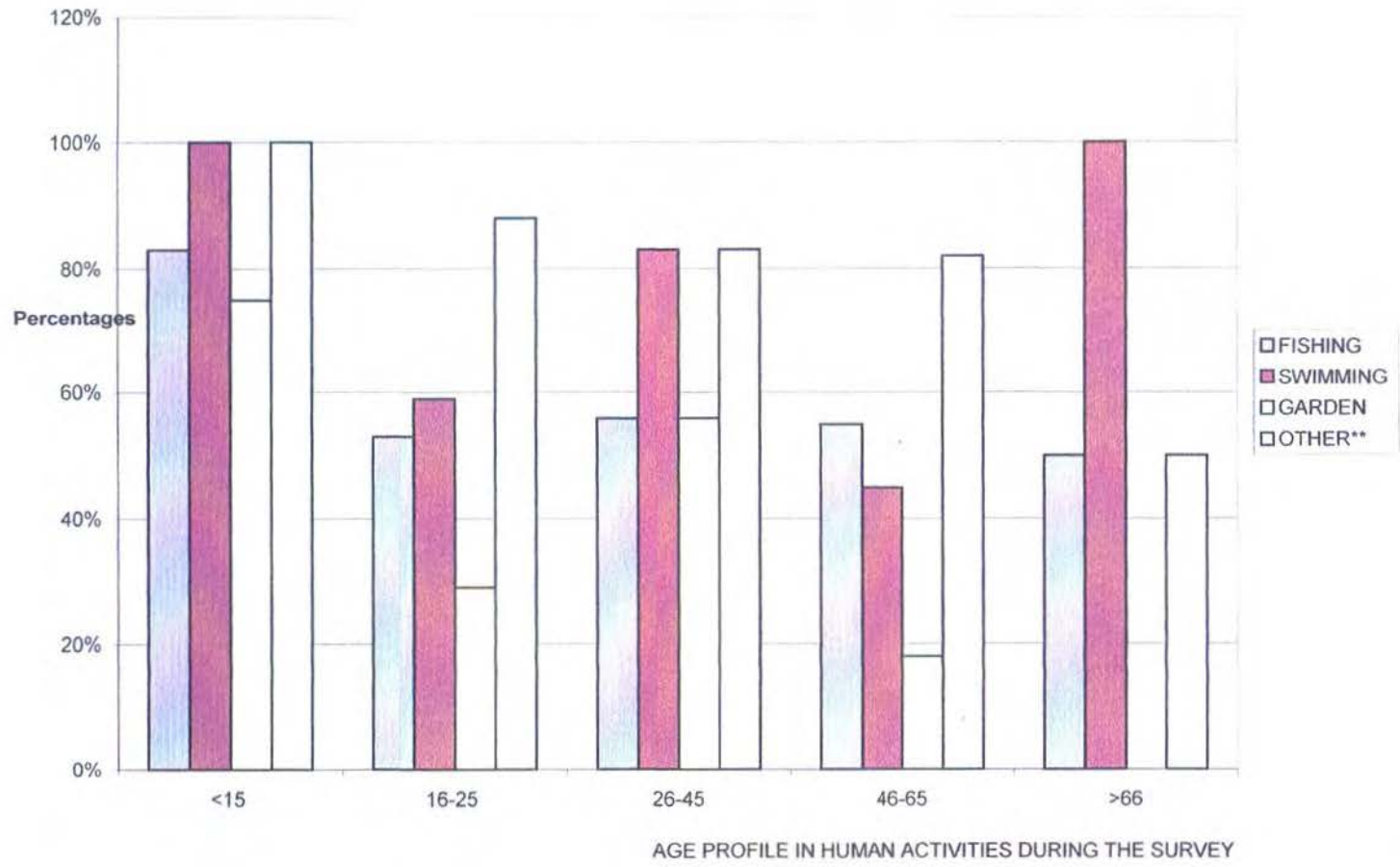


Table 7 and Figure 2 represent the percentages obtained in human activities i.e. fishing, swimming, and gardening, laundry, washing of tents, cars and religious activities taking place at the Wonderfontein dam. The first age group maintained 83% of people are fishing, 100% swimming, 75% gardening, and 100% mentioned that people are doing their laundry, cars, tents and religious activities at the dam. The second age group 16-25 yoa, only 53% said people are fishing from the dam, 59% swimming, 29% gardening and 88% other activities. 26-45 yoa category said only 56% of people said there are fishing from the dam, 83% of swimming activity taking place, 56% in gardening and 83% in other activities. The third age group (46-65 yoa) only 55% said yes fishing is taken place at the dam, 45% swimming, 18% gardening and 82% said other activities are taking place at the dam.

Table 8 : The percentages of people interviewed , obtained in the knowledge of environmental contamination during the survey

AGE	HAVE KNOWLEDGE	DO NOT HAVE KNOWLEDGE	KNOWLEDGE ON ENVIRONMENTAL CONTAMINATION DUE TO MINING ACTIVITIES
<15	0	100%	0
16-25	82%	18%	0
26-45	39%	22%	0
46-65	63%	36%	0
>66	0	100%	0



