

Metallic elements in Odonata from South Africa

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“Conservation is a cause that has no end. There is no point at which we will say our work is finished”.

- Rachel Carson

Abstract

Adult dragonflies are aerial predatory arthropods that occur globally. However, no research on adult dragonflies as potential indicators of metallic elements in the environment or metallic element concentrations and relative contribution patterns between sites, species, size classes, habitat types, and relation to possible pollution sources has been published. There is also no information available about dragonflies and their responses to toxic metals. However, metallic elements are toxic in elevated concentrations to all organisms.

I analysed 105 adult male dragonflies from 21 sites in South Africa for 33 metallic elements including thallium, thorium, zinc, mercury, arsenic, lead, chromium, cadmium, strontium, nickel, phosphorus, sodium, magnesium, potassium, rubidium, silver gold, and platinum. The results indicated that all species of dragonflies, regardless of body size are suitable indicators. Furthermore, differences between habitat differences did not affect the metallic element concentrations on the scale of this study. Interesting patterns were found between sites. Sites located near wastewater treatment plants showed elevated concentrations. Thallium, thorium, zinc, arsenic, phosphorus, sodium, magnesium, potassium, rubidium, and lead were found at elevated concentrations (relative to the other sites) in dragonflies from sites near mining. Dragonflies from sampling sites near potential pollution sources, but seemingly had isolated water sources, showed lower metallic element concentrations when compared with other sites. These observations point to avenues of investigation that can be followed up. Dragonflies are excellent indicators, especially of their immediate environments, because they are aerial predators, large enough for individual sampling and analyses, readily identifiable to species level, and obtaining ethical approval is easier than for vertebrates. Based on these results I conclude that dragonflies would be excellent indicators of environmental metallic elements.

Keywords: Dragonflies, metallic elements, pollution, mining, South Africa.

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Chapter 1: Introduction and literature study

“In nature nothing exists alone”

– Rachel Carson

1.1 Introduction

The human population is growing and there is an increasing need for food, housing, products, and everyday necessities. The environment is also becoming more polluted every day because of increasing human activities. Ecotoxicology as a science focuses on the toxic chemicals that make their way into the natural environment and their interactions with biota. The main research elements of ecotoxicology are identifying the sources of the toxicants, where they end up (fate), and the biological effects they have on living organisms at the population, community, ecosystem, and biosphere levels of organization (Calow, 1993; Newman, 2015).

Ecotoxicology is a multidisciplinary science and has numerous goals; consequently, there are a number of disciplines associated with this science. They include toxicology, ecology, biochemistry, and analytical chemistry (Rattner, 2009). The interactions that organisms have with their environment and the response the organisms have when exposed to toxic chemicals are all relevant when considering ecotoxicology. Much research has been published on aquatic and terrestrial amphibians, reptiles, birds, and mammals. However, interest in using lower invertebrates such as spiders has been growing (Jung and Lee, 2012; Tojal et al., 2002; Wilczek et al., 2003). Most eco-toxicologists specialize in a single or a particular combination of disciplines rather than addressing all. Ecotoxicology is considered a young research field. It was only when more advanced analytical, biochemical, ecological and statistical technology became available, and the awareness about toxic effects in the environment has been raised, that this field of study started to make impact (Rattner, 2009).

Much of the impetus to the field of ecotoxicology can be ascribed to the pioneering efforts of Rachel Carson who reacted to the ‘silent spring’ with no birdsong, the birds having been severely depleted by extensive use of DDT by publishing ‘Silent spring’ (Carson, 1962). Despite heavy government and industry resistance, she persisted with her efforts in bringing the truth to the fore, of the severe consequences of over-use of pesticides on human health and the environment. Her work and personal appearances (together with the public controversies) raised awareness to such an extent that it could not be ignored. In addition to many civil society organisations that fought for the protection of human health and the environment from the effects of chemicals, the US Environmental Protection Agency (USEPA) was created by President Richard Nixon, in 1970 (Burger, 1997; Bouwman et al., 2013). Although the pesticide controversy in the US is not directly related to my study, the fundamental concerns raised and the scientific space (ecotoxicology) was largely defined by the actions of this remarkable woman.

1.1.1 Routes of exposure

All organisms are effected by environmental pollution. Most organisms are exposed to pollutants through more than one route. Primarily, organisms are exposed to elevated concentrations of pollutants via inhalation and ingestion (Kampa and Castanas, 2008). However, dermal contact represents a major route of exposure for aquatic and soil dwelling

organisms (Roelofs et al., 2016). Organisms ingest trace amounts of essential elements such as iron, calcium, and magnesium through daily dietary intake. However, with air, soil and water pollution, increasingly higher concentrations of toxic elements that are available for uptake can be accumulated (Panayotou, 2016). Plants absorb and bio-accumulate elevated concentrations of metallic elements within roots and stems. Humans and other organisms consume the plants that have bio-accumulated metallic elements concentrations (Jarup, 2003).

Freshwater is a threatened natural resource. It can be polluted by a number of sources. Most animals drink water from streams and rivers; in areas where water filtration is not available humans do as well. The consumption of polluted fresh water contributes to chemical pollution exposure. Furthermore, air pollution contributes greatly to exposure via inhalation. According to Kampa and Castanas (2008) there are four major categories of air pollution namely; gaseous pollutants, persistent organic pollutants, 'heavy metals', and particulate matter. Air pollution should be taken seriously since it can affect the respiratory system, cardiovascular system, nervous system, urinary system, and digestive system, amongst others (Kampa and Castanas, 2008).

1.1.2 Sources

The sources of pollutants are not always clear. In many cases, exposure and uptake can occur far from pollution sources (Briggs, 2003, Evans et al., 2000). However, there are known sources of anthropogenic activities that contribute to air, water, and soil pollution (Briggs, 2003).

Mining activities contribute heavily to air, soil, and water pollution (Salomons, 1995). Acid mine drainage is of particular concern considering aquatic species. For example, acid mine drainage has a negative effect on the egg hatching success of branchiopod egg banks within wetlands close to mines (Henri et al., 2014). In another example, there are significant occupational exposures in people working in the mining and production of cadmium, chromium, lead, mercury, gold, and silver industries (Duruibe et al., 2007).

Industries that produce pharmaceuticals contribute to water pollution such as with synthetic hormones, antimicrobials, and anti-retroviral drugs (Kümmerer, 2009). New chemicals are being synthesised on a continuous basis. Many could be toxic, have impacts on aquatic ecosystems, and may cause endocrine disruption in humans and animals (Kim et al., 2008). Furthermore, the pollutants may lead to antibiotic resistant bacteria (Sanderson et al., 2004). In addition, it is not only the industries that cause water pollution, but the everyday household products used as well. Water polluted during domestic use of pharmaceuticals and personal care products end up at waste water treatment plants and often, from there, into fresh water sources, and onto agricultural land as sludge with the purpose of being used as fertilizer (Ternes et al., 2004).

The global human population is growing and more land is needed to grow crops (Bongaarts, 1994). However, the need for better harvest leads to increased use of fertilizers and pesticides (Köhler and Triebkorn, 2013; Tilman, 1999). Fertilizers play a key role in the cultivation of crops. However, when used in excess, the elements can drain through to the water table and end up in rivers and dams. Nutrient rich water can effect aquatic biodiversity and can affect the health of organisms dependant on the water sources (Carnelo et al., 1997).

1.2 Metallic elements

Metallic elements are a number of chemical elements that are usually shiny as solids. In the periodic table, metallic elements can be found in groups 1-2 and 4-16 (Table 1.1). Metallic elements such as silver, nickel, copper, and gold form part of the earth's crust. Most metallic elements are derived from mining activities. Some metallic elements such as iron, calcium, and magnesium are essential for life and play an important role in the welfare and health of living organisms. However, not all metallic elements are essential to life and include elements such as lead, mercury, and cadmium. Characteristics of selected metallic elements and to what group they belong to can be seen in Table 1.2. Furthermore, the uses, sources, and toxicity of each metallic element are discussed in Table 1.3.

1.2.1 *Characteristics of metallic elements*

Most metallic elements share several common properties:

- Usually shiny
- High melting point
- Conductor of heat
- Conductor of electricity
- Solid at room temperature (apart from mercury)
- Low ionization energies
- Low electronegativity
- Malleable – able to be pounded into sheets
- Ductile – can be pulled into wire
- High density (except for lithium, potassium, and sodium)
- Corrodes in air or seawater
- Loses electrons in reactions

Table 1.1 Periodic table of elements.

The image displays a periodic table of elements, color-coded by groups. A callout box highlights the element Boron (B) with the following details:

- RELATIVE ATOMIC MASS (1):** 10.81
- GROUP IUPAC:** 13
- GROUP CAS:** IIIA
- ATOMIC NUMBER:** 5
- SYMBOL:** B
- ELEMENT NAME:** BORON

A legend box defines the color-coding for element categories:

- Metal:** Blue
- Semimetal:** Orange
- Nonmetal:** Green
- Alkali metal:** Light blue
- Alkaline earth metal:** Medium blue
- Transition metals:** Dark blue
- Lanthanide:** Light purple
- Actinide:** Dark purple
- Chalcogens element:** Light green
- Halogens element:** Medium green
- Noble gas:** Dark green

Additional information in the legend box includes the **STANDARD STATE (25 °C; 101 kPa)**:

- Ne** - gas
- Hg** - liquid
- Fe** - solid
- synthetic

The periodic table itself shows elements from Hydrogen (1) to Oganesson (118), with some elements in parentheses indicating they are synthetic or have very short half-lives. The Lanthanide and Actinide series are shown as separate rows at the bottom.

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www.periodni.com

(1) Atomic weights of the elements 2013, Pure Appl. Chem., **88**, 265-291 (2016)

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LANTHANIDE														
57 138.91 La LANTHANUM	58 140.12 Ce CERIUM	59 140.91 Pr PRASEODYMIUM	60 144.24 Nd NEODYMIUM	61 (145) Pm PROMETHIUM	62 150.36 Sm SAMARIUM	63 151.96 Eu EUROPIUM	64 157.25 Gd GADOLINIUM	65 158.93 Tb TERBIUM	66 162.50 Dy DYSPROSIUM	67 164.93 Ho HOLMIUM	68 167.26 Er ERBIDIUM	69 168.93 Tm THULIUM	70 173.05 Yb YTTERBIUM	71 174.97 Lu LUTETIUM
ACTINIDE														
89 (227) Ac ACTINIUM	90 232.04 Th THORIUM	91 231.04 Pa PROTACTINIUM	92 238.03 U URANIUM	93 (237) Np NEPTUNIUM	94 (244) Pu PLUTONIUM	95 (243) Am AMERICIUM	96 (247) Cm CURIUM	97 (247) Bk BERKELIUM	98 (251) Cf CALIFORNIUM	99 (252) Es EINSTEINIUM	100 (257) Fm FERMIUM	101 (258) Md MENDELEVIUM	102 (259) No NOBELIUM	103 (262) Lr LAWRENCIUM

(EniG. Periodic Table of the Elements. 2017)

Table 1.2 Metallic element characteristics (Emsley, 2003).

Metallic element	Symbol	Atomic number	Characteristics	Type of metal	Comments
Boron	B	5	Dark powder that is unreactive to oxygen, water, acids and alkalis	Semi-metallic	• Humans consume about 2 milligrams daily. • Boron compounds may possibly be used in the treatment of brain tumours. • It is essential for plants and a small increase in boron can produce better crops while protecting the plants from pests. • The largest known boron deposits are in Turkey.
Sodium	Na	11	Sodium is a soft metal with a silvery-white appearance. It is also highly reactive	Alkali metal	• Sodium is more important to animals than it is to plants. • Sodium deficiency can cause muscular spasms in humans.
Magnesium	Mg	12	Silvery white, lustrous soft metal	Alkaline earth metal	• Magnesium is a very important component in the chlorophyll molecule. • Once magnesium catches fire, it is almost impossible to extinguish. • Magnesium is the third most abundant element in the earth's crust.
Aluminium	Al	13	Pure aluminium is a soft and malleable metal	Other metals	• Most abundant metal in the earth's crust (Al^{3+}). • The third most abundant element. • Not essential to life but some plants do absorb it and can make up to 1% of its dry weight. • Once aluminium enters the blood stream, the body finds it difficult to remove. • Aluminium has been used to stop bleeding and is available in the form of styptic pencils.
Phosphorus	P	15	There are three types of naturally occurring phosphorus; white, red and black.	Non-metal	• "Phossy jaw" is a well-known industrial disease caused by breathing in phosphorus vapour over extended periods. • Phosphorus as phosphate is mostly found in the ocean and in excess can cause an outbreak in

			White phosphorus glows in the dark and consists of a spontaneous combustible property		slimy green algae in freshwater also known as eutrophication.
Potassium	K	19	It's a soft, light, silvery-white metal	Alkali metal	• One of its isotopes is radioactive and it has been suggested that this element is one of the main causes of natural genetic modifications in animals and plants. • Potassium chloride injections are fatal.
Calcium	Ca	20	Calcium has a silvery-white metallic colour as a solid It is a relatively soft metal	Alkaline earth metal	• One of the most abundant metal elements in the earth's crust. • The third most abundant metal. • Mainly found in sedimentary rocks or in shells of marine creatures that have been bio-accumulating over millions of years. • Most abundant metal in the human body. • Calcium cannot be found alone in nature. • Calcium is found mostly as limestone, gypsum, and fluorite.
Thallium	Tl	81	Soft, silvery-white metal	Transition metal	• The antidote for thallium poisoning is a dose of Prussian blue dye ink. • It is ten times more abundant than silver.
Vanadium	V	23	Shiny, soluble, silvery metal	Transition metal	• South Africa is one of the largest sources of vanadium in the world. • It is usually obtained as a by-product of other ores. • As little as 2% vanadium can increase the strength of steel.
Chromium	Cr	24	Hard silvery metal	Transition metal	• Chromium is an element found in the precious gem alexandrite and gives the gem its blue green iridescence colour.
Manganese	Mn	25	Hard, brittle, silvery metal	Transition metal	• South Africa is one of the major countries that mine manganese ores. • The most manganese is found on the ocean floor.

Iron	Fe	26	Lustrous, silvery and soft (workable)	Transition metal	• There is an entire part of human history named after iron: The Iron age. • Iron is the most mined ore on earth.
Cobalt	Co	27	Lustrous, silvery-blue, hard metal	Transition metal	• Most of cobalt on earth is in the earth's core. • It was once popular for making "sympathetic ink". It remains unseen until it is warmed.
Nickel	Ni	28	Silvery, lustrous, malleable and ductile metal	Transition metal	• It can cause dermatitis, better known as "nickel itch". • Most nickel is inaccessible because it is deep inside the iron-nickel molten core.
Copper	Cu	29	Orange-gold, malleable and ductile metal	Transition metal	• The second best electrical conductor. • We ingest copper through our diets. • Plants absorb copper that mostly accumulate in their roots. • Where most metals are extracted from an ore, copper is one of the few metals that occur in nature in a directly usable metallic form known as native metals.
Zinc	Zn	30	Bluish-white metal	Transition metal	• Zinc oxide is used as an active ingredient in sunblock.
Arsenic	As	33	The metal form is usually grey, brittle, tarnishes and burns in oxygen	Semi-metallic	• Has been used as a poison and as a weed killer. • It has one isotope - arsenic-75. • Soil contaminated by arsenic can be cleaned up by growing <i>Pteris vittata</i>. This plant absorbs arsenic and makes up 5% of its dry weight.
Selenium	Se	34	Selenium has two forms: Silvery metal and red powder	Non metals	• Selenium deficiency is linked to low sperm counts. • It is one of the rarer elements on the surface of the planet. • Selenium can be used as an antagonist as it counters effects other toxic metals such as mercury and arsenic.
Rubidium	Rb	37	Soft, white metal that is silvery when first cut	Alkali metal	• Rocks and earth layers that contain rubidium can be dated by the rubidium-strontium dating method.

					Rubidium is more expensive than gold and platinum per kilogram.
Strontium	Sr	38	Silvery, white and relatively soft	Alkaline earth metals	Crystals of strontium titanite shine brighter than diamonds because they have a high refractive index.
Molybdenum	Mo	42	Lustrous, soft silvery metal	Transition metals	Molybdenum was alloyed with steel by Japanese blacksmiths to form a stronger and corrosion resisting sword.
Palladium	Pd	46	Lustrous, silvery-white, malleable and ductile metal	Transition metal	- It is the least dense of all four platinum group metals and has the lowest melting point. - It resists corrosion.
Silver	Ag	47	Soft, malleable and ductile metal	Transition metal	- Metallic silver occurs naturally as crystals. - Sterling silver is 93% silver and 7% copper.
Cadmium	Cd	48	Silvery, soft metal	Transition metal	In 1966, England a man died of cadmium poisoning and several others were treated for it when they were exposed to cadmium during the removal of a construction tower.
Antimony	Sb	51	Metal form is bright. Silvery, hard and brittle	Semi-metallic	Antimony was likely the cause of cot deaths in 1990, since it was present in mattresses treated with antimony for flame resistance.
Barium	Ba	56	Metallic barium is soft and silvery	Alkaline earth metal	- Abundant in the earth's crust. - The ores of barium are heavy. - Forms insoluble salts. - Some algae such as <i>Closterium</i> thrive in barium rich water and even store it as barium sulphate crystals.
Platinum	Pt	78	Lustrous, silvery-white, malleable, ductile metal	Transition metal	- Three-quarters of the world's platinum reserves comes from South Africa. - Around 50% of all platinum is used in the manufacturing of jewellery.
Gold	Au	79	Soft, shiny yellow solid metal	Transition metal	- Gold in the ocean is worth more than \$1500 trillion. - It can occur as grains, sheets, flakes, crystals and as a solid metal.

					• There are many different hues of gold. • South Africa is one of the largest producers of gold. • Many cities and towns around the world were established due to a gold rush.
Mercury	Hg	80	Heavy silvery-white liquid metal	Transition metal	• Mercury is a liquid at room temperature unlike other metals that are solid. • Mercury is poisonous in all forms and can cause diseases nicknamed; “hatter’s shakes” and “mercury madness”.
Thallium	Tl	81	Soft, silvery-white metal	Other metal	• Thallium can be absorbed through the skin. • Handling thallium with bare hands can lead to the loss of fingernails. • It was once prescribed by doctors as a treatment to remove ringworms from the scalp, it made a patient lose all hair so that the ringworms can be removed more easily.
Lead	Pb	82	Soft, weak, ductile, dull silver-grey metal that has a high malleability, low melting point, and is resistant to corrosion	Other metal	• White lead paint used in paintings turns bluish over time. • Gout in the eighteenth and nineteenth centuries was most likely caused by high concentrations of lead in port wine (lead was used to sweeten wine).
Bismuth	Bi	83	Heavy with a silvery colour and faint pink tinge. Too brittle to be used as a pure metal	Other metal	• Bismuth is the active ingredient in the medication used to treatment for several gastric disorders. • Copper and lead smelting produces bismuth as a by-product. • Bismuth occurs as the metal itself. • The glossy effect in lipstick and nail varnish is due to the additive of a bismuth compound.
Thorium	Th	90	Silvery metal that is radioactive yet soft and ductile	Actinides	• All forms of thorium are radioactive. • Could be used as a source of fuel in nuclear reactors. • It is a highly abundant metal, being more than 200 times abundant than silver in the earth’s crust.

Uranium	U	92	Silvery, malleable, ductile and radioactive	Actinides	• “Littleboy”, the atomic bomb which was dropped over Hiroshima in 1945 comprised of uranium. • Uranium today is mostly used in nuclear reactors for the generation of electricity. • Uranium is ten times more abundant than both mercury and silver together.
Titanium	Ti	22	Silvery metal that is hard and lustrous	Transition metal	• Used in the medical field to reattach broken bones since the body does not reject the metal and resists corrosion. • Titanium dioxide is used as an active ingredient in sunblock due to its high refractive index, preventing UV-rays from penetrating the skin. • Two-thirds of all titanium production goes to the production of aircraft frames and engines.

1.3 Sources and toxic effects of selected metallic elements

Humans as well as wildlife are exposed to toxic chemicals that are released into the environment. Two types of poisoning usually occur if the organism or human is exposed to elevated concentrations of metallic elements. Acute poisoning occurs when an organism is exposed to high concentrations over a short time period. The result is usually death. Chronic exposure occurs when an organism is exposed to lower concentrations over an extended period. The result is mostly sub-lethal and can cause a long and painful decrease in health and mental stability (Emsley, 2003).

1.3.1 Mercury

Mercury is one of the most toxic heavy metals. However, it is in the water-soluble forms that causes most mercury poisonings (Salonen et al., 1995). Methyl-mercury, the bioavailable form of mercury, is especially toxic since it can diffuse through tissues such as the placenta where it can cause central nervous system damage and foetal deformities (Emsley, 2003).

Volcanic eruptions are responsible for almost half of atmospheric mercury emissions (Hansen and Danscher, 1997). Mercury is also released into the environment through leaks in the earth's crust, earthquakes and erosion (Magos and Clarkson, 2006). The other half is made up of coal and gas power plants, gold mining, cement, battery, caustic soda, pig iron and steel production as well as waste disposal and smelters (Boylan et al., 2003; Tchounwou et al., 2003; Zahir et al., 2005).

We consume small amounts of mercury through our diet each day but normally in such low doses that it does not affect our well-being. Furthermore, critical determining factors such as the duration and timing of exposure could very well result in diet-exposed mercury to be detrimental to human health (Tchounwou et al., 2003). However, according to Clarkson et al. (2003) the concentration of mercury found in food intended for consumption is increasing and it's a cause for concern (Guallar et al., 2002). Today, mercury is mostly known as a toxic element but this has not always been the case. Mercury has been prescribed as a laxative, diuretic, corrosive sublimate, disinfectant, skin ointment, as well as an antibacterial and antiseptic agent (Bernhoft, 2011). The use of mercury for medical purposes has declined, and it has been banned in most countries (Emsley, 2003).