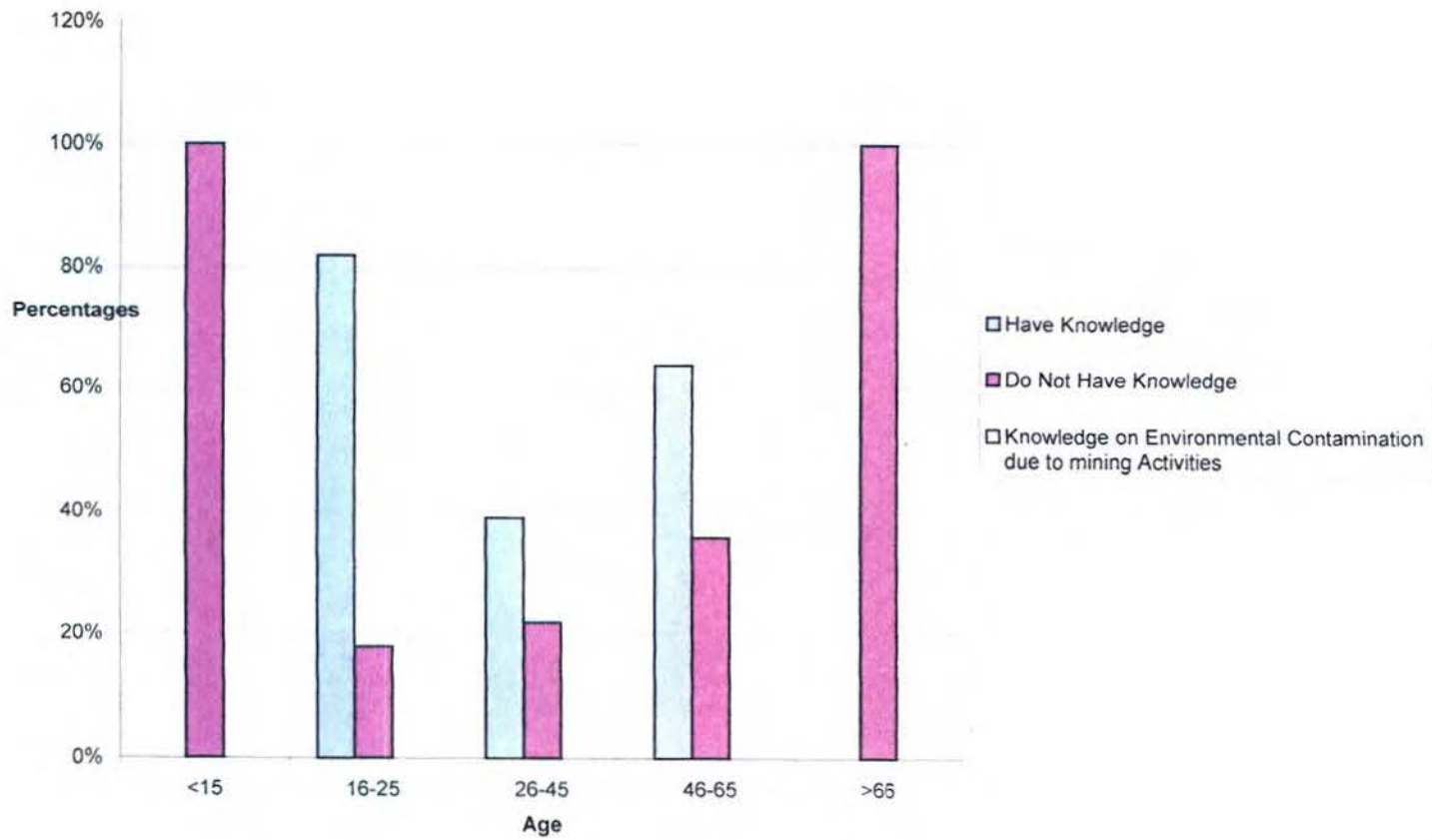


Figure 3: Percentage of people interviewed, obtained in Knowledge of Environmental Contamination during the survey

Table 8 and Figure 3 represent the percentages obtained in the knowledge on environmental contamination, the <15 yoa respondents do not have the knowledge but 82% of the 16-25 have, while 18% of the same age group do not have the knowledge. 39% of respondents between 26-45 have knowledge while 22% do not have the knowledge on environmental contamination. 63% respondents between 46-65 have the knowledge while 36% do not have the knowledge. The last age category >66 yoa, all respondents do not have the knowledge. Out of all 60 respondents none of them have the knowledge on environmental contamination due to mining activities.

AGE	HAVE KNOWLEDGE	DO NOT HAVE KNOWLEDGE
>15 yoa	42%	58%
Total no. of respondentS	12	12
16-25 yoa	82%	18%
Total no. of respondent	17	17
26-45 yoa	61%	39%
Total no. of respondent	18	18
46-65 yoa	100%	-
Total no. of respondent	11	11
>66 yoa	100%	-
Total no. of respondent	02	02
Overall Total no. of respondent	60	

Table 9: The percentages of people interviewed obtained in knowledge obtained in knowledge of the effects of pollution during the survey.