Today we know that mercury is mostly absorbed through the lungs, skin, and digestive tract, especially since mercury poisoning takes place by inhalation of mercury vapour, or by ingesting any form of mercury (Bernhoft, 2011). It affects every organ since it disrupts proteins and enzymes on a cellular level. However, the brain is the most destructive target (Emsley, 2003).

The symptoms of mercury poisoning can vary depending on numerous factors including the type and severity of poisoning. Studies done on minks (*Mustela vison*) and river otters (*Lutra canadensis*) indicated that smaller meat eating organisms are more sensitive to methyl-mercury toxicity than larger species (Wren et al., 1988).

Acute mercury poisoning symptoms include; severe headache, nausea, vomiting, stomach pains, diarrhoea and can leave a metallic taste in the mouth (Bernhoft, 2011). Excess salivation, loosening of teeth and swelling of salivary glands can follow a few days after exposure. Smaller doses over a longer time may have severe effects on the brain. Chronic mercury poisoning symptoms include; fatigue, weakness, shaky hands, insomnia and loss of

memory (Berglund et al., 1988). Sufferers can also display psychological symptoms such as irritability, depression, and paranoia. The highest concentration of mercury is usually found in the kidneys, liver, spleen, and brain (Emsley, 2003).

Humans were exposed to mercury through prescription medication and by industries using mercury such as the hat making industry. Other diseases that develop as a result of mercury poisoning include acrodynia (pink disease), Hunter-Russell syndrome, and Minamata disease (Ekino et al., 2007).

1.3.2 Lead

Lead is a non-essential element that accumulates in the body, especially in bones in the form of lead phosphate (Ethier et al., 2007). This element is known to be highly poisonous and is extremely persistent within biological tissues (Emsley, 2003).

A major source of lead is from present and past mining and industrial activities (Fisher et al., 2006; Mielke, 2002). Lead rarely occurs in its native, metallic form but rather as a chalcophile, meaning it is generally found combined with sulphur. Lead ores are mined all over the world. Galena (PbS) is the main lead ore and is mined in Australia, USA, China, Peru, Canada Mexico and West Germany. Other ores of lead include Cerussite (PbCO₃) and Anglesite (PbSO₄) (Emsley, 2003).

Lead can be exposed to humans and other organisms by inhalation, ingestion, or skin absorption (Emsley, 2003). Low concentrations of lead can bio-accumulate in plants since the element can be absorbed through the roots (Fewtrell et al., 2004). In addition, water that flows through old pipes also has low concentrations of this element (Flora et al., 2012). However, lead passes through the digestive tract of organisms quite easily and only about 10% of all the lead consumed is eventually absorbed (Emsley, 2003). Another way in which lead can be digested is by using old tableware that has a leaded glaze (Flora et al., 2012). Lead was also used to sweeten wine that can cause outbreaks of Picton colic, Devon colic, and dry gripes that are all forms of lead poisoning (Phillips, 1984). The manifestation of the outbreaks was more or likely the typical lead poisoning symptoms: stomach cramps, constipation, weariness, anaemia, insanity and lingering death eventually (Emsley, 2003; Fewtrell et al., 2004). Luckily, this practice has since been discontinued.

Leaded gasoline is still used in some parts of the world, people and other organisms in the areas are exposed to air polluted with lead (Fewtrell et al., 2004; Mielke, 2002). It can then easily be absorbed by the body via the lungs. Most of the uses of lead have been discontinued since the late 19th century when lead's toxicity was recognized, but some lead is still being used for car batteries, lead-based paints, protection of underground cables, protection against radiation, and in the computer and television screen industries (Emsley, 2003; Fewtrell et al., 2004; Flora et al., 2012; Mielke, 2002). One of the most concerned sources of lead is from lead ammunition bullets, fishing sinkers, and jigs that have negative effects on water bird populations (Fisher et al., 2006; Pain, 1990). Lead is also used in industrial processes such as lead smelting and coal combustion (Flora et al., 2012).

Lead is a known neurotoxin that accumulates in soft tissues and bones, but it does affect every organ and system of the body (Emsley, 2003). Gangoso et al. (2009) found that long-term exposure of lead poisoning in Egyptian vultures causes a reduction in bone mineralization. Lead also damages the nervous system, causes blood disorders (anemia and increased blood pressure), nephropathy, colic-like abdominal pains, and possibly weakness in the fingers,

wrists, or ankles (Emsley, 2003; Fewtrell et al., 2004; Kalia and Flora, 2005; Mushak, 1992). Exposure to lead can also cause miscarriages, reduce fertility in males, delayed puberty in girls and sleep disturbances (Emsley, 2003). The lead poisoning symptoms is a result of the build-up of aminolaevulinic acid (ALA) after the lead has deactivated the enzymes that are responsible for the making of haemoglobin (Emsley, 2003).

Mild lead poisoning symptoms include; vomiting, headaches, restlessness, illusions, incoherence, occurring in intoxication, fever stomach pains and cramps, constipation, weariness, lethargy, anaemia, convulsion and insanity (Emsley, 2003; Flora et al., 2006; Pearce, 2007). According to Flora et al. (2006) symptoms following lead exposure such as dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations can be signs of a disease called encephalopathy (Emsley, 2003).

1.3.3 Cadmium

Cadmium is a cumulative poison and a non-essential element to life (Goering et al., 1995). Cadmium is a by-product of zinc and lead. Before its toxicity was known, it was used in a number of ways.

Cadmium is present in all zinc ores and people working in zinc and lead mines are especially exposed to it (Vallee, 1959). Cadmium also has a lower boiling point than zinc and thus industries that process zinc must take care not to expose workers to the cadmium fumes (Emsley, 2003). Cadmium fumes can be inhaled, especially by people working in industry, the inhaled fumes can have devastating consequences and in some cases cause death. According to Emsley (2003) Canada, USA, Australia, Mexico, Japan, and Peru are the main zinc mining countries that in turn also produce the most cadmium from zinc smelters.

Between 7000-8000 tonnes of cadmium enters the atmosphere each year of which 90% is from human activity (Emsley, 2003; Godt et al., 2006). Little to non-cadmium is recycled and most cadmium containing products end up on dumping sites and eventually in the surrounding environment (Järup, 2003). The cadmium settles in water and soil from which we grow our crops and herd our cattle. Small amounts of cadmium are consumed through our diet and surprisingly by smoking tobacco. Tobacco smoking is possibly one of the main sources of cadmium exposure in the general population today (Erie et al., 2007). Crops grown on land, used for grazing that is fertilized by human waste, or grown near old mines or metal-processing industries absorb higher concentrations of cadmium. Even normal fertilizer contains some cadmium (Godt et al., 2006). Thus, the organisms consuming the crops bio-accumulate cadmium in organs such as the kidneys since it tends to form strong bonds with enzymes (Nordberg et al., 2007). Cadmium that passes through the digestive system mimics zinc, an essential element, but only as little as 10% of the cadmium is absorbed into the tissue (Emsley, 2003).

Before the health effects associated with cadmium was known it was used in a number of ways, the most noticeable use was for its bright yellow colour in paints, pigments, and to colour glass (Godt et al., 2006). The pigments were used by artists, and as a stabilizer in the rubber and plastic industry. One of the most famous uses is for nickel-cadmium batteries, but that too have been replaced with nickel-metal hydride and lithium-ion batteries. Cadmium was also used in corrosion-resistant plating on steel and is used in the control rods of nuclear reactors (Godt et al., 2006). While most applications for cadmium were discontinued, one of its new uses is for cadmium telluride solar panels.

There is no single way to identify cadmium poisoning in humans and most of the time cadmium accumulates over a long period. However, cadmium has been identified as a human carcinogen by the International Agency for Research on Cancer (1993). The accumulated cadmium causes some health problems, especially in woman older than 50 years of age with low iron. In most cases it seems that the high concentrations of Cadmium that have accumulated over time only increases the risk of developing diseases and health problems such as kidney disease, early atherosclerosis, hypertension, and cardiovascular diseases (Emsley, 2003). Kidney failure is a potential result of high cadmium concentrations since the build-up of cadmium in the kidney can damage the filtering system and prevent the reabsorption of proteins, glucose and amino acids (Nordberg et al., 2007).

Cadmium affects the reproductive system of both males and females. Cadmium affects the ovaries, early embryo development and can result in irregular oocyte development (Thompson and Bannigan, 2008). During sperm production, zinc plays an important role in DNA polymerase and since cadmium mimics zinc, when absorbed, it can damage the reproductive tissues in testicles (Akinloye et al., 2006; van Wijngaarden et al., 2008).

A disease known as Itai-Itai with the symptoms of weakened bones and joints that make movement extremely painful is a result of a diet high in Cadmium (Inaba et al., 2005). This disease leads to renal abnormalities, including proteinuria and glucosuria and (Emsley, 2003). The treatment of this disease can also cause potential health problems since the treatment agent that removes the cadmium can also remove the essential element zinc.

Inhalation of cadmium fumes and dust can cause a shortness of breath and advance to metal fume fever. It can also cause destruction of mucous membranes, chemical pneumonitis, pulmonary edema, and death (Seidal et al., 1993)

1.3.4 Arsenic

Inorganic arsenic is extremely toxic to insects, bacteria, and fungi. It is also extensively distributed throughout the environment (Hughes, 2002).

Arsenic exposure to humans or other organisms occur through inhalation, absorption and ingestion (Ratnaike, 2003). Environmental arsenic contamination takes place by mining, industry, or from natural geological sources where it ends up contaminating underground water (Ratnaike, 2003). Most unintentional arsenic poisoning takes place by drinking arsenic contaminated water derived from geological sources (Acharyya et al., 2000; Choong et al., 2007; Jomova, et al., 2011; Matschullat, 2000)

Arsenic and its compounds have a number of uses. Even though most of the common uses are being replaced by more environmentally friendly metals and elements, some uses persist in certain parts of the world. The uses of arsenic include pesticides, wood preservatives, paints, cotton desiccants, fungicides, herbicides, and insecticides (Ratnaike, 2003). In addition, arsenic compounds are also used to manufacture semiconductors, light emitting diodes, and components of lasers and microwave circuits (Ratnaike, 2003). Metallic arsenic is mostly used in alloys of lead to aid in the car batteries and ammunition industry.

Organic arsenic compounds are less toxic than pure- or inorganic arsenic, and is added to pig and poultry feed to aid in weight gain, to improve feed efficiency, and to prevent disease outbreak in the animals that are bred for human consumption (Ratnaike, 2003).

Arsenic is extremely poisonous in high quantities. Its toxicity inactivates around 200 enzymes in the human body (Ratnaike, 2003). Not only is it used in pesticides, herbicides and insecticides to kill pests and unwanted diseases in crops and fruit orchards, but it also affects the people working among those sprayers, the effects were so severe that brain damage sometimes accord (Piao et al., 2005).

Acute arsenic poisoning symptoms include gastrointestinal discomfort, nausea, vomiting, abdominal pain, bloody urine, severe diarrhoea and excessive salivation, convulsions, coma, and death (Hughes, 2002; Ratnaike, 2003). Over time, it can cause acute psychosis, skin rashes, toxic cardiomyopathy, and seizures. In extreme cases, haematological abnormalities arise such as renal failure, respiratory failure, and pulmonary oedema (Ratnaike, 2003).

Chronic arsenic toxicity over an extended time can lead to multisystem diseases such as respiratory disease (restrictive and obstructive lung disease, malignant disease, neutropenia) peripheral vascular disease (black foot disease), cardiovascular disease, and diabetes mellitus (Lien et al., 1999; Ratnaike, 2003). In some cases, it can also lead to malignancy associated with organs such as the skin, lungs, liver, kidneys, and bladder. (Hughes, 2002; Lien et al., 1999; Ratnaike, 2003).

1.3.5 Chromium

Chromium is an essential element but only in trace amounts. If concentrations exceed the recommended intake, it can result in toxic effects.

Chromium ores are mined in many countries around the world, including South Africa. It was mined as a black iron-chromium mineral in Turkey (Emsley, 2003). Because of its properties of being resistant to corrosion it has been used in many industries including chrome-plating, metal ceramics, French polishing, calico printing and chrome tanning industries (Dayan and Paine, 2001). Chromium in different valence states represents popular pigments (red and green) used in the dyeing and leather tanning industries (Emsley, 2003). Other uses of chromium ores include the manufacturing of refractory bricks and as alloys, especially stainless steel (Barnhart, 1997; Dayan and Paine, 2001; Fairhurst and Minty, 1989).

Untreated waste from the industries can end up in rivers and cause environmental pollution, but it is the people that work in the industries and chromium ore mines that are at most risk (Emsley, 2003).

For the people working in the industries, the risk of exposure and the pain associated with the result of exposure are great. Chromium in various valence states can be inhaled, consumed orally, or dermally exposed (Gad, 1989). The most famous disease associated with chromium exposure is chrome ulcers (World Health Organization, 1990). The symptoms of this disease are extremely painful and include the appearance of holes in the skin that expose raw flesh and itches unbearably (Emsley, 2003). The exposure to chromates has also caused stomach ulcers, bronchial asthma (World Health Organization, 1990), and is believed to be linked to carcinogenic potential (Costa and Klein, 2006), as well as kidney and liver damage (Gad, 1989).

1.3.6 Copper

Copper is an essential element to life, especially since there are copper-dependant enzymes, but copper can be toxic in high concentrations and cause health problems if below adequate concentrations are ingested (Emsley, 2003).

The most famous form of copper is in its metal form that we mine globally. Smelting copper ores have been practiced for thousands of years to make jewellery, weapons, tools and many other household objects (Emsley, 2003). Today, we use copper in much the same way, but with a few new additional uses, such as for alloys, electrical equipment, electrical wiring, construction (roofing and plumbing) and industrial machinery (Barceloux and Barceloux, 1999; Winge and Mehra, 1990). The use of copper in coins today and throughout history is also one of its many uses. Copper in the form of Bordeaux mixture is used as a fungicide. It is added to water to prevent the growth of algae (Teviotdale et al., 1989).

Essential copper is ingested through our diet, especially meat products where it is found as a copper-protein (Emsley, 2003). Water that flows through copper pipes contain low copper concentrations, slightly higher than water that does not flow through copper pipes. However, the water flowing through copper pipes can cause human health hazards if the concentrations exceed the required intake (Brewer, 2010). Plants absorb copper and humans can be exposed to copper through consumption of plant material or the smoking of tobacco products (Emsley, 2003).

Most acute copper toxicities are either a result of the ingestion of copper-contaminated water that have been contaminated with copper, or from the accidental or deliberate ingestion of high concentration copper salts (Uriu-Adams and Keen, 2005). Excess concentrations of copper can have devastating effects such as tissue damage and can lead to excessive oxidative stress (Uriu-Adams and Keen, 2005) since the copper will bind with enzymes and prevent zinc and iron from binding (Cecconi et al., 2002). When copper is ingested, the body will try to prevent acute toxic effects leading the person to a course of action of vomiting which is one of the body's natural defence mechanisms. Other typical symptoms and effects include abdominal pain, nausea, headache, lethargy, diarrhoea, tachycardia, respiratory difficulties, haemolytic anaemia, gastrointestinal bleeding, liver and kidney failure and death (Davanzo et al., 2004; Srivastava et al., 2005). Excess copper concentrations can also lead to Wilson's disease (Uriu-Adams and Keen, 2005). Even though most copper toxicity occurs through ingestion, it can also be taken up through the skin (Hostynek and Maibach, 2003) and respiratory tract (Nemery, 1990). Copper sulphate can cause death (Emsley, 2003).

Since copper is an essential element, concentrations that exceed required dosages can cause health problems such as; increased levels of cholesterol, high blood pressure, impaired growth, weakened immune system, bone abnormalities, anaemia symptoms, neutropenia, hypopigmentation, osteoporosis, hyperthyroidism, and problems with the digestion of glucose (Dunlap et al., 1974; Emsley, 2003; Hart et al., 1928; Williams, 1983).

1.3.7 Nickel

Nickel in low concentrations is an essential element. However, it can cause health problems in concentrations not meeting the healthy recommended dosages and can be highly toxic at concentrations exceeding the recommended dosages (Cempel and Nikel, 2006).

According to Denkhaus and Salnikow (2002), the primary method of nickel exposure to humans is through inhalation and ingestion. Nickel and its compounds end up in the air, soil and water as a result of numerous industrial practices and uses such as the combustion of coal, diesel oil, and fuel oil (Dean et al., 1972). The burning of dumping sites and the accumulation of wastewater contributes to polluting the environment (Dean et al., 1972). Mining, smelting, and industrial sources are the biggest hazards to the environment regarding the pollution of nickel (Lin, 1999). In fact, most nickel derived from mining is from iron-nickel sulphide mines (Emsley, 2003). Since nickel is easy to work with and resists corrosion, it is used in many industries and machinery. These include electroplating and electroforming processes, gas turbines, rocket engines, rechargeable nickel-cadmium batteries, as well as industrial catalysts in the hydrogenation of oils and fats (Denkhaus and Salnikow, 2002; Grandjean, 1984). Nickel is also a well-known alloy that is used in the manufacturing of appliances and machinery as well as in the metallurgical, chemical and food processing industries (Cempel and Nikel, 2006). Cempel and Nikel (2006) also mentioned that nickel as a powder is added to paints to serve as a protective layer for sensitive electronic instruments. Nickel alloys and plating is used in many accessories that humans use every day, such as wristwatches, spectacle frames, jewellery, and in money coins (Kitaura et al., 2003).

Our daily consumption of food provides the human body not only with sufficient concentrations of essential elements and minerals but also absorbs low concentrations of non-essential elements. The non-essential elements often mimic the properties of the essential elements, allowing them to be absorbed within biological tissue. It was thought that the daily absorption of nickel from food had no real threats to human health. However, recent studies show that food and water contain higher concentrations of nickel than it used to have (Haber et al., 2000). According to Emsley (2003), nickel has been used to make tools and weapons, in some cultures it was even regarded as an improvement on steel since it did not rust.

The best-known effect of exposure to nickel solutions is dermatitis (Kitaura et al., 2003). Nickel solutions are used to produce objects that we use daily such as watches and jewellery. Even mixed with other metals such as stainless steel, nickel can cause an itch called “nickel itch”, erythema, eczema and lichenification of the hands and other areas when exposed or rubbed against the skin (Cempel and Nikel, 2006).

Chronic exposure causes nickel to accumulate within the human body and can lead to conditions such as lung fibrosis, cardiovascular- and kidney diseases as well as carcinogenic related health issues (Denkhaus and Salnikow, 2002). The people working in mining, smelting and manufacturing industries that use nickel, are primarily exposed by inhaling nickel dust which can cause lung and nasal cancer (Coogan et al., 1989; Sunderman et al., 1986), this is the result when nickel binds to atoms during DNA polymerase instead of zinc and magnesium (Denkhaus and Salnikow, 2002). Not only does nickel dust cause serious health complications, but inhaling nickel carbonyl gas can cause death if sufficient concentrations are inhaled and left untreated (Emsley, 2003).

1.3.8 Selenium

As in the case of many other elements, either too much or not enough selenium can cause health problems. In excess, it can lead to toxic effects and ultimately death.

Selenium is an essential element for humans, animals, and plants. Fearing a selenium deficiency in animals and plants, supplements can be added to feed and fertilizers to counter this. The concentrations should be monitored and controlled with caution (Emsley, 2003).

Selenium usually occurs together with sulphides of metals such as copper, zinc, and lead. This makes most mining and smelting activities a source of selenium as a by-product of other metal ores (Eisler, 2000). Selenium is used in metal alloys where it can be used in storage batteries and rectifiers. It can also be recycled from industrial waste, where selenium is an excellent conductor of electricity and used in the manufacturing of photocopiers, photoelectric cells, and light meters (Heinz et al., 1996). Selenium is used in the glass industry to decolorize glass and to make ruby coloured glasses and enamels (Lemly, 2002). However, the main source of environmental pollution of selenium is from coal-burning power stations, metal smelters and municipal incinerators burning paper, cardboard and tyres (Eisler, 2000; Hamilton, 2004; Heinz et al., 1996; Lemly, 2002).

Selenium deficiency can lead to serious health problems and unwanted side effects such as bad breath and body odour. Our diet should be adequate in providing enough selenium. However, if a person does suffer from selenium deficiency it can result in conditions such as anaemia, high blood pressure, infertility, growth depression, cancer, arthritis, premature ageing, and multiple sclerosis (Reilly, 1996; Underwood, 1971). The conditions can progress and develop into serious diseases such as white-muscle disease, Keshan disease, and Kashin-Beck disease (Underwood, 1971). Studies show that a selenium supplement can reduce the symptoms and prevent some of the conditions.

Like most elements, if selenium is consumed or exposed to organisms at high concentrations it can lead to serious health hazards such as foetal mutations and death. High concentrations of selenium can induce carcinogenesis, cytotoxicity and genotoxicity (Hamilton, 2004). This leads to deoxyribonucleic acid (DNA) structures damage, reduced cell growth, diabetes, heart conditions, and various types of cancer (Selvaraj et al., 2012; Selvaraj et al., 2013).

1.3.9 Aluminium

Aluminium is a non-essential element; however, plants do absorb it since it is a naturally abundant metal (Newman, 2015). The human body does not absorb this metal easily but when it does enter the blood stream it is extremely persistent and toxic (Kaehny et al., 1977).

Aluminium is a naturally occurring metal and is often abundant in soil. It is easily absorbed by plants, especially by tea bushes where aluminium is also present in their fertilizers (Ganrot, 1986). Aluminium is used by municipalities to purify water. This contributes to moderate exposure of aluminium through our food and water consumption (Sorenson et al., 1974).