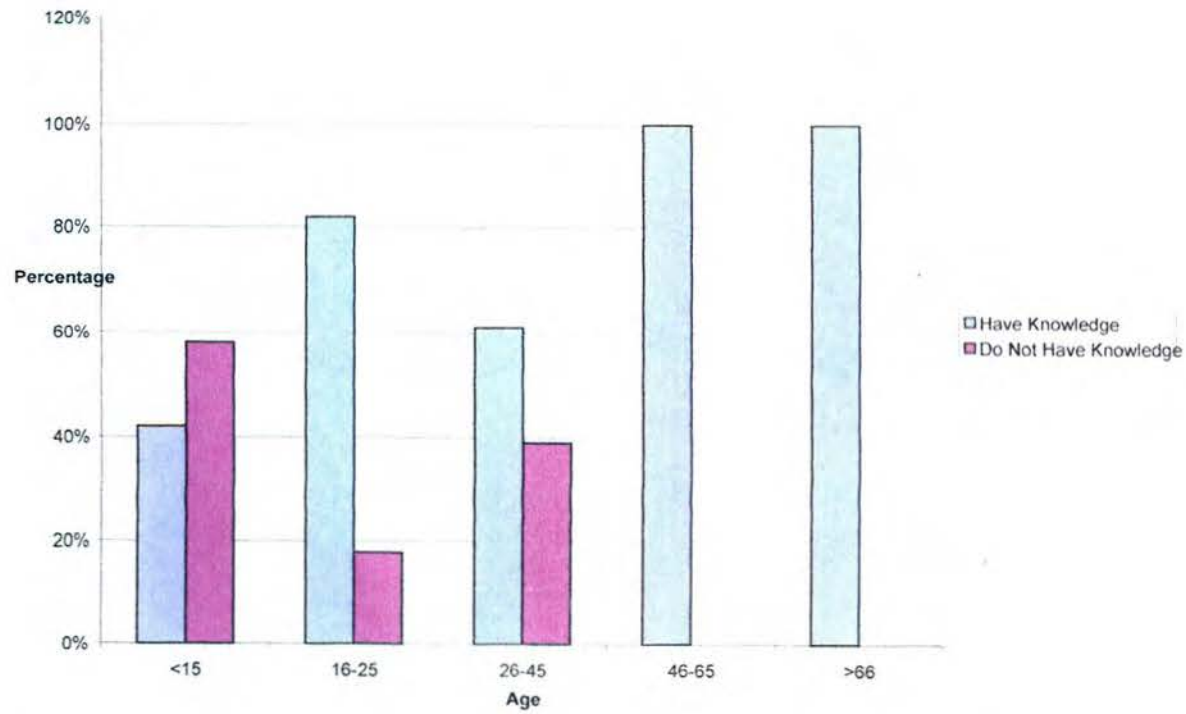


Figure 4. : The Percentages of people interviewed obtained in Knowledge of the Effects of Pollution during the survey

Table 9 and Figure 4 represent the percentages obtained in the knowledge of the effects of pollution. 42% of respondents of the age < 15 knew what pollution is whilst 58% did not. 82% and 61 % of the 16-25 and 26- 45 yoa had sufficient knowledge on pollution respectively and 18% and 39 % lacked this knowledge. 100% of the respondents between 46-65 and above 66yoa had the general understanding of what pollution is and the effects thereof.

5.3 CONCLUSION AND RECOMMENDATIONS

Heavy metal pollution is increasingly becoming a problem to communities that live in the vicinity where mining effluents are deposited, including Khutsong in this study. The survey conducted also indicated that approximately 70% of the community had general knowledge on environmental contamination while 30% did not but 100% did not have any knowledge on mining effluents, heavy metal pollution and their public health effects on humans and livestock. The positive influence of environmental metals in this study on serum, faeces, liver, kidney and muscle tissues indicates the need for studies on the possible effects of high environmental levels of these heavy metals on various aspects of animal health and animal production as the survey indicated that people use water for various needs as swimming, gardening, washing etc. Also, routine bio-monitoring of this potentially polluted area should be encouraged in order to keep environmental pollution under control.