Aluminium is lightweight, strong, resistant to corrosion, and a good conductor of heat making this metal ideal to use in the manufacturing of bicycles, aircrafts, saucepans, soda cans, cooking foil, metal tubing, boats, window frames, door handles, solar mirrors, heat-reflecting blankets, and power cables (Ganrot, 1986). Aluminium foam is also used in spacecrafts and to line traffic tunnels (Emsley, 2003). Alloys with other metals such as copper are even stronger than pure aluminium and used in construction (Emsley, 2003).

Throughout history, aluminium has not only been used for its benefits of being a strong lightweight metal but also for medical and chemical purposes. It was discovered that aluminium can be used to stop bleeding, and since then it has been modified into the form of

styptic pencils (Ganrot, 1986). Another way that aluminium was used and is still practiced today is in the paper making industry where it is used as a preservative (Emsley, 2003).

Even though aluminium is rarely absorbed by the body when passing through the digestive tract, it does have a serious toxic effect if it enters the blood stream. Acute aluminium poisoning can be linked to symptoms such as nausea, fatigue, skin irritations, constipation and colic pain (Rifat et al., 1990). Chronic aluminium poisoning can lead to conditions such as anorexia, paralysis, constant fasciculation as well as liver and kidney damage (Rifat et al., 1990; Shadnia et al., 2005). There are reports that link aluminium toxicity to Alzheimers and Parkinson's disease (Crapper et al., 1973). However, the accusations are debatable.

1.3.10 Gold

Gold is a non-essential element and has no biological role. Gold is famous for being the metal used in jewellery. Even though gold is not easily absorbed into the body, it can cause health hazards if high concentrations do manage to enter the blood stream (Emsley, 2003).

The main source of gold and probably the most famous is from mining activities. Gold is mined in many countries around the world including South Africa. Gold can be collected from alluvial deposits, or separated from the ores of silver, copper, and other metals (Emsley, 2003).

Throughout history, gold has captivated the mind of humans and it has been used in many ways. Gold was used to make lavish jewellery, decorations, statues, and furniture. It was also used in cosmetics and medical practices (Antonovych, 1981; Lewis and Walz, 1982; Merchant, B., 1998). Today, gold is used to make most of the ornaments and accessories such as in history with the added use of gold in glass, components in electronics, and gold fillings and crowns in dentistry (Levison et al., 1984). Gold is also alloyed with other metals such as copper, platinum, and palladium (Wiesenfeld et al., 1984).

Gold has been used to treat various illnesses and conditions, some with no success. However, gold has been used to treat rheumatoid arthritis (Merchant, 1998). The gold salts that are admitted for the treatments can build up in the kidneys, liver, spleen, marrow, skin, hair and nails. The build-up of gold salts can lead to pruritus, rash, cheilitis, pulmonitis, eosinophilia, nephrotoxicity and nephrotic syndrome, chronic popular eruptions, contact sensitivity, blood, dyscrasias erythema nodosum, allergic contact purpura, and pityriasis rosea, plus lichenoid and exfoliative dermatitis (Antonovych, 1981; Penneys et al., 1974). Although gold is used to treat an illness, if it is administered incorrectly or used for long periods, it can have serious side effects.

Table 1.3 Uses, sources, and effects of some metallic elements (Emsley, 2003).

Compound		Uses and Sources	Other forms	Effect
Metallic element	Symbol			
Sodium	Na	• Mining • Chemical industry • Salt • Glass production • Metallurgy • Water treatment • Component in Soda drinks • Textile and leather industries • Firefighting foams • Dye manufacturing • Component in air bags of cars • Bleaching • Fertilizers • Soap • Preservative	• Sodium chloride • Sodium carbonate • Sodium carbonate bicarbonate • Sodium hydrogen carbonate • Sodium hydroxide • Sodium azide • Sodium borohydride • Sodamide	• Some forms can be toxic; however, it is mainly and essential element.
Magnesium	Mg	• Fertilizers • Component in plastic • Cattle feed • Heat-resistant bricks • Mining • Alloys • Automobiles and aircrafts • Electronics • Dye industries • Batteries • Beverage cans	• Magnesium bromide • Magnesium sulphate • Magnesium hydroxide • Magnesium oxide • Magnesium silicate • Magnesium chloride	• Essential to life. • If too much magnesium is exposed to the body it can lead to muscle weakness, lethargy and confusion. • Magnesium deficiency can also cause problems such as irritation, depression and personality changes.

Aluminium	Al	• Abundant in nature • Mining • Industries (car manufacturing, window frames, door handles, metal tubing, power cables, boats, aircrafts, cooking foil and drink cans) • Alloys • Insulation • Solar mirrors • Paper treatment • Water purification • Production and use of Aluminium foam	• Duralumin • Aluminium sulphate • Aluminium hydroxide • Aluminium oxide • Aluminium silicate	• Toxic, not essential to life.
Phosphorus	P	• Food additives • Mining • Fertilizers • Cleaning agents • Used in industry as a rust remover/preventer • Flame retardants • Insecticides • Weed killers • Oil additives • Dye manufacturing • Leather tanning • Toothpaste	• Calcium phosphate • Organophosphate • Adenosine Triphosphate • Guanosine monophosphate • Phospholipids • Hypophosphorus acid • Phosphoric Acid • Phosphorus pentoxide • Phosphorus trichloride	• Only white phosphorus is poisonous.

Potassium	K	• Mining • Production of porcelain, ceramics and glass • Fertilizers • Liquid soaps and detergents • Tanning leather • Pharmaceuticals • Fire matches • Fireworks • Gunpowder	• Potassium chloride • Potassium carbonate • Potassium nitrate • Potassium aluminium sulphate • Potassium magnesium chloride • Potassium hydroxide	• Essential element to life but in high doses it can cause extreme illness. • Potassium chloride injections are used.
Calcium	Ca	• Alloying agent (aluminium, beryllium, copper, lead and magnesium) • Used to make cement and mortar • Glass industry • Toothpaste • Mineral supplements	• Calcium carbonate • Calcium phosphate • Calcium oxide	• Essential element to life, but toxic in oxalate form.
Thallium	Tl	• By product of other metals • Glass production • Chemical research • Pesticide • Coal-fired power stations • Metal processing industries • Rat poison	• Thallium oxide • Thallium sulphide • Thallium bromide-iodide • Thallium nitrate	• Toxic, it can be absorbed through the skin.
Vanadium	V	• By product of other ores • Venezuelan oil • Steel production • Alloys • Polymer production	• Vanadium pentoxide • Lead chloride vanadate • Vanadium sulphide • Potassium uranyl vanadate • Vanadium pentoxide • Vanadium trichloride • ferrovanadium	• Essential element to humans and certain other species. • In certain forms, it can cause some health problems.

Chromium	Cr	- Alloys - Catalysts - Pigments - Wood preservatives - Tanning - Anticorrosive - Production of refractory bricks	- Chromates - Chromite - Chromium sulphate - Chromium oxide	Toxic and carcinogenic, but essential in the right concentrations.
Manganese	Mn	- Fertilizers - Animal feed - Mining - Glass production - Alloys - Rubber production	- Manganese dioxide - Manganese carbonate - Manganese oxide - Manganese sulphate	Essential element but in some forms at high concentrations it can be a health hazard.
Iron	Fe	- Pharmaceuticals - Alloys - Industries - Construction - Manufacturing of weapons, jewellery, cutlery, household appliances, motor vehicles, - Many more	Iron oxide	- Essential to all life on earth, but is poisonous in excess and toxic in high amounts. - Iron deficiency leads to anaemia.
Cobalt	Co	- Mining - Alloys - Catalysts - Colour agents (porcelain, pottery, stained glass, tiles, enamel jewellery) - Food preservative	- Cobalt arsenide - Cobalt sulphide	Essential element, but in high concentrations it is suspected that it can cause health problems.
Nickel	Ni	- Alloys (stainless steel and nickel plating) - Battery production - Mining - Gas turbines	- Nickel sulphide - Nickel carbonyl - Nickel arsenide	Toxic and carcinogenic.

		• Rocket engines • Coinage • Food and chemical processing industries • Paints		
Copper	Cu	• Wiring and electronics • Plumbing • Mining • Construction • Alloys • Water purification • Agrochemical pesticides- Used to control the growth of algae, bacteria and fungi	• Copper sulphate • Copper acetate • Copper carbonate • Copper oxide	• Essential life element, but toxic at high concentrations.
Zinc	Zn	• Essential metal • Protective coatings and galvanizing to prevent corrosion • Alloys • Mining • Industry • Batteries • Pigments • Rubber industry • Used as a phosphor • Paints	• Zinc sulphate • Zinc chloride • Zinc oxide • Zinc stearate • Zinc carbonate • Zinc silicate	• Toxic, although less so than most metals. In low concentrations, it is essential to life.
Arsenic	As	• Metalloid • Metal alloys • Pesticides and herbicides • Wood preservatives • Plant desiccants • Mining (gold and lead) • Production of glass • Volcano		• Toxic and carcinogenic.

Selenium	Se	• Non-metal that behave similar to arsenic • Electronics • Glass • Pigments • Alloys • By-product of mining (gold, copper, and nickel) • Animal feed • Food supplements • Used to vulcanize rubber • Anti-dandruff shampoo • Coal power stations	• Methyl selenium • Sodium selenite • Sodium selenate • Cadmium selenide • Cerium selenide • Methyl selenide • Dimethyl selenide	• Essential element for humans but in high doses it can be poisonous.
Rubidium	Rb	• Used for research • Manufacturing of a special type of glass	• Rubidium chloride • Rubidium-silver iodide • Rubidium carbonate	• No known biological role.
Strontium	Sr	• Mining • Warning flares • Fireworks • Glass manufacturing	• Strontium sulphate • Strontium oxide • Strontium carbonate	• Non-toxic element.
Molybdenum	Mo	• Pencils • Mining • Electric and electronic devices • Glass manufacturing • Alloys • Lubricant and anti-corrosion additives • Microwaves • Used in the fossil fuel industry • Used in the formation of synthetic fibres and rubbers • Pigments	• Molybdenum sulphide • Molybdenum oxide • Ammonium molybdate • Molybdenum disulphide • Sodium molybdate	• Essential element in low concentrations but can be toxic in higher doses. • It can cause foetal deformities.

Palladium	Pd	- Alloys - Fuel - Electrical appliances - Chemical industry - Fertilizers - Terephthalic - production of polyester	- Palladium chloride - Palladium cyanide - Palladium antimonide	Low toxicity, but can be poisonous at high doses and carcinogenic.
Silver	Ag	- Mining - By product of lead, zinc, copper and gold mining - Alloys - Photography - Electrical and electronic industries - Cutlery - Jewellery - Mirrors - dentistry	- Silver nitrate - Silver acetate - Colloidal silver - Silver sulphide - Silver antimony sulphide - Silver chloride - Silver arsenide	Toxic in high amounts and non-essential to life.
Cadmium	Cd	- Alloy production - Electroplating - Galvanizing - Pigments - Batteries - Plastic - Produced during zinc ore processing - Cigarette - Mining (zinc and lead mines)	- Cadmium sulphide - Cadmium selenide - Cadmium carbonate - Cadmium chloride - Cadmium oxide	Toxic and carcinogenic.
Antimony	Sb	- Alloys - Flame retardant - By product of lead		Toxic.
Barium	Ba	- Mining activities - Alloys - Weighting agent	- Barium sulphate (sulphate) - Barium carbonate - Barium hydroxide	Believe to be non-toxic in low concentrations.

		• Manufacturing of oil and grease additives • Refining of beet sugars • Dehairing agent • Manufacture of section types of glass • Used in fireworks	• Barium oxide • Barium nitrate • Barium chlorate	
Platinum	Pt	• Mining • Alloys • Jewellery • Catalytic convertors • Chemical industry • Electrical industry • Glass industry • Aircraft industry	• Platiniridium • Platinum sulphide • Platinum arsenide	• It is a non-toxic metal, but some of its associated compounds are poisonous.
Gold	Au	• Mining • Alloys • Dentistry • Jewellery • Glass • Bullion • Electronics	• Gold sodium thiomalate	• Non-essential element, but toxic at high concentrations.
Mercury	Hg	• Electronics • Dental amalgams • Chlorine-alkali production • Gold mining • Paints • Industrial catalyst • Compound in electrolysis • Biocide • Burning of fossil fuels (coal and oil) • Cinnabar ore mines • Alloys	• Mercurous chloride • Mercuric chloride • Mercury sulphide • Mercury antimony sulphide • Mercuric sulphide • Mercury(I) chloride • Mercury(I) hydride • mercury(II) chloride • Mercury(II) oxide • mercury(II) sulphide • Mercury(II) selenide	• Toxic, especially methyl mercury.

		Volcanoes	mercury(II) telluride	
Lead	Pb	- Storage batteries - Ammunition - Leaded petrol - Imaging devices - Mining - Insecticide - Cosmetics (hair gels) - Sheeting, cables, solders, lead crystal glassware, bearings. - Architecture (roof cladding and stained glass windows)	- Lead acetate - Lead oxide - Lead carbonate - Lead chloride - Lead chloride carbonate - Lead chloride phosphate - Lead antimony sulphide - Lead arsenate - Lead phosphate	Poisonous.
Bismuth	Bi	- Medicine - Cosmetics - Mining (lead and copper) - Alloys - Synthetic fibre industry - Rubber industry	- Bismuth subnitrite - Bismuth subcarbonate - Bismuth subcitrate - Bismuth sulfide	No biological role and has no real environmental threat.
Thorium	Th	- Gas mantles - Metallurgical industry - Oil refinery - Tungsten of light-bulbs - Photoelectric cells - Alloys - Nuclear fuel - Nuclear reactors	- Thorite - Uranothorite - Thorianite - Thorium oxide	No biological role since it is radioactive and moderately toxic.
Uranium	U	- Bomb manufacturing - Mining - Nuclear reactors - Nuclear fuel - Nuclear powered submarines and naval vessels	- Uraninite - Pitchblende - Autunite - Carnotite - Samarskite - Torbernite	No biological role since it is radioactive and poisonous.

		•	• Betafite • Uranyl ions	
Titanium	Ti	• Medical implants and devices • Aircraft industry • Chemical plants • Power plants • Alloys • Oil rigs • Submarines • Ships • Paint • Plastics and papers • Ceramics and enamels	• Titanium dioxide • Titanium tetrachloride	• Non-poisonous metal even though it has no biological role.

1.4 Bio-indicators

Bio-indicators are living organisms that give us insight to the general health of an ecosystem. Certain organisms are more sensitive to pollution than other organisms in the same environment, and if pollutants are present, the organism may change its morphology, physiology, or behaviour to survive the conditions. In some cases, either the individual or even the entire population may die (Newman, 2015).

1.4.1 *Vertebrates*

Vertebrates are usually much larger organisms and ethical implications of using vertebrates are extremely difficult and time consuming. In most studies the use of feathers (Wepener and Meyer, 2015), blood (van Wyk et al., 2001), hair (Rashed and Soltan, 2005), scales (Khanna et al., 2007), eggs (Bouwman et al., 2012; Bouwman et al., 2008) or other biological tissue is preferred. Biological tissues will be collected and used in analyses instead of the entire organism. In addition, the life duration of most vertebrates extends over years and although this may favour bioaccumulation of toxic chemicals over many years it does however, make the process of evaluating the effects on a reproduction viewpoint difficult. Furthermore, it will be a time consuming process to evaluate the organisms as biological indicators taking into account reproductive success and effect on offspring. Using vertebrates does have advantages - heavy metal toxicity can be suspected by observing symptoms such as dermatitis and organ failure (Newman, 2015).

1.4.2 *Invertebrates*

Invertebrates such as arthropods make up an estimated 69% of all species on earth (Holm, 2017). Non-vertebrates are becoming a popular biological indicator to assess for many changes in the environment. Most of the organisms have a relatively short life span and can be “early warning” indicators. Unlike birds and mammals, invertebrates are noticeably smaller and their decreased numbers after capturing will have less of an impact on the environment (Gess and Gess, 2014). These organisms have a close relationship with their abiotic surrounds (whether it is with soil, water, food or air), and as a result are exposed to toxic chemicals.

Sentinel species accurately reflect ambient contaminant concentrations, and can be routinely and conveniently collected and analysed as part of monitoring programs (Beeby, 2001). Corbet et al. (1999) investigated the use of invertebrates in monitoring pollutants; they found that many groups such as nematodes, enchytraeids, Lumbricina, Gastropoda, Collembola, Arachnida, and Isopoda could be used in the study of bioaccumulation and ecotoxicology. Reish & Gerlinger (1997) tested for metallic elements in Polychaeta and found that copper, along with mercury to be the most toxic metals. Nummelin et al. (2007) tested for metallic elements in a number of different predatory Arthropods such as Gerridae, Odonata larvae, Myrmeleontidae larvae, and Formicidae. They found each taxon effective as bio-indicators of different metals, but that no single species is the ultimate indicator for environmental pollution. Individuals of many arthropods are not easy to collect in quantities large enough for analyses or there is a short seasonal supply. Often, their origin is unknown due to their flying ability, and in some cases, a taxonomic expert is needed to determine the individual up to species level (Nummelin et al., 2007). Nonetheless, invertebrates have been used as indicator species for environmental pollution.

Bees

Bees are one of the more popular insects on earth. They play key roles in the production of food via the pollination of flowers (Gess and Gess, 2014), in turn aiding the fruit industry and honey production. Bees have in addition aided in environmental monitoring research. They have been used as bio-indicators of environmental pollution since the early 1930s when J. Svoboda suggested that bees could give us insight into the environmental impact that selective industries have on certain areas. They found an increase of the radionuclide strontium 90 in the environment (Svoboda, 1962). Since then, many new studies have been conducted using bees, honey, and the pollen found on bees as bio-indicators for heavy metal pollution (Crane, 1984; Rashed et al., 2009; Erbilir and Erdoğan, 2005; Leblebici and Aksoy, 2008; Fredes and Montenegro, 2006) and radioactive pollution (Tonelli et al., 1990).

The advantages of using bees and their products as bio-indicators:

- They are abundant, especially with beekeeping practices.
- Bees are found around the globe.
- Bees are very active throughout the day, making frequent trips to flowers each day (Celli and Maccagnani, 2003).
- They can occupy an area up to seven square kilometres around the hive, giving a good indication of the pollution in the surrounding area (Rashed et al., 2009).
- Bees are easy to collect with entomological nets.
- Comparing honey from around the world is relatively easy.
- Ethical clearance is easily obtained.

Bees can indicate environmental pollution in two ways, either by a high mortality rate as a result of toxic chemicals in the environment (insecticides), or via the heavy metal, fungicides and herbicides residues in honey, pollen, and larvae (Celli and Maccagnani, 2003).

Spiders

Araneae (spiders) can be poisonous but many of the common spiders in homes are actually harmless. Spiders keep pests like midges under control. Spiders can be found around the world and in high numbers. They distribute to new areas, but not as flying insects would. They have a short life span, which means that they have to either adapt or flee when the environment becomes unfavourable. Since they are predaceous, bioaccumulation of toxic chemicals is possible (Clausen, 1986). Spiders have been used as indicators of environmental metal pollution by Wilczek et al. (2003). According to Wilczek et al. (1999), the concentration of metals depend on the species of spider, which can be influenced by the hunting vigour, the prey of choice, and the way they feed. The advantages of using spiders as indicators for environmental pollution are evident. However, they still lack the exposure of pollutants in other habitats like water.

The advantages of using spiders as bio-indicator:

- Spiders are found around the globe.
- Taxonomically relatively easy to identify.
- Known to tolerate toxic chemicals within the environment.
- Predatory species that favours bioaccumulation.
- Close association with soil.
- Ethical clearance is easy to obtain.

- Relatively easy to collect.
- Spider webs can possibly be used to analyse for toxic chemicals.
- Relatively short life span.

Other arthropods used as bio-indicators for environmental pollution

Carabidae (ground beetles), Gerridae (water striders), and Formicidae (ants) have all been used as indicators of environmental pollution. They occupy a specific niche and each species is perfectly adapted to those surrounding. Tables 4, 5, and 6 contain the different advantages, as well as disadvantages as to why they have been used as indicators of environmental pollutions.

Table 1.4 A summary of biological and ecological characteristics of water striders that make them suitable indicators of environmental conditions (Holm, 2017; Picker et al., 2004).

Water striders	
Advantages	Disadvantages
• Relatively long-lived • Predatory invertebrates • Easy to collect • Relatively easy to identify to species level • Low dispersal range	• Low dispersal range • Difficult to handle • Small organisms (will need multiple samples) • Only favours water above 22°C

Table 1.5 A summary of biological and ecological characteristics of ground beetles that make them suitable indicators of environmental conditions (Holm, 2017; Picker et al., 2004).

Ground beetles	
Advantages	Disadvantages
• Easy to collect with pitfall traps • Close association with soil • Predatory invertebrates	• Locating individuals can be difficult

Table 1.6 A summary of biological and ecological characteristics of ants that make them suitable indicators of environmental conditions (Holm, 2017; Picker et al., 2004)

Ants	
Advantages	Disadvantages
• High tolerance to toxic chemicals • Abundant • Scavengers and predatory invertebrates • Easy to collect • Easy to locate	• Numerous individuals would have to be collected • Male ants are short-lived

1.4.3 Ideal indicator?

It is clear that there is no single ultimate indicator for environmental pollution. Numerous aspects influence the suitability of an organism to be an indicator. However, more than one aspect should be considered when choosing a species to use in a relative study. Different species have different ecological requirements. Some are habitat specialists where their presence, absence, increase, and decrease of numbers can indicate environmental disturbances. While not all taxa are habitat specialists, nor found over large numbers of habitats, some taxa would be better indicators than others (Dufrene and Legendre, 1997).

1.4.4 Bioaccumulation and bio-magnification

Organisms exposed to toxic chemicals through their environment tend to either perish from the exposure or bio-accumulate it within tissues and organs. Bioaccumulation occurs when toxic chemicals in the air, water, and soil are taken up. When toxic chemicals are released to the environment, it may become bioavailable. Plants absorb many toxic chemicals and may eventually be consumed by primary consumers. Bio-magnification on the other hand refers to the increased concentration of toxic chemicals through trophic levels. Thus, toxic chemicals are transferred from prey to predator and increase with each trophic level. The organisms at the top of the food web are often the most severely affected by the pollutants because it is not only exposed to the toxic chemicals through air and water, but also that through their diet (Newman, 2015).

1.4.5 Bioaccumulation of heavy metals

Since some metals have a tendency to bind to amino acids and adipose tissues, metal concentrations in organisms increase over time. Some of the metals are not easily absorbed by the digestive tract and are excreted through urine, faeces, and reproduction. The organs that tend to bio-accumulate the toxic chemicals the most are the liver and kidneys. Metallic elements in the form of dust can bio-accumulate in the respiratory system. However, eventually all organs are affected in some way. Thus, organisms are exposed to the increasing concentrations of metallic elements from all sources and tend to bio-accumulate the metals more often than not (Newman, 2015).

1.4.6 Bioaccumulation by super predators

Organisms that only feed on plant material inhabit lower trophic levels and are primarily exposed to toxic chemicals through their plant-based diet, water, sediments, and air (Swackhamer and Skoglund, 1993). Predatory species tend to have higher concentrations of toxic chemicals than herbivores, since they inherit the toxicants that have built up in their prey (Oliver and Niimi, 1988). Studies have shown that the same toxic chemicals which bio-accumulated within prey, will often bio-accumulate within their predators (Madenjian et al., 1994). Super predators such as predatory birds and fish are exposed to even higher concentrations since they consume species that already have accumulated toxic chemicals. It is important to take into account the food preferences and metal concentrations when doing animal risk assessments (Heikens et al., 2001). Their primary exposure is through their dietary uptake (Thomann et al., 1992). Wolfe et al. (1998) stated that if organisms high up in the food web, such as birds that prey on fish, consume adequate concentrations of MeHg through prey and water, can lead to severe damage to the nervous, excretory and reproductive systems (Newman, 2015).

1.4.7 Effects of metallic elements on populations and species

Humans accumulate trace level elements that have been obtained from dietary intake (Emsley, 2003). It is when environmental pollution from mining, industries, power stations and such like becomes bioavailable and accumulate within organisms that the effect of the metallic elements becomes problematic. The concentration of metallic elements can often approach toxic levels resulting in reproductive failure and mortality. The consequences can lead to population declines or the disappearance of entire populations (Hamr et al., 1995). If keystone species are affected, it may lead to a chain of events where more species are affected. This was the case when Kolaja (1977) indicated the effect that dichlorodiphenyltrichloroethane (DDT) accumulation has on peregrine falcons and ducks.

1.4.8 Fluctuating asymmetry (FA)

Fluctuating asymmetry occurs when a bilateral structure has small irregularities that result in each bilateral side not being perfectly symmetrical. The deviations are thought to be an indication of environmental stress and genetic instability during development. This irregularity has been used to assay the individual's ability to not only service but to find mating partners and reproduce. Furthermore, fluctuating asymmetry can be used as a biomarker in detecting pollution within water bodies (Tomkins and Kotiaho, 2002).

1.5 Odonata

1.5.1 Taxonomy

Odonata are divided into two suborders - damselflies (Zygoptera) and dragonflies (Anisoptera). Dragonflies and damselflies can easily be distinguished from one another by the differences in eye structure, the shape of their wings, and overall build (Tarboton and Tarboton, 2015). Dragonflies are not considered one of the most important invertebrate taxa, given that they have no real economic value. Unlike bees, dragonflies do not produce any food sources or aid in the pollination of flowers. In some countries, they are consumed and used in magical or medicinal practices. Dragonflies have also been acknowledged to influence the population of disease vectors (Kalkman et al., 2008) such as mosquitos. Today, they are more commonly used in natural monitoring programs and dragonfly watching. Dragonfly enthusiasts take pride in trying to recognize as many different types of Odonata as they can. This is especially popular in countries such as Japan where dragonflies are not only abundant but respected (Lemelin, 2007).

Dragonflies have been around for millions of years and some of the best fossils, in either tree resin (amber) or fine slate (petrified mud), include dragonflies in which some examples date back to the Carboniferous periods (Holm, 2017). Morphologically, they have changed little except in body size. According to fossil records, dragonflies today are much smaller than their ancestors. Odonata are taxonomically one of the best-described taxa in the world, except for butterflies. Numerous studies have been conducted to understand their distribution and they have received much attention from the public (Kalkman et al., 2008). As a result, of the interest in Odonata, many regions have reliable identification literature available in the form of field guides. To date, over 5680 species of Odonata have been described, of which 2941 belong to the suborder Anisoptera (dragonflies), while between a 1000 to 1500 species of dragonflies still await description (Kalkman et al., 2008).

1.5.2 Life cycle

Larval stage

Adult dragonflies select an abode of preference and lay their eggs in selected fresh water habitats where the males protect their territories vigorously. Females of certain species depend on vegetation for oviposition and camouflaging. Once the eggs are laid it can take anything from a month to several months before the larvae hatch, depending on the species (Corbet and Brooks, 2008). The long-lived larval stage can set the platform for accumulation of pollutants, and cause subsequent developmental irregularities (CARLE, 1979). The larvae are sedentary (CARLE, 1979) and the dispersal of larval forms is very much restricted to the moist and freshwater locations the eggs were laid in.

Most dragonfly larvae are found in clean freshwater. Unlike larvae, adult dragonflies do occur and even oviposit at poor quality aquatic sites. The eggs may not develop in such conditions (Corbet, 1999). With this said, larvae have been found to tolerate brackish conditions; two species have even been found living in salt marshes and mangroves (Kalkman et al., 2008). Dragonfly larvae complete their juvenile life stages in the water and are dependent on this water source for sustenance. Dragonflies have a high trophic position where larvae feed on all kinds of small animals up to the size of tadpoles, small fish (Kalkman et al., 2008), and in some cases other dragonfly larvae. The dragonfly larvae undergo multiple metamorphosis stages and can take anything from a few weeks to seven years to develop (Kalkman et al., 2008). Odonata larvae are mostly opportunistic foragers and prey upon other stream invertebrates including the larvae of Diptera and planktonic Crustacea, some species are also known as the main predators of water striders (Gerridae) (Corbet, 1999). Dragonfly larvae are also prey to a number of predators including fish, amphibians, reptilians, and birds.

Adult stage

Adult dragonflies are considered jewels of nature. With the adult male dragonflies being vibrant in colour, human fascination is no wonder. They are vigorous flyers and ruthless hunters, but many people do not appreciate the ecological values of dragonflies. Dragonflies have a relatively short lifespan as adults relative to vertebrate predators. They can live as adults between one to nine months depending on species and location. Some species remain close to where they emerged as adults, while others disperse over larger areas or are migratory. The ability of the adults to move around, in some cases over oceans, ensures re-establishment of dragonflies in suitable new habitats. Adult dragonflies fall prey to many predators including Arachnida, Insecta, Amphibia, Reptilia, Mammalia, and Aves (Corbet, 1999). Dragonflies are not only predators during their larval stage, but also during adulthood, preying on Anisoptera, Zygoptera and species such as *Anax junius*, *Pantala flavescens* and *Mnais pruinosa* (Corbet, 1999).

1.5.3 Exposure and uptake

Adult dragonflies move around in search of prey or mating partners. Some species as mentioned before stay close to water, typically the source from where they emerged. Some tend to distribute to other freshwater sources, in search of mating partners, prey, or to find more favourable habitat. Dragonflies are found all over the world except Antarctica. Some dragonfly species such as *Crocothemis erythraea* are found across South Africa as well as in the southern parts of Europe, the Middle East, and the western parts of Asia (Tarboton and Tarboton, 2015). *Crocothemis erythraea* males are territorial and tend to stay near where they

emerged. This low dispersal characteristic makes it an excellent candidate for the use as an indicator for environmental pollution.

During the transition from larvae to adult, the dragonflies leave behind an exuviae that is in some cases seen as a record of their aquatic life stage, giving insight to how their first stage of life transpired (Gladysz et al., 2016). In recent years, Odonata has become increasingly popular in the study of environmental indicators, with most research focusing on the use of larvae and exuviae. However, dragonflies can also be used for the analyses of pollutants. Lesch et al. (2017) found traces of persistent organic pollutants (POPs) in adult male dragonflies from water sources in South Africa that are known to be polluted. Kormondy (1965) found zinc in the exuviae that was absorbed into the exoskeleton; zinc was also not metabolically absorbed during the larvae stage. Simaika and Samways (2011) found that pH and water temperatures affect dragonfly assemblages more than other environmental factors.

Male dragonflies from Neotropical regions have a strong relationship with freshwater. As adults, they are territorial along fresh water systems; as larvae, they are confined to the water body (Dutra and De Marco, 2015). This association with fresh water makes them an ideal candidate for the use as an environmental pollution indicator. The body size of an organism also influences many characteristics such as prey preferences and dispersal ability.

1.5.4 Dragonfly larvae as indicators

To date, the only published record of adult dragonflies used as indicators for environmental pollution is by Lesch et al. (2017) who found that adult male dragonflies could be used to indicate the concentrations of perfluoroalkyl substances in the environment. With that said, adult dragonflies have been used as bio-indicators to assess the quality of water bodies and vegetation (Barbosa et al., 2001; Clark and Samways, 1996; Clausnitzer and Jödicke, 2004; D'amico et al., 2004; Hornung and Rice, 2003; Júnior et al., 2015; Lee Foote and Rice Hornung, 2005; Lenat, 1988; Metcalfe, 1989; Osborn, 2005; Sahlén and Ekestubbe, 2001). The use of aquatic invertebrates to assess the water quality, and numerous studies has been done by using dragonfly larvae as indicators of water pollution (Bulánková, 1997; Nummelin et al., 2007; Sharma and Rawat, 2009; Tollett et al., 2009; Van Praet et al., 2014). Although used as indicators for environmental water quality, studies on the toxicity of certain metallic elements on dragonfly larvae in laboratory experiments indicated that some metallic elements are more toxic than others are. Tollett et al. (2009) found that copper exposure significantly decreased larval survival whereas the larvae tolerated cadmium and lead better. In addition, larvae have an exoskeleton and studies found that metallic elements accumulate in their exoskeletons (Giesy et al., 1981; Gupta, 1995; Meyer et al., 1986) and are lost when shedding their exuviae, thus not transferring all metallic elements exposed and absorbed from larvae stage to adult stage. The use of dragonfly larvae has its advantages and disadvantages as listed in Table 7.

Table 7. Advantages and disadvantages of using dragonfly larvae as indicators of environmental pollution (Corbet and Brooks, 2008; Corbet et al., 1999).

Advantages	Disadvantages
• Usually abundant in fresh water • Easy to collect with entomological net • Can be collected during winter months • Provides a good indication of the toxic chemicals within the water • Predatory organisms • Can be sampled during day and night	• Difficult to identify species • They are restricted to the freshwater locations the eggs were laid in • Only accounts for the toxic chemicals within the water • While sampling, other aquatic organisms can also become trapped • Disruption of peace and habitat

However, there remains a big opportunity to investigate adult dragonflies as indicators of chemical pollutants. This is the subject of my study

1.6 Aim and objectives

The aim of the study is:

- To evaluate adult dragonflies as potential indicators of metallic elements in the environment.

This will be achieved by completing the following objectives:

- To collect adult dragonflies at a number of sites across South Africa
- To measure concentrations metallic elements in each individual dragonfly
- To compare the metallic element concentrations and relative contribution patterns between sites, species, size classes, habitat types, and relation to possible pollution sources
- To deliberate the potential and value of adult dragonflies as bio-indicators of environmental pollution

“Science is part of the reality of living; it is the what, the how, and the why of everything in our experience.”

– Rachel Carson

Chapter 2: Materials and methods

“The most alarming of all man’s assaults upon the environment is the contamination of air, earth, rivers, and sea with dangerous and even lethal materials”

– Rachel Carson

2.1 Introduction

For this study, adult male dragonflies were sampled during the summer months of 2016 and 2017 at various locations across South Africa (Fig. 2.1). The sites were selected based on several factors namely: the availability of water, access, and the presence of Odonata. This study was approved by the North-West University Ethics committee and was graded as a Category 0 application since it only involved lower invertebrates. No permits were needed to sample dragonflies.

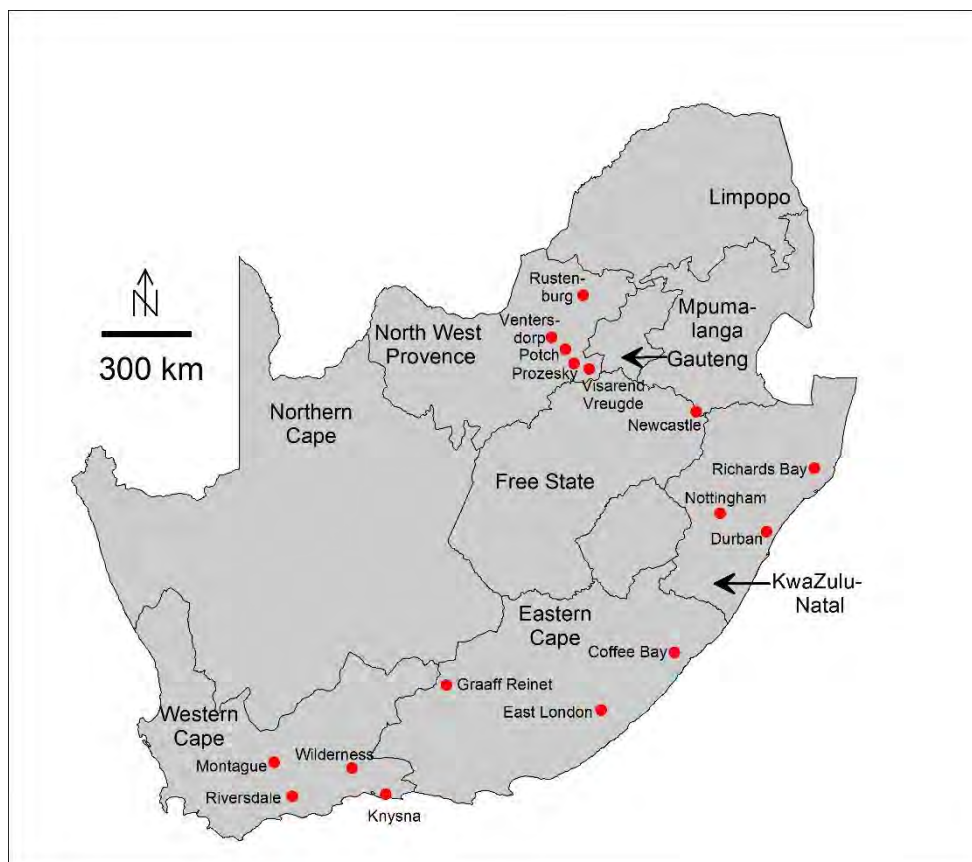


Figure 2.1 Sampling locations in South Africa.

2.2 Collection and preservation

Capturing of adult dragonflies was done between the 10:00 and 16:00 from November 2015 to May 2016, and from November 2016 to May 2017. I became proficient in capturing adult dragonflies during a previous study (Lesch et al., 2017). Clean entomological nets were used to capture single perching adult male dragonflies. The dragonflies were placed individually into new, small Ziploc plastic bags that were then placed into a small plastic container that was marked with the date and site name. Special care was taken not to damage the wings of the dragonflies by carefully placing them individually in small Ziploc bag. The plastic container was

held in a cold storage box to ease the stress on the organism and to slow down its metabolic rate.

The samples were held in a cold storage box or in a refrigerator at $+4^{\circ}\text{C}$ during transportation and traveling between sites. The low temperatures slowed down the dragonfly's metabolic rate and led to the euthanasia of the individuals. Once back at the laboratory the dragonflies were placed in a freezer at -4°C for up to a month. The dragonflies were then placed in falcon tubes and left over night at -86°C before being freeze dried for 24 hours. The samples were then kept in dark dry storage until the time for heavy metal examination and evaluation was affixed and concurrent.

2.3 Species sampled

Adult dragonflies are abundant across South Africa and are often found near freshwater systems during the warmer months (September-April) around mid-day. Most individuals are easily identifiable up to species level by means of observation without catching them. I used the guide, "Dragonflies & damselflies of South Africa" (Tarboton and Tarboton, 2015) for identification and nomenclature, as well as for general biological information.

Fifteen different species were used in this study (Table 2.1). Most of the species sampled in this study do not only occur in South Africa but can be found across Africa and some species distribution can stretch as far as the Middle East, Asia and some parts of Europe. Their sizes vary from very small (21-27mm), small (30-37mm), medium (37-44mm) to large (50-58mm). Due to the size differences, adults prey on different sized flying organisms. Some of the larger species such as *Zosteraeschna minuscula* will even prey on other adult dragonflies. This predatory life-style puts dragonflies high up on the food web.

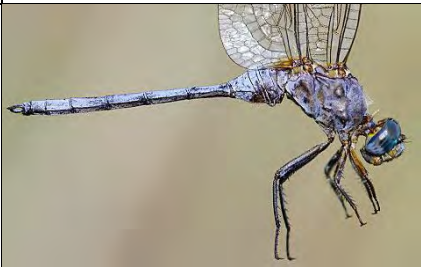
Male dragonflies often perch or patrol around freshwater habitats. Some species such as *Crocothemis erythraea* protect territories from other males and guard females when they oviposit their eggs (Tarboton and Tarboton, 2015).

Details of the species are listed in Table 2.1. Here, I provide information on scientific and colloquial names, an image, and notes on distribution, habitats, size, behaviour, as well as where they were collected for this study.

I became proficient in identifying adult dragonflies from a previous study (Lesch et al., 2017).

Table 2.1 Details of the species that were collected. Nomenclature and information were mainly from Tarboton and Tarboton (2015).

Species	Species number	Image	Distribution	Habitat preference	Size and behaviour	Sampled at:
<i>Crocothemis erythraea</i> Broad Scarlet Breë Bloisie	6		- Europe - Pan-Africa	- Streams - Rivers - Ponds - Marshes - Floodplains - Lakes	Small Territorial	- Durban - WFS 5 - New Castle
<i>Brachythemis lacustris</i> Red Groundling Rooigrondwagttertjie	10		- Eastern Africa - North-eastern Africa - Western Africa - Southern Africa - Central Africa	- Large and small rivers - Streams	Very small Non-territorial	Boskop
<i>Brachythemis leucosticta</i> Banded Groundling Gebande Grondwagttertjie	3		- Eastern Africa - Southern Africa	- Dams - Pans - Temporary rain puddles	Small Nomadic	- Richards Bay - Durban - Coffee Bay

<i>Tramea limbata</i> Ferruginous Glider Enkelbandswewer	7		• Middle East • Asia • Madagascar • Mauritius • Other Indian Ocean Islands • Pan-Africa	• Fresh and brackish waters that are in open unshaded shallow pools, ponds and lagoons, and in grassy marshes • Large lakes with submerged vegetation	Medium Full migrant	• Durban
<i>Palpopleura jucunda</i> Yellow-veined Widow Geelaarweetjie	14		• Tropical Africa • Central Africa • North-eastern Africa • Eastern Africa • Southern Africa	• Swampy habitats • Streams • Rivers • Lakes • Marshes	Very small Non-territorial	• Potch Dam Rivier
<i>Orthetrum chrysostigma</i> Epaulet Skimmer Epouletskepper	4		• Southern Europe • Middle East • Canary Islands • Pan-Africa	• Open water with sandy beaches or rocky verges • Standing and/or slow-flowing fresh and brackish waters	Medium sized Territorial	• Riversdale • Graaf Reinet • Montague • Prozesky

<i>Othetrum caffrum</i> Two-striped skimmer Strepieskepper	1		• Pan-Africa	• Ponds • Pans • Slow flowing rivers • Swamps • Marshes • Permanent and temporary pools • Small dams and springs	Medium sized Territorial	• Nottingham • WFS 5 • Wilderness • Potch Dam Agter • New Castle • Knysna • Mooi Rivier 1 • Potch Dam Rivier
<i>Trithemis annulata</i> Violet Dropwing Purpervalvlerkie	9		• Southern Europe • Middle East • Western Asia • Indian Ocean Islands • Pan-Africa	• Lakes • Dams • Sluggish rivers • Marshes • Still-water ponds • Brackish water habitats	Small Nomadic	• Mooi Brug • Boskop • Rustenburg • Potch Dam Agter • Mooi Rivier 1 • Prozesky
<i>Nesciothemis farinosa</i> Eastern Blacktail Swartstertskepper	2		• Middle East • Pan-Africa	• Reed-, sedge- and grass-lined margins of still and running water • Tropical moist scrubland • Intermittent rivers • Swamps • Freshwater lakes • Intermittent freshwater lakes	Medium Territorial	• Richards Bay • Graaff Reinet • Wilderness

				• Freshwater marshes • Intermittent freshwater marshes		
<i>Trithemis furva</i> Navy Dropwing Blouvalvlerkie	8		• Pan-Africa	• Rivers • Streams • Subtropical or tropical moist scrubland • Freshwater marshes • Intermittent freshwater marshes	Small Territorial	• Mooi Brug • Potch Dam Agter • Mooi Rivier 1 • Visarend Vreugde • Prozesky
<i>Trithemis arteriosa</i> Red-veined Dropwing Rooinerfie	12		• Mediterranean • Middle East • Pan-Africa	• Rivers • Streams • Ponds • Dams • Intermittent rivers • Freshwater lakes • Intermittent freshwater lakes • Freshwater marshes • Intermittent freshwater marshes • Irrigation canals and ditches	Small Nomadic	• East London • Coffee Bay

<i>Sympetrum fonscolombii</i> Nomad Swerwertjie	5		• Pan-Africa • Europe • Madagascar • Asia	• Permanent and ephemeral shallow water • Well exposed still standing waters • Man-made barrages • Lakes • Tanks • Dams • Ponds • Permanent and seasonal rivers and wadis	Small Full Migrant	• Riversdale • WFS 5 • New Castle
<i>Trithemis kirbyi</i> Orange-winged Dropwing Oranjevlerk-valvlerkie	11		• Pan-Africa • Middle East • Asia • Indian ocean islands	• Pools • Ponds • Swimming pools • Water tanks • Wadis • Brooks • Streams • Rivers	Small Territorial Nomadic	• Rustenburg
<i>Trithemis dorsalis</i> Highland Dropwing Hooglandvalvlerkie	13		• Southern Africa • Eastern Africa • Central Africa	• Streams • Rivers • Pools in bush or savannah.	Small Non-territorial	• East London

<i>Zosterateschna minuscula</i> Friendly Hawker Vrolike Venter	15		- Namibia - South Africa	- Still standing and slow running waters - Open waters - Wetlands - Ponds - Dams - Small mountain streams with pools	Large Territorial	Prozesky
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Table 2.2 Sampling site locations and descriptions.