This study suggests further research be done on:

- The public health effects of heavy metals in humans and livestock of Khutsong

and recommends that:

- Environmental regulations be enforced on mining effluents being released to the environment;
- The community of Khutsong be educated on heavy metal pollution and their health effects; and
- General meat inspections in abattoirs handling livestock from Khutsong to include routine heavy metal analysis.

CHAPTER 6

6.1 QUESTIONNAIRES ON HEAVY METAL RESEARCH AROUND THE POTENTIALLY POLLUTED AREA OF KHUTSONG IN SOUTH AFRICA.

Form no:.....

Date:

SECTION A: General Information

(A1) Name and Surname of respondent.....(OPTIONAL)

(A2) Village of residence:.....

(A3) Gender of respondent

M	F
---	---

(A4) Age of respondent: *Tick appropriate box.*

1	2	3	4	5	6
<15	16-25	26-45	46-65	66-85	>85

(A5) Level of education of respondent:

1	2	3	4	5	6	7
Nil	Primary school	Std 6-7 Grade 8-9	Std 8-9 Grade 10-11	Matric	Post matricyrs	Other -----

(A6) Source of Income

1	2
Yes	No

SECTION B: Land Use with regard to livestock

(B1) Where do livestock graze on a daily basis?

1	2	3	4
Grazing camps	Veld	Both	Other.....

(B2) Are fenced camps used for grazing control and grazing management?