Location (Site 10)	Capture site description
Rustenburg	Dragonflies were collected in an old man-made farm dam that had about 30 cm depth of water and relative healthy vegetation growing within it. Dragonflies perched on the dam wall and on the overhanging vegetation within the dam.
Coordinates	General site description
25°43'31.0"S 27°17'19.1"E	The area surrounding the sampling site was mostly agricultural farm land with residential areas in between the farms. There was also a mine about 4 km from the sample site.
Sampling date	
8 February 2016	
Species sampled	
<i>Trithemis annulata</i> <i>Trithemis kirbyi</i>	
Aerial view	Ground view
	

Location (Site 12)	Capture site description	
East London	Strong flowing river that had healthy vegetation.	
Coordinates	General site description	
32°57'50"S 27°55'01"E	The area had some residential suburbs surrounding it. The river flows through East London and passes many industrial areas as well that could be sources of pollution.	
Sampling date		
22 February 2016		
Species sampled		
<i>Trithemis arteriosa</i> <i>Trithemis dorsalis</i>		
Aerial view		Ground view
		Main Rd East London, Eastern Cape 

Location (Site 17)	Capture site description	
Ventersdorp	The sampling site was a small temporal natural wetland close to the road. Mostly grassy vegetation.	
Coordinates	General site description	
26°15'35.1"S 26°51'22.4"E	The site is located on a farm with cattle, chicken broiler houses, and some sheep. It is about 6 km out of town and surrounded by more farms.	
Sampling date		
9 February 2016		
Species sampled		
<i>Sympetrum fonscolombii</i>		
Aerial view		Ground view
		

Location (Site 15)	Collection site description	
New Castle	The sampling site was on a guest farm. The freshwater system was a man-made pond with much vegetation and some ducks.	
Coordinates	General site description	
27°43'58.0"S 29°53'32.6"E	The sampling area is just outside Newcastle and has mostly agricultural farm land surrounding it. There is no clear source of pollution. There are however, some heavy industrial activities on the opposite side of town, about 5 km away from the sampling site.	
Sampling date		
10 April 2016		
Species sampled		
<i>Orthetrum caffrum</i> <i>Sympetrum fonscolombii</i> <i>Crocothemis erythraea</i>		
Aerial view		Ground view
		

Location (Site 5)	Collection site description
Durban Botanical Darden	Samples were collected in the Botanical gardens of Durban located in the middle of the city. There was a nearby pond and some man-made fountains.
Coordinates	General site description
29°50'50.3"S 31°00'26.6"E	The Durban Botanical Garden is situated in the heart of Durban city itself. The city is densely populated with many industries. There is always construction underway nearby. Durban Harbour is about 4 km away.
Sampling date	
8 April 2016	
Species sampled	
<i>Brachythemis leucosticta</i> <i>Crocothemis erythraea</i> <i>Tramea limbata</i>	
Aerial view	Ground view
	

Location (Site 1)	Collection site description	
Nottingham	The sampling site was on a guest farm in the middle of Nottingham. Small shrubs and thick stands of grass are on the edges of the dams.	
Coordinates	General site description	
29°18'23.8"S 30°03'38.1"E	The surrounding area is mostly agriculture and tree plantations. Some farms also serve as wedding and function venues. There are no obvious mining or industrial activities near the sampling site.	
Sampling date		
8 April 2016		
Species sampled		
<i>Orthetrum caffrum</i>		
Aerial view		Ground view
		

Location (Site 2)	Collection site description
Richards Bay	Strong flowing river with trees and shrubs.
Coordinates	General site description
28°41'46.6"S 32°00'50.2"E	The river flows through Enseleni Nature Reserve. There are some farms in the surrounding the area. It is situated outside Richards Bay and has very little residential areas close by. Richards bay airport is about 15 km away.
Sampling date	
9 April 2016	
Species sampled	
<i>Nesciothemis farinosa</i> <i>Brachythemis leucosticta</i>	
Aerial view	Ground view
 An aerial satellite image showing a wide, muddy river flowing through a dense forest. A road crosses the river, and a red circle highlights a specific area on the right bank. Coordinates 28°41'47.4"S 32°00'50.2"E are marked on the image.	 A ground-level photograph taken from a bridge or elevated road, looking down at the river. The river is surrounded by lush green trees and vegetation. A concrete structure is visible on the left bank.

Location (Site 16)	Collection site description
Knysna	A small stream that flows from the mountain to the marshlands. It was surrounded by grass and shrubs. The water was clear.
Coordinates	General site description
34°02'40.7"S 23°04'17.6"E	The surrounding area was used for leisure activities and as a result had lots of trash scattered around. Residential and industrial activities were close by.
Sampling date	
11 February 2017	
Species sampled	
<i>Orthetrum caffrum</i>	
Aerial view	Ground view
	

Location (Site 3)	Collection site description	
Riversdale	A relatively big farm dam with other dams in the area not far away. The site had little vegetation within the water body and had long grass and rocky vegetation about 2 meters away from the water's edge.	
Coordinates	General site description	
34°06'23.6"S 21°09'33.6"E	The farm is situated outside of town with no clear mining and industrial activities. The surrounding land is used for agricultural activities.	
Sampling date		
13 February 2017		
Species sampled		
<i>Orthetrum chrysostigma</i>		
Aerial view		Ground view
		

Location (Site 7)	Collection site description
Wilderness	The sampling site covered one dam and two wetland type water bodies. All sites had a healthy vegetation growth.
Coordinates	General site description
33°57'43.3"S 22°34'42.4"E	The sampling sites was located deep in the mountains on a farm called Perdepoort Horse Trails. The area surrounding the farm is mostly used for either agricultural activities or residential purposed.
Sampling date	
12 February 2017	
Species sampled	
<i>Orthetrum caffrum</i> <i>Nesciothemis farinose</i>	

Aerial view



Ground view



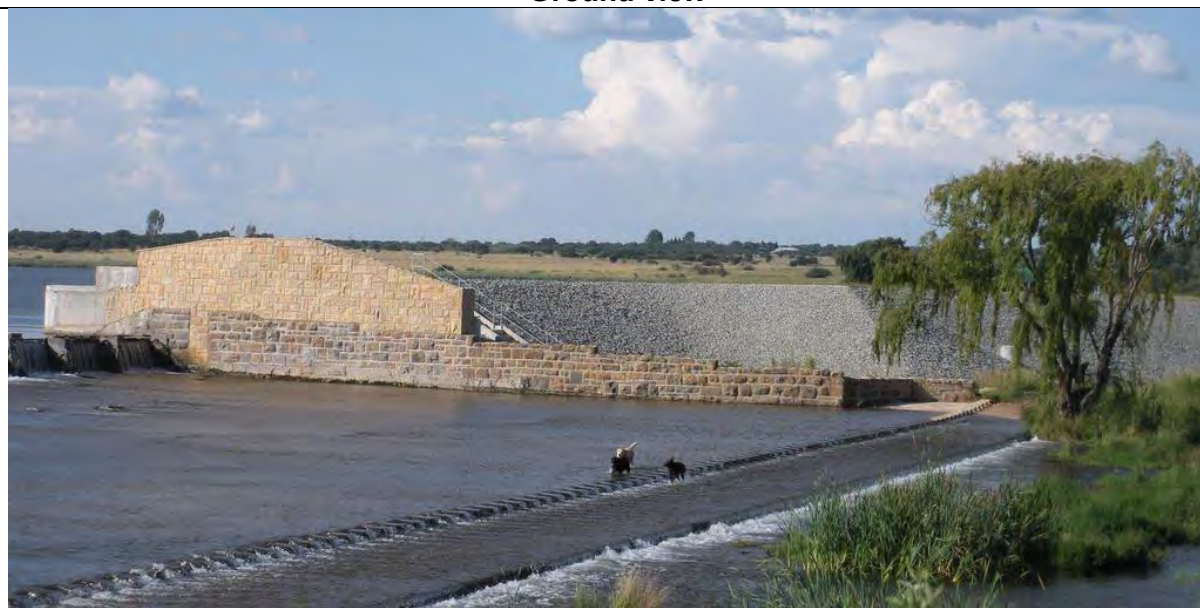
Location (Site 11)	Collection site description	
Montague	A slow flowing stream with an abundance of green grass and small bushes growing along it. There was some pollution with plastic and trash.	
Coordinates	General site description	
33°47'31.5"S 20°07'11.4"E	The stream flows through a small town and has residential houses on both sides of the stream. Cattle grazed along the stream. The stream flows underneath bridges and along the road. The town is located between vast mountain ranches.	
Sampling date		
15 February 2017		
Species sampled		
<i>Orthetrum chrysostigma</i>		
Aerial view		Ground view
		

Location (Site 19)	Collection site description
Potch Dam River	The sampling site is located at the overflow of the dam into Mooi River. The vegetation growth is healthy and abundant with plenty of other invertebrates, and birds nesting and feeding.
Coordinates	General site description
26°40'26.4"S 27°05'45.2"E	The dam is intensively used for recreational activities. Surrounding the sampling site is residential areas and some construction on the one side of the dam. Mining activities occurs within 20 km of the sampling site. There are some industrial activities upstream.
Sampling date	
10-12 March 2017	
Species sampled	
<i>Orthetrum cafferum</i> <i>Palpopleura jucunda</i>	

Aerial view



Ground view



Location (Site 14)	Collection site description	
Potch Dam Agter	Little vegetated except with grass. The immediate sampling site is mostly used for camping and fishing.	
Coordinates	General site description	
26°39'45.2"S 27°05'22.2"E	The dam is intensively used for recreational activities. Surrounding the sampling site is residential areas and some construction on the one side of the dam. Mining activities occurs within 20 km of the sampling site. There are some industrial activities upstream.	
Sampling date		
11-12 March 2017		
Species sampled		
<i>Orthetrum caffrum</i> <i>Trithemis annulata</i> <i>Brachythemis lacustris</i>		
Aerial view		Ground view
		

Location (Site 6)	Collection site description
WFS 5	Well-vegetated dam with a high diversity of species. Water is clean and clear. There was a variety of water plants growing along the edges.
Coordinates	General site description
26°21'51.2"S 27°16'25.1"E	The site is close to a small residential area and is a popular bird watching spot. It is located near mines and part of the Wonderfontein Spruit system.
Sampling date	
10 May 2016	
Species sampled	
<i>Orthetrum caffrum</i> <i>Crocothemis erythraea</i>	

Aerial view



Ground view



Location (Site 18)	Collection site description
Mooi River 1	Well-vegetated area with an abundance of bird species.
Coordinates	General site description
26°20'14.1"S 27°08'28.4"E	The river flows through agricultural land and has well-vegetated shores. Mining activities are present In the area.
Sampling date	
24 November 2016	
Species sampled	
Orthetrum caffrum Trithemis furva Trithemis annulata	
Aerial view	Ground view
	

Location (Site 9)	Collection site description	
Boskop	The site had grassy vegetation and is a camping site.	
Coordinates	General site description	
26°33'34.0"S 27°07'19.4"E	Boskop Dam is a dam located to the north of Potchefstroom. The dam's water sources consists of a number of small rivers (Mooi River and Wonderfontein Spruit) and streams. These rivers and streams flow through agricultural land and mining activities.	
Sampling date		
10 May 2016		
Species sampled		
<i>Trithemis annulata</i> <i>Brachythemis lacustris</i>		
Aerial view		Ground view
		

Location (Site 20)	Collection site description	
Visarend Vreugde	Visarend Vreugde is a camping site. It had trees overhanging the river and some bare ground.	
Coordinates	General site description	
26°53'33.0"S 27°16'06.5"E	The site is located on the shores of the Vaal River. Upstream from the river are a number of industrial activities, boating and possible mining. It has a mild current year long.	
Sampling date		
14 April 2017		
Species sampled		
<i>Trithemis furva</i>		
Aerial view		Ground view
		

Location (Site 8)	Collection site description
Mooi Brug	Well-vegetated, with clear water with rocks on the bottom. The vegetation varies from grasses to aquatic plants.
Coordinates	General site description
26°25'19.7"S 27°07'03.2"E	The surrounding area is used for agricultural activities with little to no residential areas except for some farm houses. Mines are located about 15 km away and no clear industrial activities were present.
Sampling date	
10 April 2017	
Species sampled	
<i>Trithemis furva</i> <i>Trithemis annulata</i>	

Aerial view



Ground view



Location (Site 21)	Collection site description	
Prozesky	Well-vegetated, with clear water, located in a wetland.	
Coordinates	General site description	
26°45'27.4"S 27°05'53.1"E	The surrounding area has a residential area nearby and some agricultural farms. There is also a waste water treatment plant located next to the wetland. An old mine is located 4 km away.	
Sampling date		
10 April 2017		
Species sampled		
<i>Orthetrum chrysostigma</i> <i>Trithemis furva</i> <i>Trithemis annulata</i> <i>Zosteraeschna minuscula</i>		
Aerial view		Ground view
		

Location (Site 13)	Collection site description
Coffee Bay	The sample site is a natural free flowing stream that flows underneath a bridge. There were some plastic waste and other trash scattered around. Construction work on the road was underway during the sampling period.
Coordinates	General site description
31°58'38.9"S 29°08'53.8"E	The surrounding area is relatively open. The residence of the area lives in small huts. There were no large towns or cities nearby, nor any mines.
Sampling date	
23 February 2016	
Species sampled	
<i>Brachythemis leucosticta</i> <i>Trithemis arteriosa</i>	
Aerial view	Ground view
	No Photograph available

Location (Site 4)	Collection site description
Graaff Reinet	The site was along the Sundays river that flows through Graaff Reinet. The area itself is used for leisure activities such as fishing and picnics. It has thick tree and shrub vegetation with water plants growing in the shallow parts of the river.
Coordinates	General site description
32°14'45.1"S 24°31'31.6"E	On the opposite side of the sampling site was a residential suburb. On the side where sampling took place were foothills. The area was quite polluted with plastic wrappers and trash.
Sampling date	
20 February 2016	
Species sampled	
<i>Nesciothemis farinosa</i>	
<i>Orthetrum chrysostigma</i>	
<i>Sympetrum fonscolombii</i>	
Aerial view	Ground view
	

2.4 Sample preparation and analyses

The samples were thawed at room temperature and the wings carefully removed with a small pair of scissors. The wings will be used in a later project. The dragonflies were placed individually in falcon tubes and left over night at -86°C before being freeze dried for 24 hours. The samples were then kept in dark dry storage. Dragonflies were freeze dried, weighed, and ground to a fine powder. The whole sample (approximately 80 mg) was placed in a Teflon tube. A volume of 7 ml nitric acid (HNO_3) and 1 ml hydrogen peroxide (H_2O_2) was added to the tube, capped, and placed in a high-performance microwave digestion system (Milestone, Ethos UP, Maxi 44). Twenty minutes was allowed for the system to reach 1800 MW at a temperature of 200°C , which was maintained for 15 minutes. After cooling, the sample was brought up to a final volume of 10 ml and analysed on an ICP-MS (Agilent 7500 series), following EPA 3051A. A certified reference standard (CRM 477 mussel tissue) was used for quality control. All certified metals were quantified within 20% of the certified concentrations. Concentrations are expressed as mg/kg dry mass (dm). Agilent supplied estimated detection limits are 0.01 for antimony, arsenic, barium, bismuth, cadmium, cobalt, gold, lead, manganese, mercury, molybdenum, palladium, platinum, rubidium, silver, strontium, thallium, thorium, and uranium; 0.2 for copper, titanium, vanadium, and zinc; 0.3 for nickel; 0.4 for magnesium; 0.5 for sodium; 0.7 for aluminium; 1 for chromium and selenium; 10 for iron; and 50 for calcium.

2.5 Data analyses

Statistical analyses were conducted with Graphpad Prism 5. Data below detection limits were not used. Column statistics tested for normality for both untransformed and log-transformed data. Where untransformed data were not normally distributed (using the Shapiro-Wilk normality test, $p < 0.05$ if not normally distributed), log-transformed data were used in statistical comparisons. Unmatched, one-way ANOVA was used to compare elemental concentrations between sites, species, and habitat types. Statistical significance was set at $p < 0.05$. Tukey's multiple comparison was used to compare each mean with every other mean. The results are displayed in table form, and graphically as scatterplots with means and standard deviations indicated.

I also compared the different size classes. Because only small ($n = 32$) and medium ($n = 42$) size classes had enough data, t-tests were performed. Normality was tested using the D'Agostino & Pearson normality test ($p < 0.05$ if not normally distributed). Where data were not normally distributed, log-transformed data were used. I used unpaired, two-tailed t-tests. Statistical significance was set at $p < 0.05$.

Nonmetric multivariate statistics (NMS) were conducted to ordinate the metal concentrations for various groupings of samples: sites, size classes, habitat types, and species. I used MjM Software PC-ORD version 6. The data for was relativized by PC-ORD in order to present a proportional profile of metallic elements. Environmental variables used were inverse distances between collection sites and mines, industry, residential areas, and farms. If no potential source of a specific type was located within 100 km, a maximum of 100 km was entered. Inverse distances were used to investigate associations between potential pollution sites and metallic element concentrations. A concentration vector and a source distance factor would therefore ordinate close together if there was an association, instead of opposite vectoring. Six axes were allowed. Gower ignore 0, was used as relative measurement of distance. The maximum number of iterations allowed was 500. 250 runs of real data were used with random starting configurations. Monte Carlo tests were then performed also using 250 runs, with randomised data. Only two dimensions were needed for a successful ordination, with a stress of 13.607, and a final instability of 0.00000, reached after 70 iterations. Convex hulls were used on the same ordination to investigate species, size classes, and habitat types.

Chapter 3: Results

“One way to open your eyes is to ask yourself, “What if I had never seen this before? What if I knew I would never see it again?””

– Rachel Carson

3.1 Tables

Tables indicating the mean concentrations of selected metallic elements regarding species, body size, habitat type, and sampling site.

Table 3.1 Scientific names and species number of all species used in this study.

Species number	Scientific name	Species number	Scientific name
1	<i>Orthetrum cafferum</i>	8	<i>Trithemis furva</i>
2	<i>Nesciothemis farinosa</i>	9	<i>Trithemis annulata</i>
3	<i>Brachythemis leucosticta</i>	10	<i>Brachythemis lacustris</i>
4	<i>Orthetrum chrysostigma</i>	11	<i>Trithemis kirbyi</i>
5	<i>Sympetrum fonscolombii</i>	12	<i>Trithemis arteriosa</i>
6	<i>Crocothemis erythraea</i>	13	<i>Trithemis dorsalis</i>
7	<i>Tamea limbata</i>	14	<i>Palpopleura jucunda</i>
		15	<i>Zosterateschna minuscula</i>

Table 3.2 The 21 site numbers and names associated.

Site number	Site name	Site number	Site name
1	Nottingham	12	East London
2	Richards Bay	13	Coffee Bay
3	Riversdale	14	Potch Dam Agter
4	Graaff Reinet	15	New Castle
5	Durban	16	Knysna
6	WFS 5	17	Venetersdorp
7	Wilderness	18	Mooi Rivier 1
8	Mooi Brug	19	Potch Dam Rivier
9	Boskop	20	Visarend Vreugde
10	Rustenburg	21	Prozesky
11	Montague		

Table 3.3 The mean concentrations (mg/kg dm) of selected metallic elements regarding species.

Species		<i>Orthetrum caffrum</i>	<i>Nesciothemis farinosa</i>	<i>Brachythemis leucosticta</i>	<i>Orthetrum chrysostigma</i>	<i>Sympetrum fonscolombii</i>	<i>Crocothemis erythraea</i>	<i>Trithemis furva</i>	<i>Trithemis annulata</i>	<i>Trithemis kirbyi</i>	<i>Trithemis arteriosa</i>
Species number		1	2	3	4	5	6	8	9	11	12
Element		Mean									
Hg	Untransformed	0.07	0.02	0.03	0.06	0.1	0.04	4.9	0.3	0	0
	Log-transformed	-1.1	-1.8	-1.5	-1.5	-1.4	-1.5	-0.6	-0.5	0	0
Pb	Untransformed	0.9	0.4	0.4	0.2	1.6	0.9	1.3	0.8	1.1	0.8
	Log-transformed	-0.03	-0.5	-0.5	-0.7	0.1	-0.3	0.02	-0.2	-0.02	-0.1
Cd	Untransformed	0.08	0.05	0.2	0.05	0.07	0.1	0.04	0.03	0.03	0.06
	Log-transformed	-1.1	-1.5	-1.1	-1.4	-1.2	-1.1	-1.5	-1.6	-1.6	-1.3
As	Untransformed	0.08	0.09	0.08	0.08	0.2	0.06	0.04	0.02	0.005	0.01
	Log-transformed	-1.1	-1.1	-1.2	-1.2	-0.8	-1.3	-1.5	-1.6	-2.4	-2.2
Cr	Untransformed	2	1.4	1.6	1.2	3.5	1.8	2.8	2.8	5.4	2.6
	Log-transformed	0.3	0.1	0.2	0.08	0.5	0.2	0.4	0.4	0.7	0.4
Cu	Untransformed	37	26	28	27	34	24	38	31	28	30
	Log-transformed	1.6	1.4	1.4	1.4	1.2	1.4	1.6	1.5	1.4	1.5
Ni	Untransformed	0.2	0.3	0.4	0.2	0.4	0.2	0.4	0.7	0.7	0.6
	Log-transformed	-0.6	-0.5	-0.5	-0.7	-0.4	-0.7	-0.4	-0.4	-0.1	-0.3
Se	Untransformed	1.1	1.1	0.9	1.4	1.5	1.4	1	0.7	0.9	0.6
	Log-transformed	0.03	-0.1	-0.2	0.1	0.09	0.09	-0.01	-0.2	-0.09	-0.2
Al	Untransformed	27	25	33	25	62	24	49	42	46	56
	Log-transformed	1.4	1.3	1.4	1.3	1.7	1.3	1.7	1.6	1.6	1.7
Au	Untransformed	2	0.1	0.06	0.9	0.04	0.02	32	1.7	0	0
	Log-transformed	-1.8	-0.9	-1.3	-1.5	-1.7	-1.7	-1	-1.2	0	0

Table 3.4 The mean concentrations (mg/kg dm) of selected metallic elements regarding sites.

Site name		Nottingham	Richards Bay	Riversdale	Graaff Reinet	Durban	WFS 5	Wilderness	Mooi Brug	Boskop	Rustenburg	Montague	East London	Coffee Bay	Potch Dam Agter	New Castle	Knysna	Ventersdorp	Mooi Rivier 1	Potch Dam Rivier	Visarend Vreugde	Prozesky
Site number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Element		Mean																				
Hg	Untransformed	0	0	0.01	0	0.03	0.01	0.0006	0	0.08	0	0.04	0	0.006	0	0.1	0.2	0.06	0.006	0.02	2.7	1.3
	Log-transformed	-1.4	0	-1.6	0	-1.4	-1.3	-1.8	0	-0.6	0	-1.8	0	-1.5	0	-1	-0.8	-1.7	-2	-1.4	1.1	-0.6
Pb	Untransformed	0.2	0.2	0.2	0.3	0.6	0.4	0.3	0.7	0.5	1	0.3	0.7	0.5	2	1.8	6.9	2.3	1.9	1.8	0.7	0.5
	Log-transformed	-0.7	-0.6	-0.8	-0.6	-0.2	-0.5	-0.5	-0.2	-0.3	-0.07	-0.6	-0.3	-0.4	0.3	0.2	0.7	0.3	0.2	0.2	-0.2	-0.3
Cd	Untransformed	0.05	0.005	0.04	0.05	0.4	0.03	0.05	0.01	0.02	0.03	0.05	0.04	0.1	0.06	0.08	0.2	0.05	0.05	0.06	0.04	0.06
	Log-transformed	-1.3	-2.1	-1.5	-1.3	-0.5	-1.6	-1.4	-1.9	-1.8	-1.6	-1.3	-1.5	-0.1	-1.3	-1.2	-0.8	-1.3	-1.3	-1.3	-1.4	-1.3
As	Untransformed	0.0002	0	0.04	0.03	0.1	0.05	0.1	0.01	0.01	0.005	0.1	0.02	0.06	0.08	0.1	0.2	0.2	0.06	0.07	0.03	0.08
	Log-transformed	-1.9	-1.6	-1.4	-1.3	-1	-1.6	-1	-1.9	-1.8	-2.2	-0.9	-2	-1.4	-1.1	-1	-0.8	-0.8	-1.3	-1.2	-1.6	-1.2
Cr	Untransformed	0.8	1.3	1.1	2.5	1.4	1.6	1.3	1.8	2.3	5.5	1	2.3	2.1	3	2.8	2.9	3.6	3.2	2.6	2.6	2.3
	Log-transformed	-0.1	0.1	0.04	0.3	0.1	0.2	0.1	0.3	0.4	0.7	0.02	0.4	0.3	0.5	0.4	0.4	0.5	0.5	0.4	0.4	0.3
Cu	Untransformed	22	23	21	29	28	23	32	29	20	26	22	27	29	42	33	92	37	43	43	37	46
	Log-transformed	1.3	1.4	1.3	0.4	1.4	1.4	1.5	1.5	1.3	1.4	1.3	1.4	1.4	1.6	1.5	1.9	1.6	1.6	1.6	1.6	1.6
Ni	Untransformed	0.4	0.3	0.2	0.4	0.3	0.2	0.3	0.3	0.3	1.4	0.2	0.4	0.5	0.4	0.3	0.2	0.4	0.3	0.3	0.4	0.4
	Log-transformed	-0.4	-0.5	-0.6	-0.5	-0.5	-0.6	-0.6	-0.5	-0.5	0.01	-0.8	-0.4	-0.4	-0.5	-0.6	-0.9	-0.4	-0.5	-0.6	-0.4	-0.4
Se	Untransformed	0.9	0.2	1.4	0.6	1.1	1.2	1.3	0.7	0.8	0.8	1.5	0.6	0.9	0.8	1.5	1.4	2.1	0.9	1.6	1.4	1.1
	Log-transformed	-0.05	-0.6	0.1	-0.2	0.02	0.1	0.2	-0.1	-0.1	-0.1	0.2	-0.2	-0.07	-0.1	0.1	0.1	0.3	-0.06	0.1	0.2	0.02
Al	Untransformed	23	13	23	18	24	24	34	33	31	46	21	40	67	49	32	38	88	46	45	57	59
	Log-transformed	1.4	1.1	1.3	1.2	1.4	1.3	1.5	1.5	1.5	1.6	1.3	1.5	1.8	1.6	1.5	1.5	1.9	1.6	1.6	1.7	1.7
Au	Untransformed	0.09	0.08	0.02	0.0007	0.04	0	0	0	0.01	0	0	0	0	0.04	0.05	0.01	0.02	0.02	0.01	0.8	82.8
	Log-transformed	-1	-1	-2	-2.5	-1.5	0	0	0	-1.4	0	0	0	0	-1.4	-1.6	-1.9	-1.9	-1.9	-2	-1.2	0.9

Table 3.5 The mean concentrations (mg/kg dm) of selected metallic elements regarding habitat type.

Habitat type		Wetlands	Rivers	Dams
Element		Mean		
Hg	Untransformed	0.6	1	0.1
	Log-transformed	-1.1	-1.2	-1.2
Pb	Untransformed	1	1.3	1
	Log-transformed	-0.1	-0.2	-0.2
Cd	Untransformed	0.1	0.1	0.05
	Log-transformed	-1.2	-0.4	-1.4
As	Untransformed	0.07	0.07	0.1
	Log-transformed	-1.3	-1.4	-1.2
Cr	Untransformed	3.1	2.2	2
	Log-transformed	0.4	0.3	0.2
Cu	Untransformed	35	35	32
	Log-transformed	1.5	1.5	1.5
Ni	Untransformed	0.6	0.3	0.3
	Log-transformed	-0.3	-0.5	-0.6
Se	Untransformed	1.3	1	1.2
	Log-transformed	0.04	-0.08	0.05
Al	Untransformed	55	36	35
	Log-transformed	1.7	1.5	1.5
Au	Untransformed	31	0.2	0.04
	Log-transformed	-0.7	-1.6	-1.6

Table 3.6 The mean concentrations (mg/kg dm) of selected metallic elements regarding body size.

Size category		Small	Medium
Element		Mean	
Al	Untransformed	45	31
	Log-transformed	1.6	1.4
As	Untransformed	0.1	0.1
	Log-transformed	-1.5	-1.2
Au	Untransformed	11.6	0.3
	Log-transformed	-1.3	-1.5
Cr	Untransformed	2.9	1.6
	Log-transformed	0.4	0.2
Cu	Untransformed	32.2	35.9
	Log-transformed	1.5	1.5
Cd	Untransformed	0.1	0.1
	Log-transformed	-1.4	-1.3
Hg	Untransformed	1.4	0.1
	Log-transformed	-1.1	-1.4
Ni	Untransformed	0.5	0.3
	Log-transformed	-0.4	-0.6
Pb	Untransformed	1.1	1.2
	Log-transformed	-0.1	-0.3
Se	Untransformed	1	1.2
	Log-transformed	-0.06	0.04

Recoveries of certified elements were within 20% of CRM certified values. The sites and species were given a number to aid in map simplification and map reading. The numbers and their respective site name and species name can be seen in Tables 3.1 and 3.2. Means of a selection of elements, per variable, are shown in Tables 3.3-3.6. Notably high mean concentrations of 4.9 mg/kg dm of Hg and 32 mg/kg dm of Au were found in *Trithemis annulata* (Table 3.3). The mean concentrations of all elements showed statistically significant differences ($p < 0.05$) between sites, except for Hg (Table 3.4). The mean concentration of 31 mg/kg dm of Au was notably higher in the wetland habitat type (Table 3.5). Pb had a 17.8 mg/kg dm maximum concentration, and Cu a 152 mg/kg dm maximum concentration in the river habitat type, which was slightly higher than the maximum concentration found at the other two habitat types. However, only the mean concentrations of Al, Cr, Ni, and Au were significantly higher in the wetland habitat type compared with the other two. Elements such as Au and Hg had a notably higher maximum concentration in the small size category while Pb and Cu had higher maximum concentrations in the medium size category (Table 3.6). There were significant differences between size categories for Al, Cr, Ni, As, and Pb.

3.2 Site results

Scatterplots and distribution maps of all metallic elements that have been analysed.

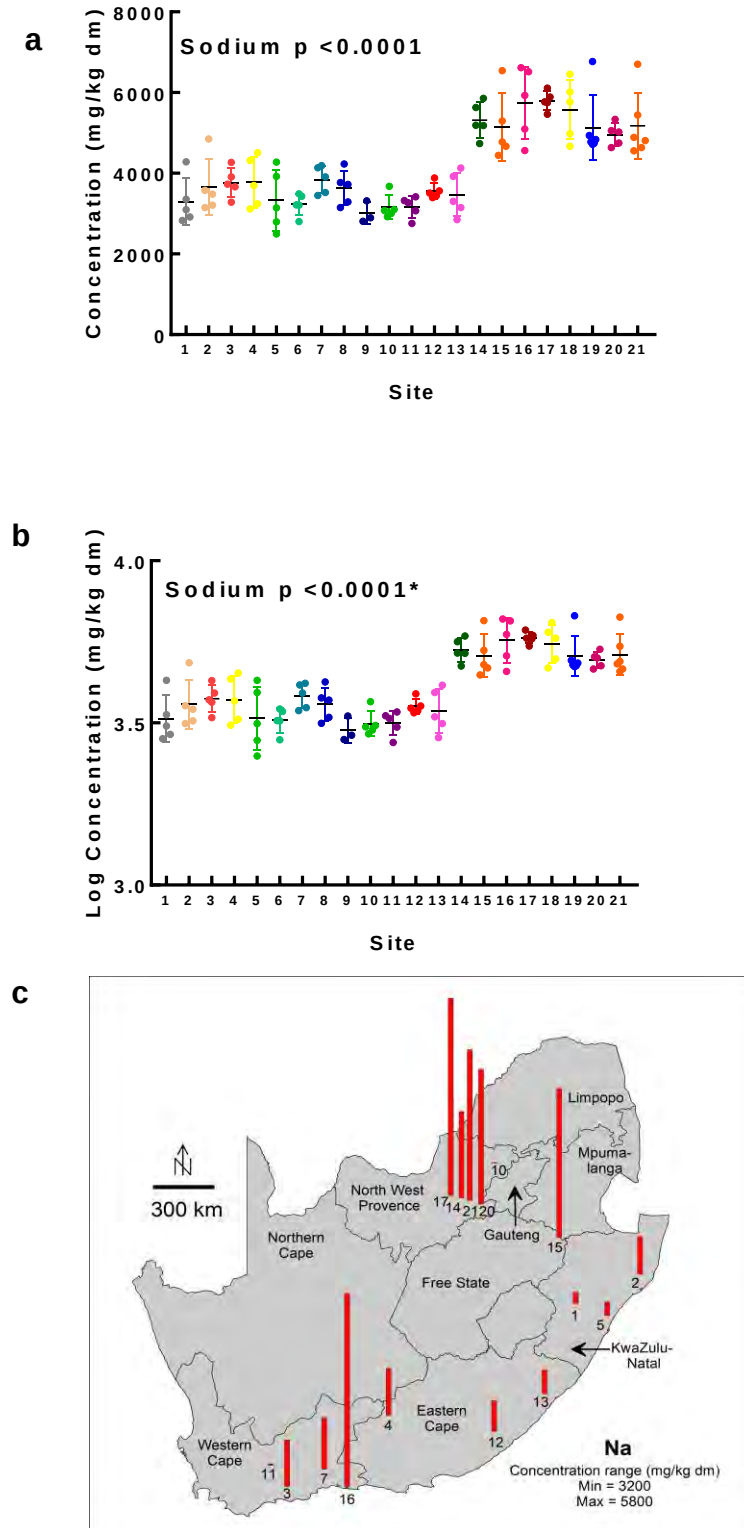


Figure 3.1 Distribution of sodium according to sites.

Figure 3.1a Scatterplot of untransformed sodium data according to sites. The means and standard deviations are indicated. The untransformed concentrations were not distributed normally.

Since the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.1b Scatterplot of log-transformed sodium data according to sites. The means and standard divisions are indicated. All concentrations were distributed normally except for Site 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$) between sites. Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 14, 15, 16, 17, 18, 19, 20, 21; 2 vs. 14, 15, 16, 17, 18, 19, 20, 21; 3 vs. 14, 15, 16, 17, 18, 19, 21; 4 vs. 14, 15, 16, 17, 18, 19, 21; 5 vs. 14, 15, 16, 17, 18, 19, 20, 21; 6 vs. 14, 15, 16, 17, 18, 19, 20, 21; 7 vs. 14, 16, 17, 18, 21; 8 vs. 14, 15, 16, 17, 18, 19, 20, 21; 9 vs. 14, 15, 16, 17, 18, 19, 20, 21; 10 vs. 14, 15, 16, 17, 18, 19, 20, 21; 11 vs. 14, 15, 16, 17, 18, 19, 20, 21; 12 vs. 14, 15, 16, 17, 18, 19, 20, 21 and 13 vs. 14, 15, 16, 17, 18, 19, 20, 21.

Sites 14-21 had on average higher mean concentrations.

Figure 3.1c Map of mean sodium concentrations.

Sites in the North West Provence all had high mean concentrations, except for Site 10 that were noticeably lower. Sites along the coastline all had lower concentrations than the sites in the centre, except for Site 16 that were noticeably higher.

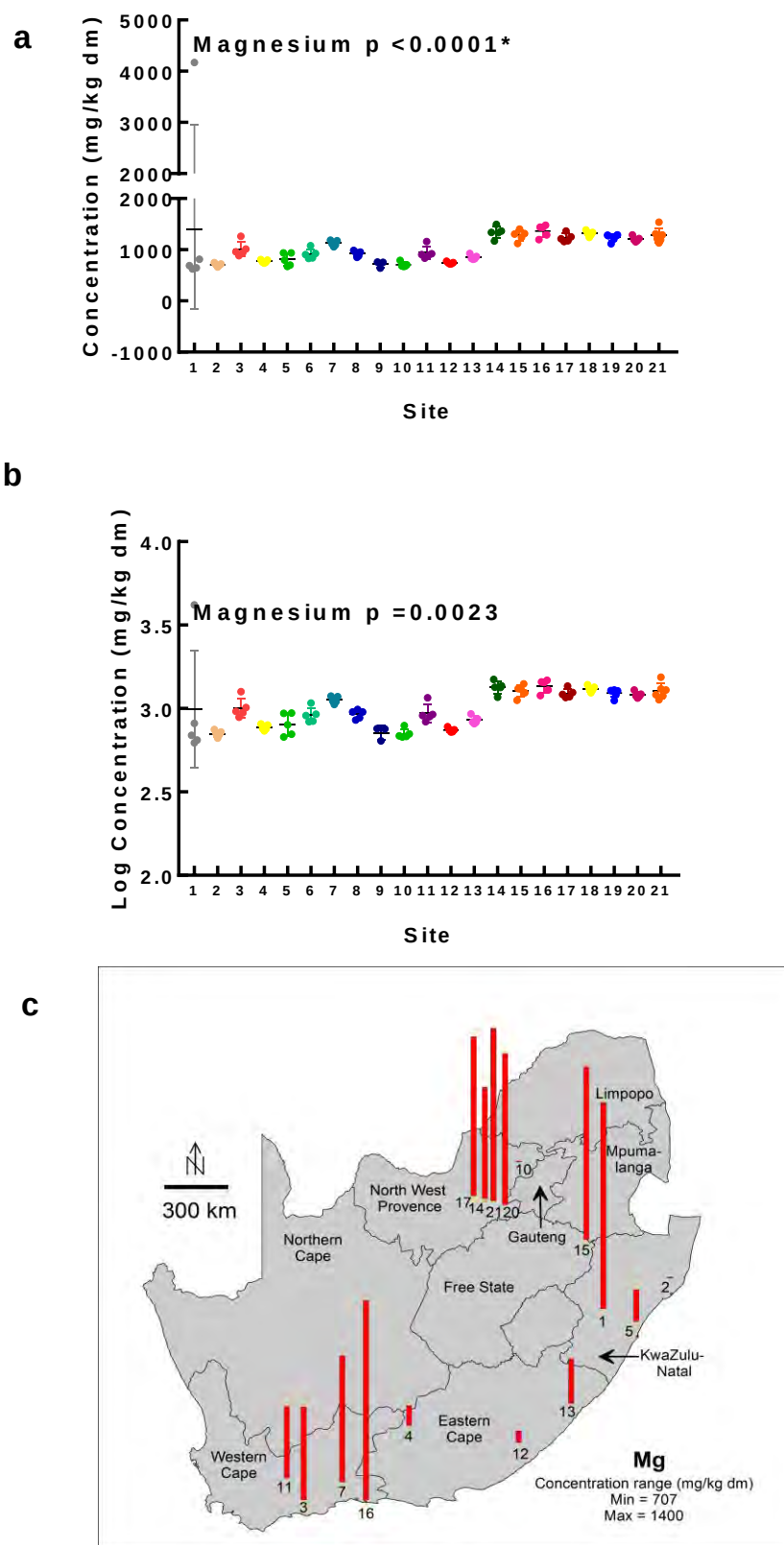


Figure 3.2 Distribution of magnesium according to sites.

Figure 3.2a Scatterplot of untransformed magnesium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1, 2, 5 and 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons showed no differences ($p > 0.05$).

Figure 3.2b Scatterplot of log-transformed magnesium data according to sites. The means and standard deviations are indicated.

Since the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.2c Map of mean magnesium concentrations.

All sites except 2, 4, 5, 10, 12 and 13 had relatively high mean concentrations. Notably sites 2 and 10 were especially low compared to the other sites. Site 1 had the highest mean concentration.

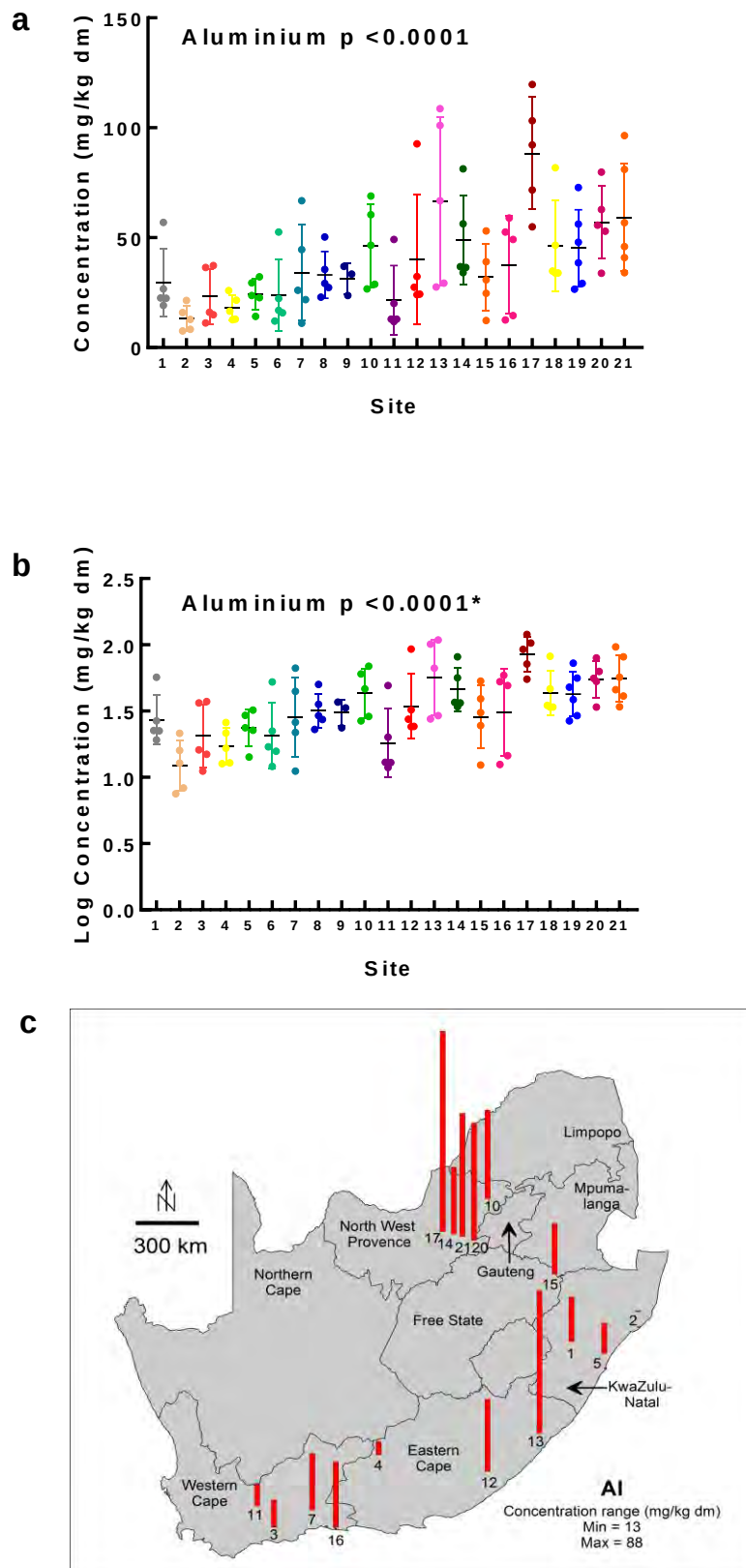


Figure 3.3 Distribution of aluminium according to sites.