1	2
Yes	No

(B3) Will you use fenced camps in order to control the areas where cattle graze if proper camps are available? Reason.

Yes	No
-----	----

.....

(B4) Must one pay for the veld where the cattle graze?

Yes	No	Don't know
-----	----	------------

(B5) Are there any other suggestions of how grazing control could be improved?

.....

(B6) Is feed supplement provided for livestock

Yes	No
-----	----

SECTION C: General water provision for Livestock and Humans

(C1) What is the source of water for livestock and humans (Tick appropriate)

1	2	3	4	5	6	7
Tap in house	Tap in yard	Borehole	Spring	Dam	River	Other*

*Specify.....

(C2) If not at homestead, how far is the water source?

1	2	3	4	5
< 1 km	1-3 km	4-5 km	> 5 km	*Other.....

Specify*.....

(C3) Is the water that is used for human consumption boiled before domestic use, is fetched from spring, dam or river ?

1	2	3
Always	Someti mes	Never

(C4) Is water fetched for the livestock and humans?

1	2	3
Yes	No	Sometimes

(C5) Who fetches water for the livestock?

1	2	3	4	5
Owner	Herdsmen	Family member	Friend	Other.....

(C6) Must one pay for the water for both livestock and humans?

Yes	No
-----	----

If YES to (C6), how much? R..... Per litre Per container

Specify:.....
.....

(C8) When does one struggle to get enough water for the livestock?

1	2	3	4.	6.	7.
Only during droughts	Throughout every winter	Som e years in winte r	Only in late winter	Neve r	Other.....

(C9) What are the main reasons for not having enough water for the livestock sometimes?

1	2	3	4	5.	6.	7
Many droughts	Small dams	Not enough dams	Not enough pumps	Pumps break frequently	Water is too far	Other.....

(C10) If applicable, are there any suggestions on how to improve the situation on providing good quality and quantity water for both humans and livestock?

Yes	No
-----	----

Specify:.....
.....
.....

SECTION D: Mining Activities and Crops

(D1).Is the community benefiting from the mining area?

1	2
Yes	No

(D2) Is there a special dumping area for waste materials from the mines

1	2	3
Yes	No	*Other

*Specify.....

(D3) Does the mining equipments cause any hazardous noise

1	2
Yes	No

IF (D3) is yes please indicate how does it affect the surrounding community

.....
.....
.....

(D4) Does the mining activities pollute ground water/ drinking water from the river/dam/spring?

1	2
Yes	No

D.5. Is there any air pollution and dust?

1	2
Yes	No

IF (D5) is yes please indicate how it affects the surrounding community

.....

.....

.....

.....

(D6) Do you have a back yard garden?

1	2
Yes	No

(D7) Do you grow vegetables?

1	2
Yes	No

(D8) What is the source of water for watering vegetables?

1	2	3	4	5	6	7
Tap in house	Tap in yard	Borehole	Spring	Dam	River	Other*

(D9) What type of plants or vegetables does one grow? (*Specify)

.....

(D10) Are the grown vegetables for subsistence or commercial farming?

1	2
Subsistence	Commercial

(D11) Is livestock allowed to graze the crop fields after harvest?

1	2
Yes	No

(D12) Is there any kind of, feed specifically planted for cattle for grazing/fodder?

Yes	No
-----	----

If YES: Specify

what:.....

If NO: Specify

whynot:.....

SECTION E: Miscellaneous

(E1) Do u drink milk straight from the cow/goats?

1	2	3
Always	Sometimes	Never

(E2).Is the milk boiled before drinking

1	2	3
Always	Sometimes	Never

(E3).Are there any swimming activities undergone at the local dam?

1	2
Yes	No

(E.4) Are there any swimming activities undergone at the local dam?

1	2
Yes	No

(E5.)Is there anything one would like the government or NGO to do concerning the heavy metal pollution in and around the Khutsong area.

1	2
Yes	No

If **YES** :Specify what:.....
.....

If **NO**: Specify why not:.....
.....

CHAPTER 7

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