Figure 3.3a Scatterplot of untransformed aluminium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.3b Scatterplot of log-transformed aluminium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 12 and 18. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs 10, 17; 2 vs 10, 13, 14, 17, 18, 19, 20, 21; 3 vs 17; 4 vs 13; 4 vs 17, 20, 21; 5 vs 17; 6 vs 17 and 11 vs 13, 17, 21.

Figure 3.3c Map of mean aluminium concentrations.

All sites had measurable mean concentrations of Al. Sites clustered around Potchefstroom all had high mean aluminium concentrations. Sites closer to the coastal areas had slightly lower mean concentrations, with the exception of Site 13.

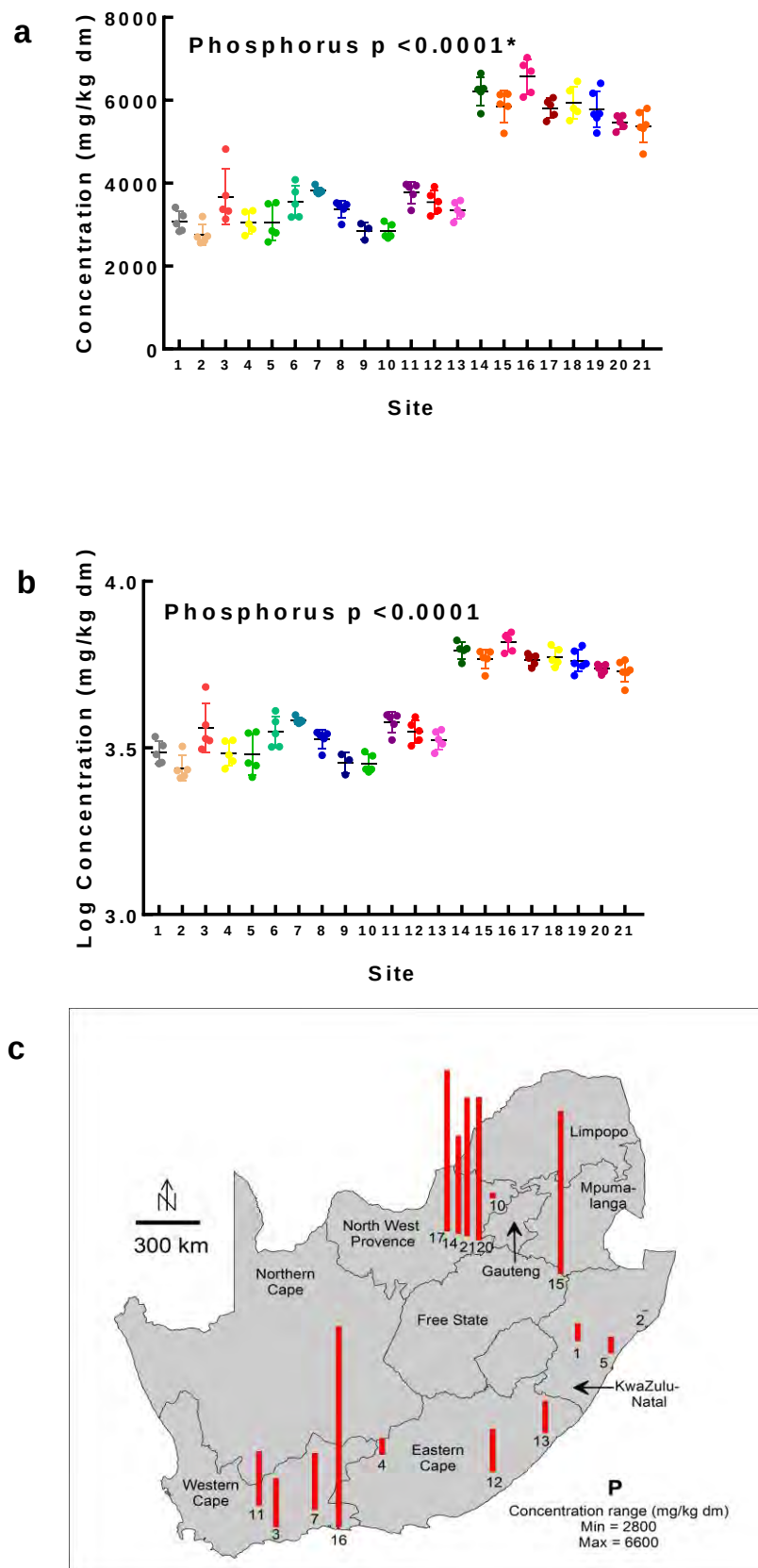


Figure 3.4 Distribution of phosphorus according to sites.

Figure 3.4a Scatterplot of untransformed phosphorus data according to sites. The means and standard deviations are indicated.

All distributions were normal. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 14,15,16,17,18,19,20, 21; 2 vs. 3, 6, 7, 11, 12, 14, 15, 16, 17, 18, 19, 20; 3 vs. 14, 15,16, 17, 18, 19, 20, 21; 3 vs. 10; 4 vs. 14, 15, 16, 17, 18, 19, 20, 21; 5 vs. 14, 15, 16, 17, 18, 19, 20, 21; 6 vs. 14, 15, 16, 17, 18, 19, 20, 21; 7 vs. 9, 10; 7, 14, 15, 16, 17, 18, 19, 20, 21; 8 vs. 14, 15, 16, 17, 18, 19, 20, 21; 9 vs. 11, 14, 10 vs. 15, 16, 17, 18, 19, 20, 21; 9 vs. 15, 16, 17, 18, 19, 20, 21; 10 vs. 11; 11 vs. 14, 15, 16, 17, 18, 19, 20, 21, 12 vs. 14, 15, 16, 17, 18, 19, 20, 21; 13 vs. 13, 14, 15, 16, 17, 18, 19, 20, 21; 14 vs. 21 and 16 vs. 19, 20, 21. Sites 14-21 on average had higher mean concentrations compared to sites 1-13.

Figure 3.4b Scatterplot of log-transformed phosphorus data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.4c Map of mean phosphorus concentrations.

Sites clustered around Potchefstroom -14, 15, 16, 17, 20, and 21 - were relatively higher than other sites. Sites that had notably lower mean concentrations were all three from KwaZulu-Natal. Other low concentrations include sites 4 and 10.

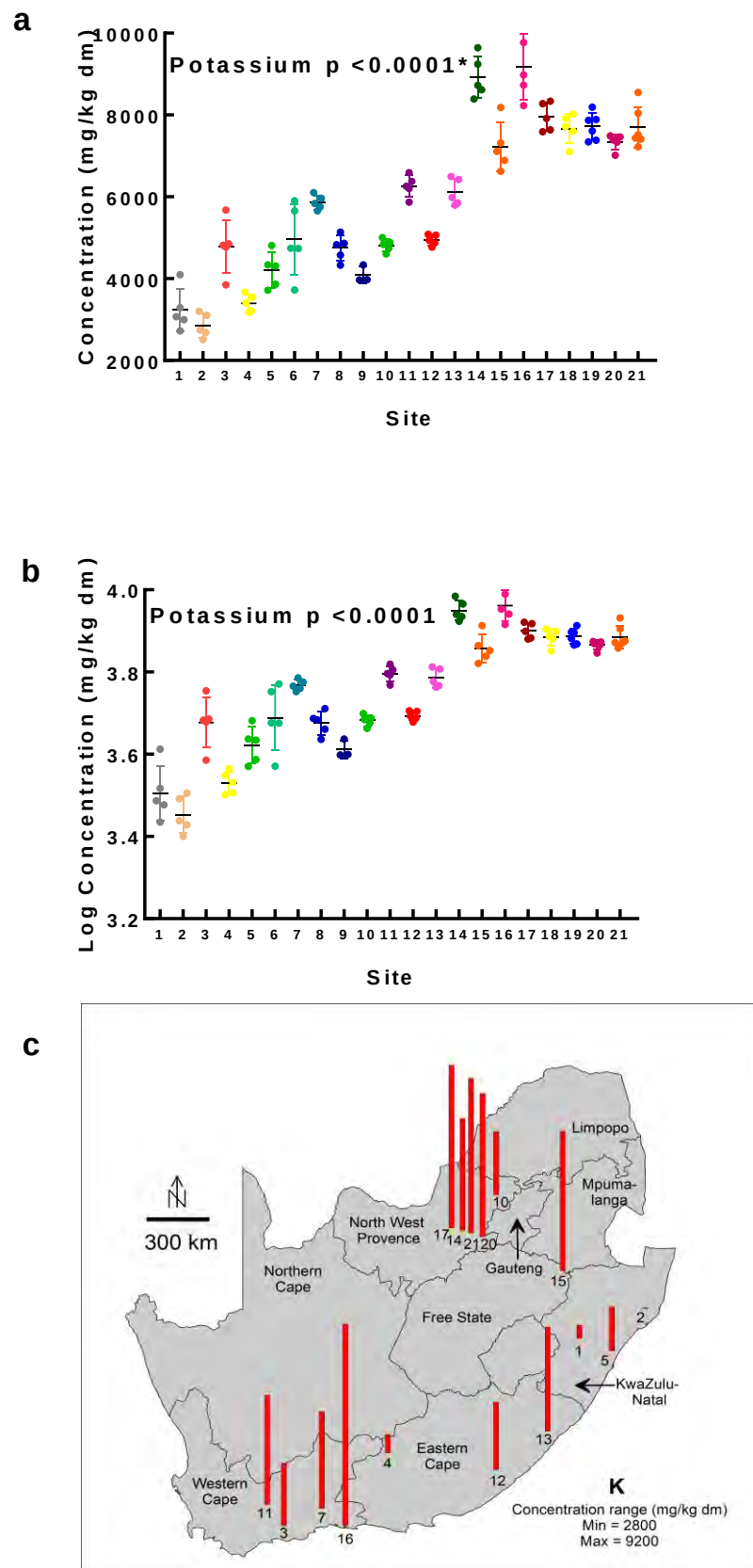


Figure 3.5 Distribution of potassium according to sites.

Figure 3.5a Scatterplot of untransformed potassium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 6 vs. 7, 8, 9, 10, 12; 7 vs. 11, 12, 13; 8 vs. 9, 10, 12; 9 vs. 10, 12; 10 vs. 12; 11 vs. 13, 15; 14 vs. 16, 17; 15 vs. 17, 18, 19, 20, 21; 17 vs. 18, 19, 20, 21; 18 vs. 19, 20, 21; 19 vs. 20, 21 and 20 vs. 21.

Figure 3.5b Scatterplot of log-transformed potassium data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.5c Map of mean potassium concentrations.

All sites had relatively high mean concentrations of potassium with the exception of sites 1, 2, and 4. Highest mean concentrations were at Site 16 and lowest were Site 2.

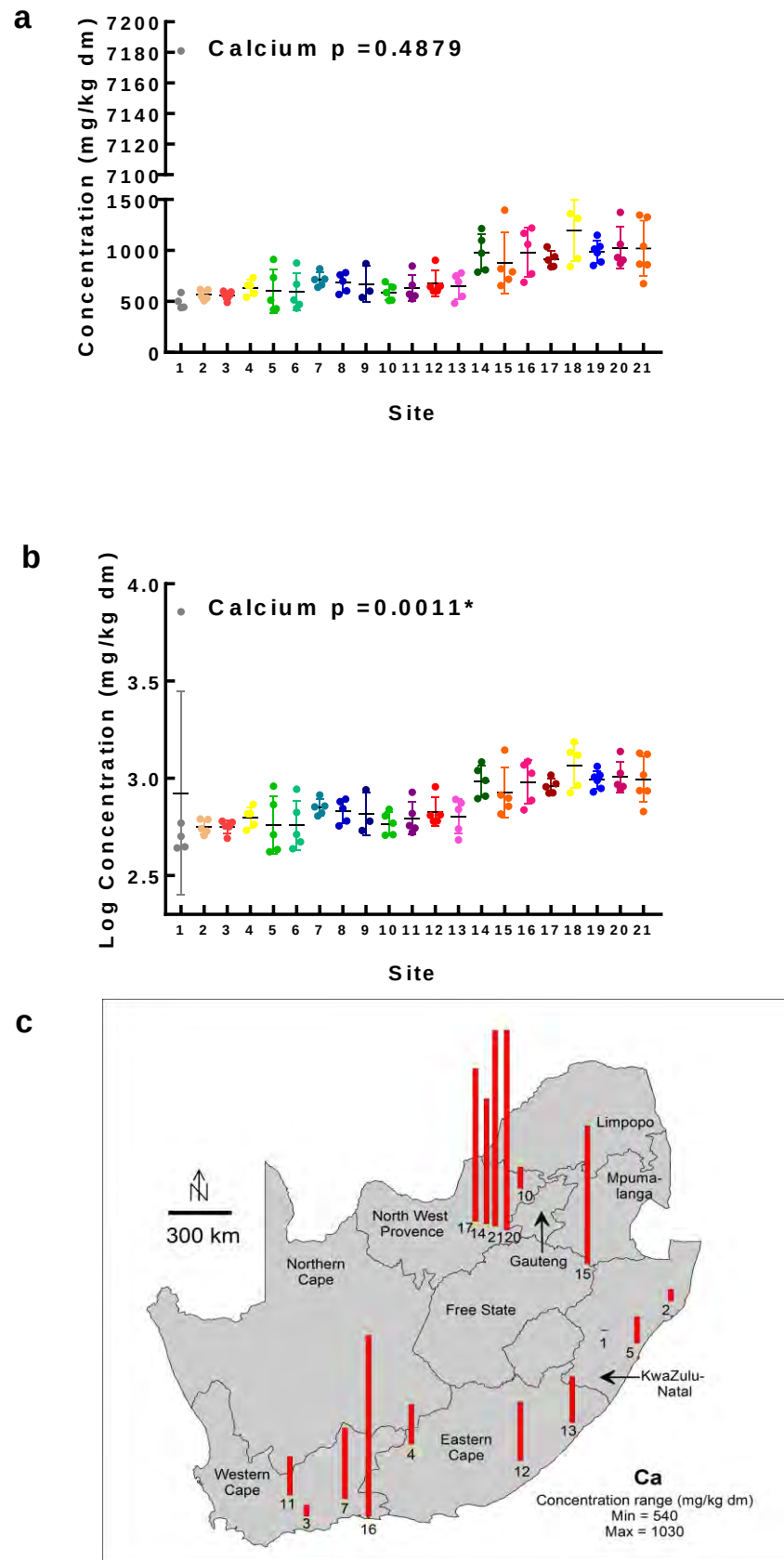


Figure 3.6 Distribution of calcium according to sites.

Figure 3.6a Scatterplot of untransformed calcium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.6b Scatterplot of log-transformed calcium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1 and 12. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons showed no differences for any comparison between sites.

Figure 3.6c Map of mean calcium concentrations.

Sites clustered around Potchefstroom - 14, 15, 16, 17, 20, and 21 - were relatively higher than other sites. Sites that had notably lower mean concentrations were all three from KwaZulu-Natal. Other low concentrations were found at sites 3 and 10.

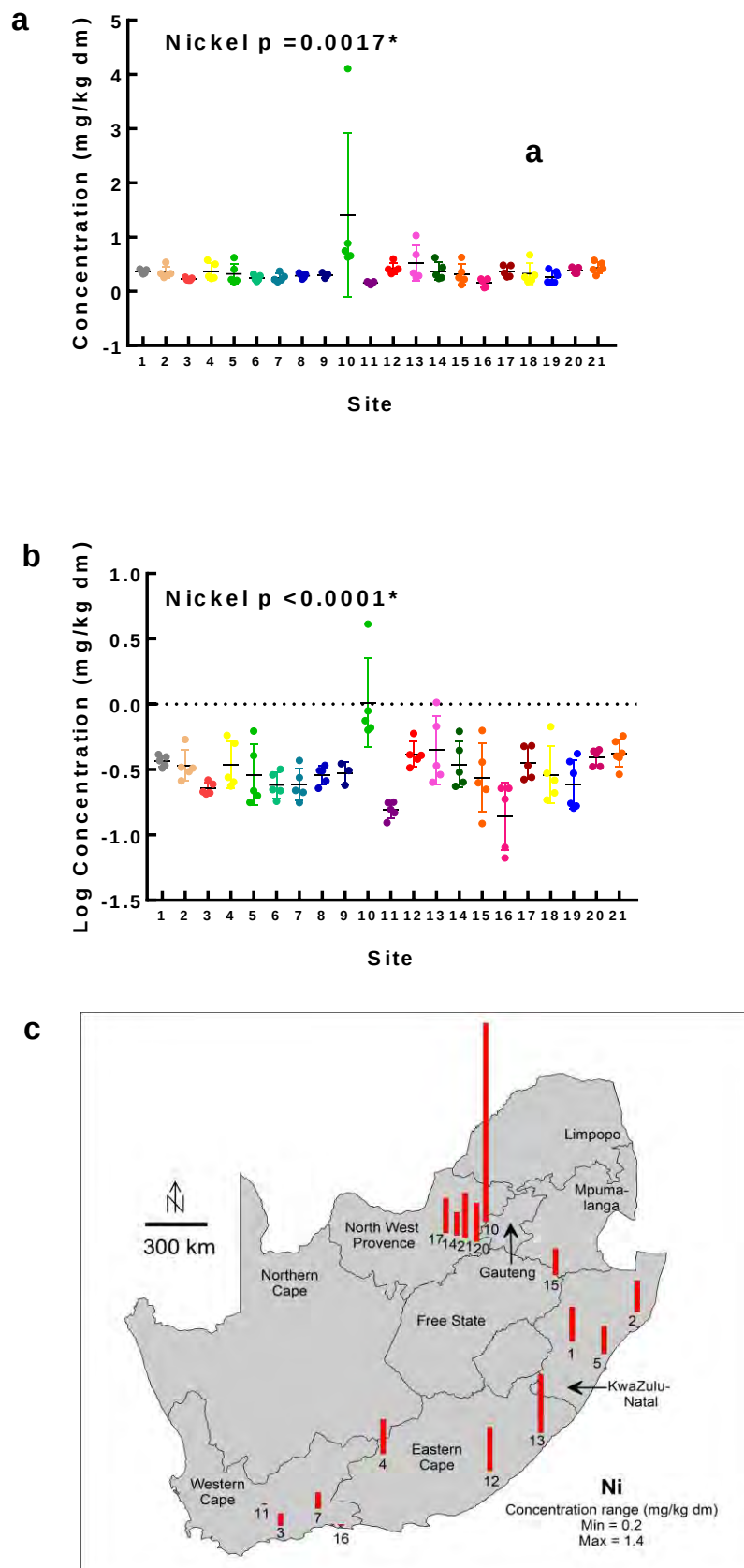


Figure 3.7 Distribution of nickel according to sites.

Figure 3.7a Scatterplot of untransformed nickel data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.7b Scatterplot of log-transformed nickel concentrations according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 5. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 10; 3 vs. 10; 4 vs. 10; 5 vs. 10; 6 vs. 10; 7 vs. 10; 8 vs. 10; 9 vs. 10; 10 vs. 11, 14, 15, 16, 17, 18, 19, 20; 11 vs. 12, 13, 21; 12 vs. 16; 13 vs. 16 and 16 vs. 17, 20, 21.

Figure 3.7c Map of mean nickel concentrations.

Mean concentrations of all sites were low in comparison to Site 10. Site 10 had a mean concentration three fold higher than the second highest mean concentration (site 13). Sites 11 and 16 were noticeably lower than other sites.

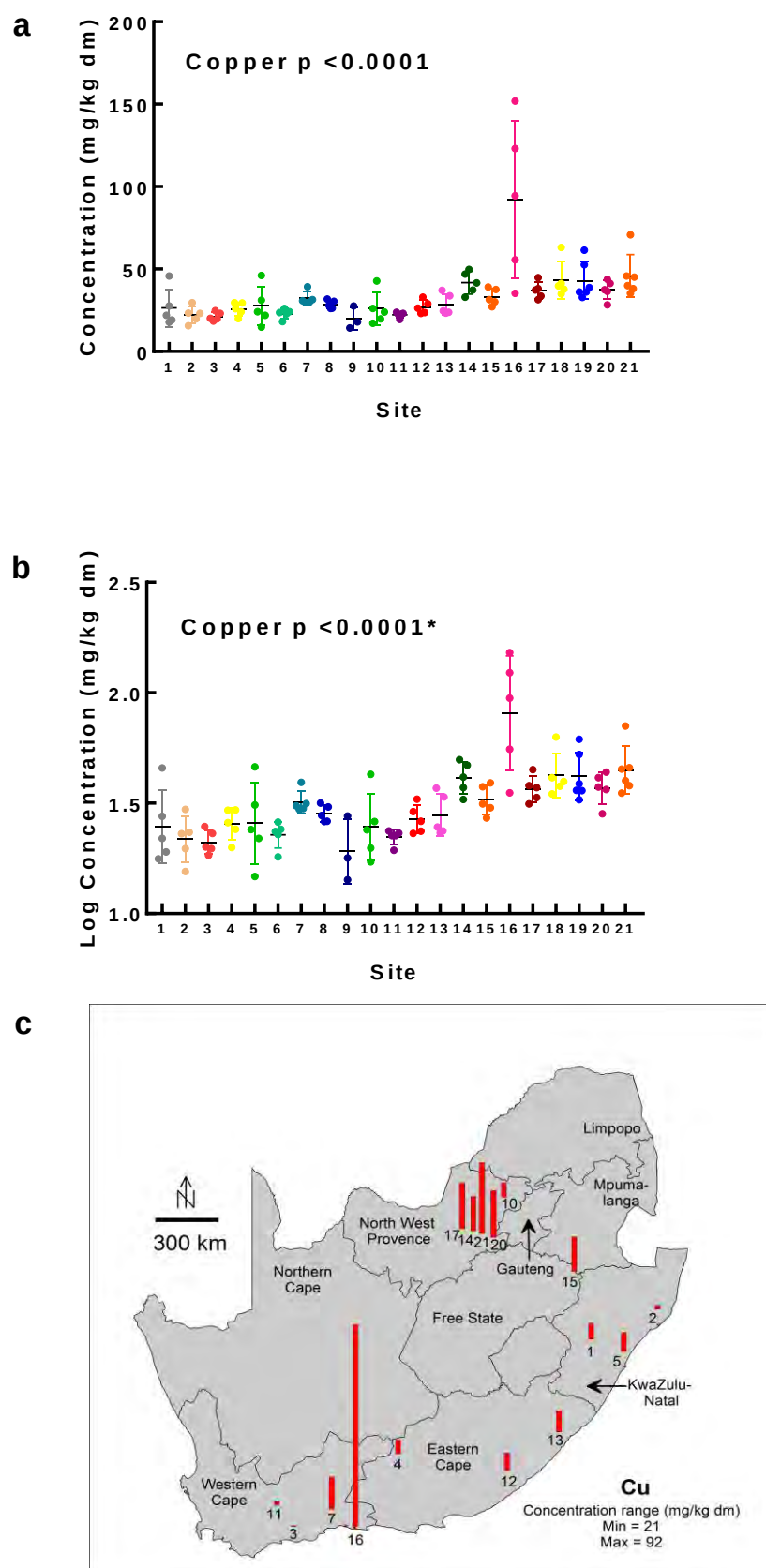


Figure 3.8 Distribution of copper according to sites.

Figure 3.8a Scatterplot of untransformed copper data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations are normally distributed, this graph will not be discussed further.

Figure 3.8b Scatterplot of log-transformed copper data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 7. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 5 vs. 16; 6 vs. 16, 18, 21; 7 vs. 16; 8 vs. 16; 9 vs. 14, 16; 9, 18, 19, 21; 10 vs. 16, 21; 11 vs. 14, 16, 18; 11, 19, 21; 12 vs. 16; 13 vs. 16; 14 vs. 16; 15 vs. 16 and 16 vs. 17, 18, 19, 20, 21.

Figure 3.8c Map of mean copper concentrations.

Site 10 had noticeably higher mean concentrations than all other sites. Sites 2, 3, and 11 were the lowest. Sites clustered around Potchefstroom (14, 17, 20, and 21) were higher than other sites but lower than 16.

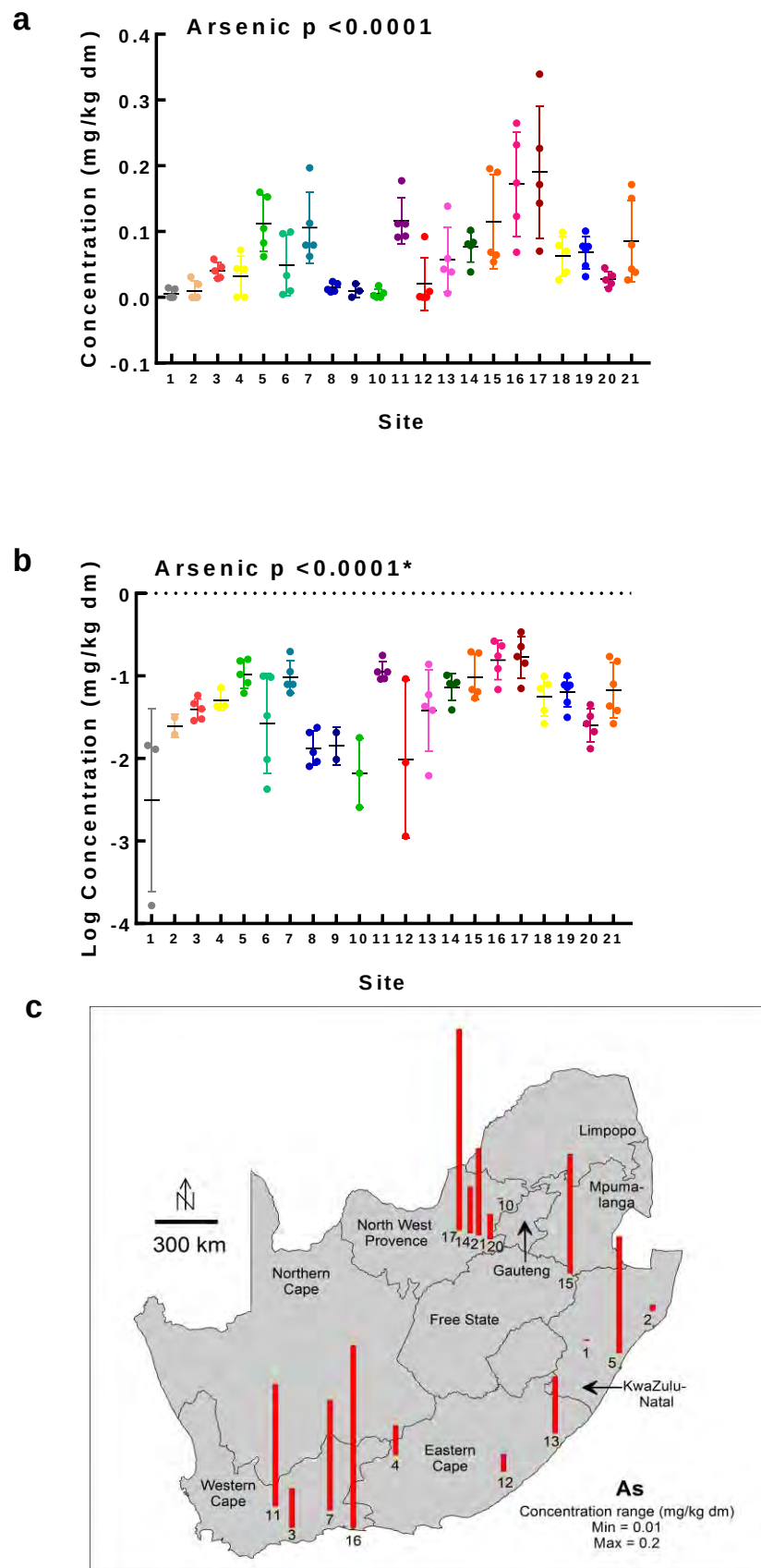


Figure 3.9 Distribution of arsenic according to sites.

Figure 3.9a Scatterplot of untransformed arsenic data according to data. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations are normally distributed, this graph will not be discussed further.

Figure 3.9b Scatterplot of log-transformed arsenic data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 3. Sites 2 and 10 had too low N values to run the test. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 3, 4, 5, 7, 11, 13, 14, 15, 16, 17, 18, 19, 21; 5 vs. 8, 10, 12; 7 vs. 10, 12; 8 vs. 11, 15, 16; 10 vs. 11, 14, 15, 16, 17, 19, 21; 11 vs. 12 and 12 vs. 15, 16, 17.

Figure 3.9c Map of mean arsenic concentrations.

All sites had measurable concentrations of arsenic and had noticeably high mean concentrations except for sites 1, 2, and 10. Sites 16 and 17 had the highest mean concentration.

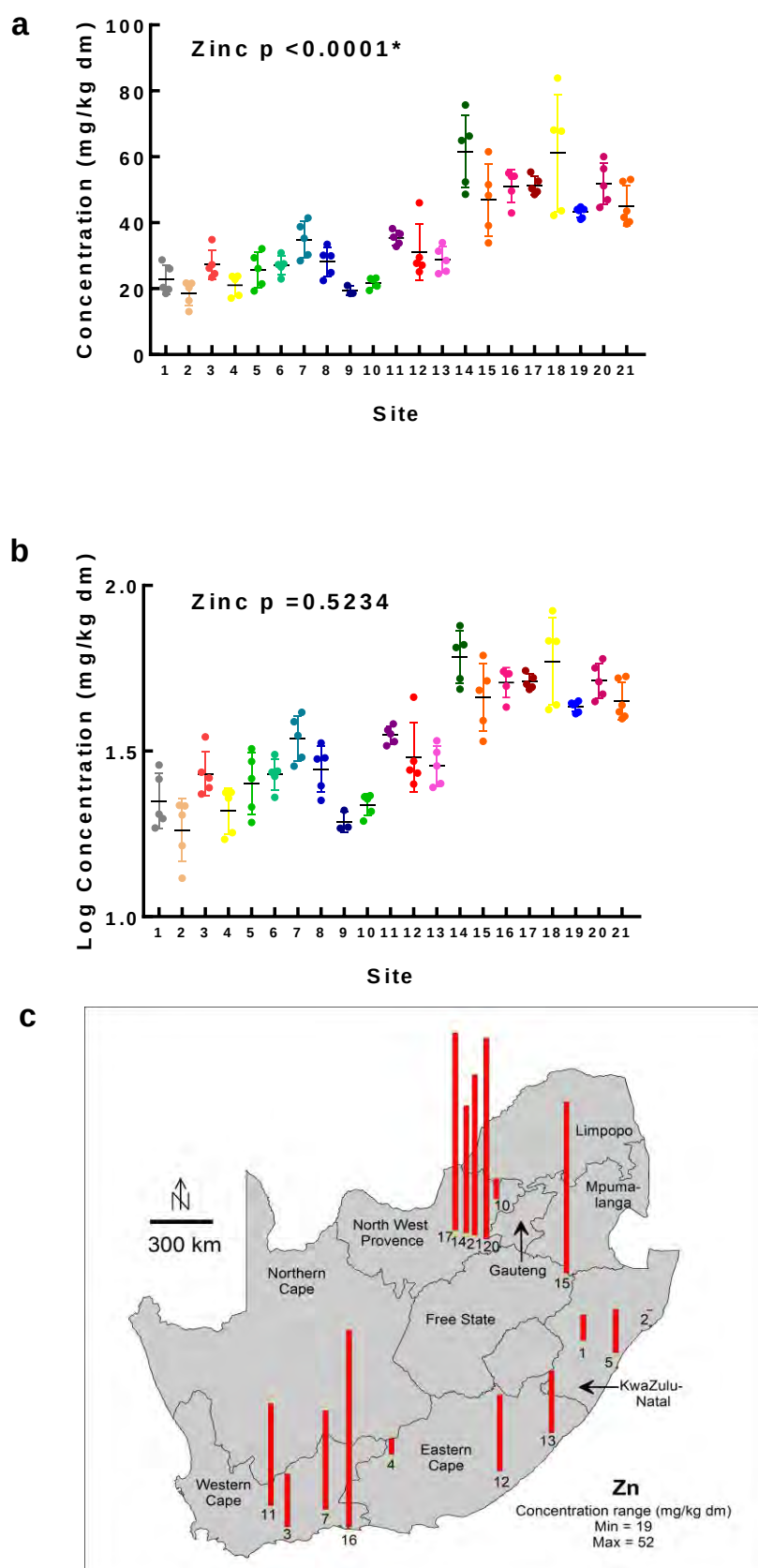


Figure 3.10 Distribution of zinc according to sites.

Figure 3.10a Scatterplot of untransformed zinc data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 12. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 12 vs. 20; 13 vs. 14, 15, 16, 17, 18, 20, 21; 14 vs. 19, 21 and 18 vs. 19, 21.

Figure 3.10b Scatterplot log-transformed zinc data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.10c Map of mean zinc concentrations.

All sites had relatively high mean concentrations except for sites 1, 2, 4, and 10. The lowest mean concentration was at Site 2. Sites 15, 16, 17 and 20 noticeably had the highest mean concentrations.

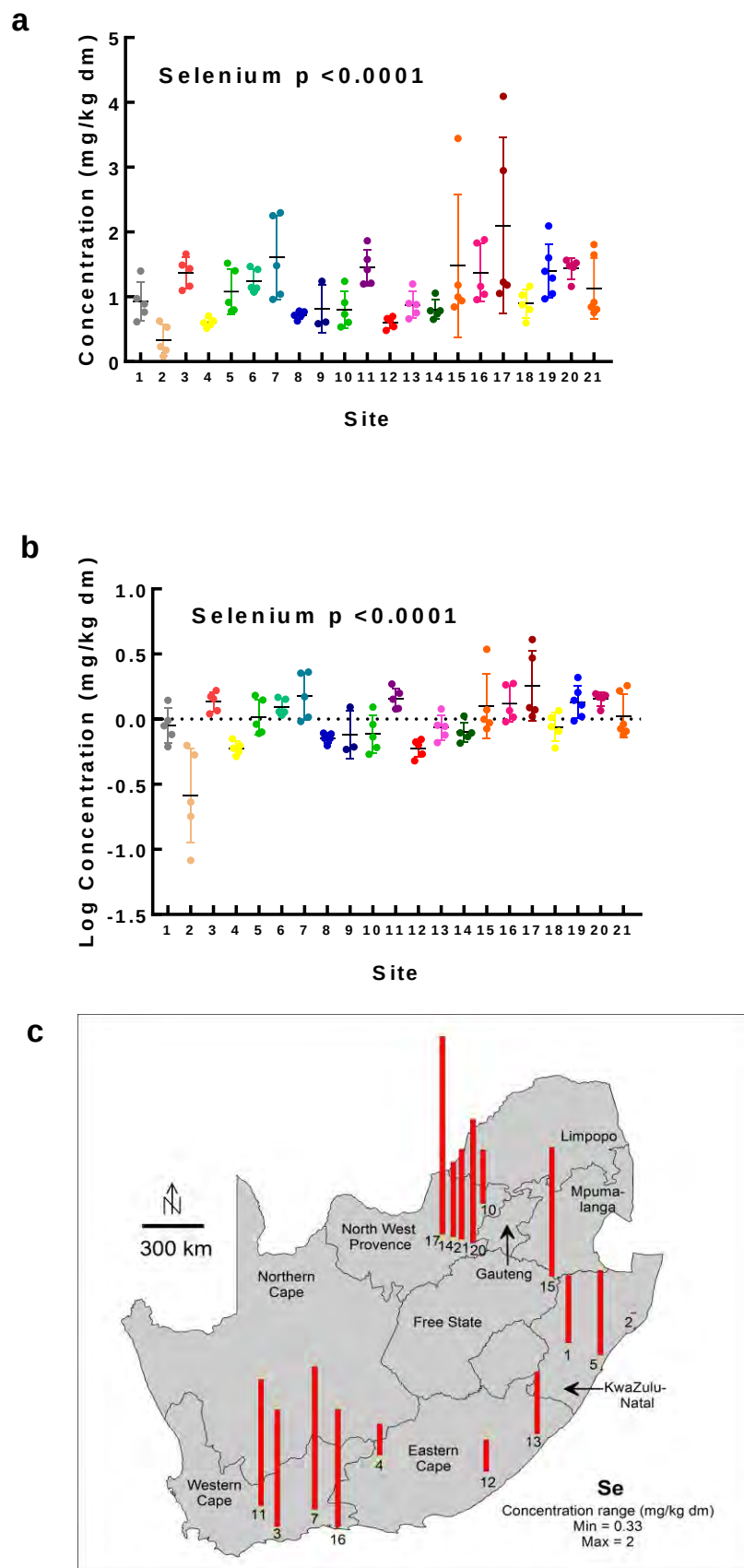


Figure 3.11 Distribution of selenium according to sites.

Figure 3.11a Scatterplot of untransformed selenium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 15 and 21. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 17; 2 vs. 7, 15, 17; 4 vs. 17; 8 vs. 17; 10 vs. 17; 12 vs. 17; 13 vs. 17; 17 vs. 18 and 14 vs. 17

Figure 3.11b Scatterplot of log-transformed selenium data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.11c Map of mean selenium concentrations.

All sites had high mean concentrations compared to Site 2. Site 2 was noticeably lower than all other sites. Site 17 had the highest mean concentration. Sites in the Western Cape were approximately all in the same ranges of each other.

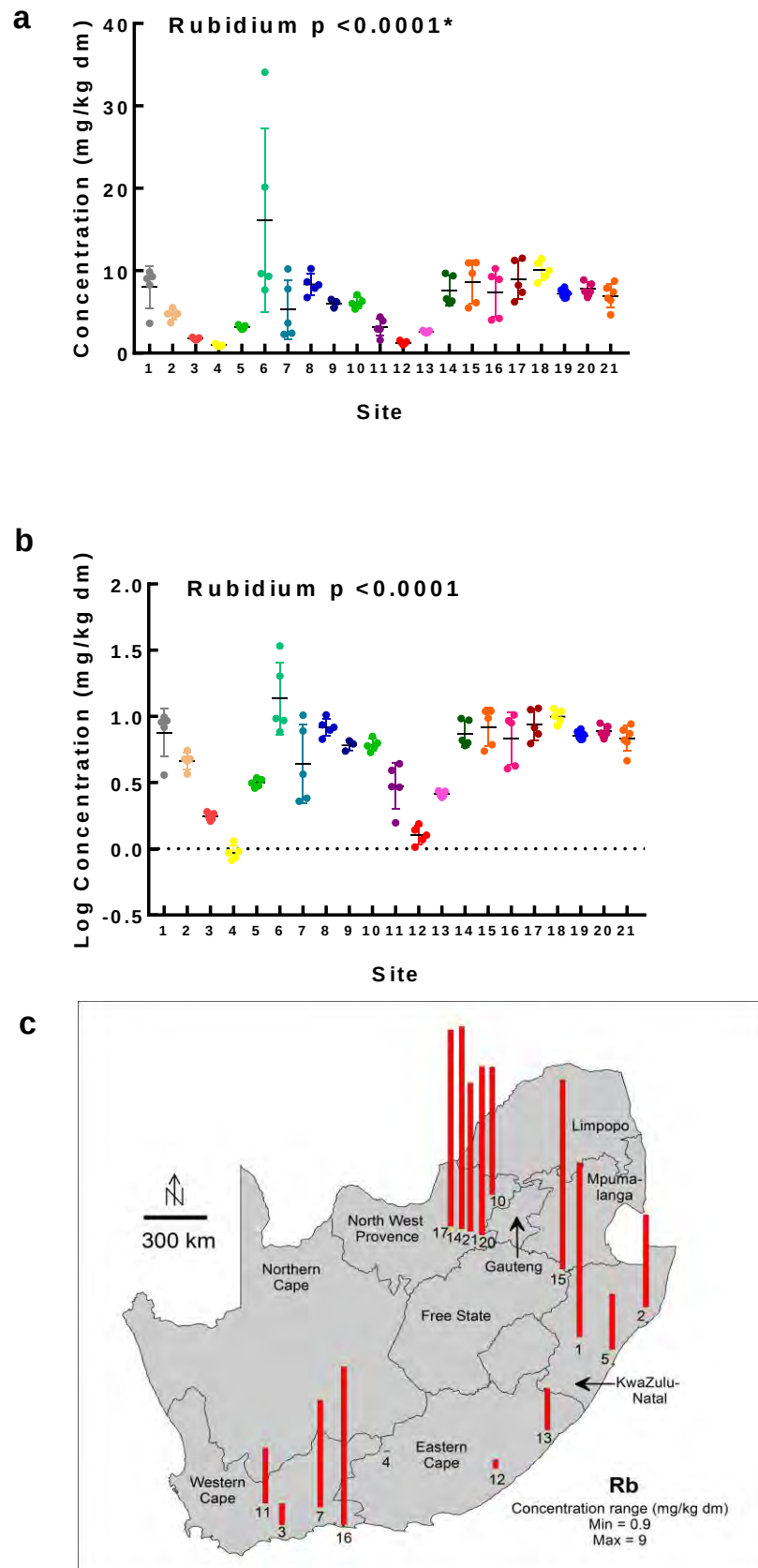


Figure 3.12 Distribution of rubidium according to sites.

Figure 3.12a Scatterplot of untransformed rubidium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 1. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 11 vs. 18; 12 vs. 15, 17, 18 and 13 vs. 18.

Figure 3.12b Scatterplot of log-transformed rubidium data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.12c Map of mean rubidium concentrations.

All sites around Potchefstroom had high mean concentrations. Sites 1 and 15 were also high. The sites that were the lowest (3, 4, and 12) were all in the southern part of South Africa.

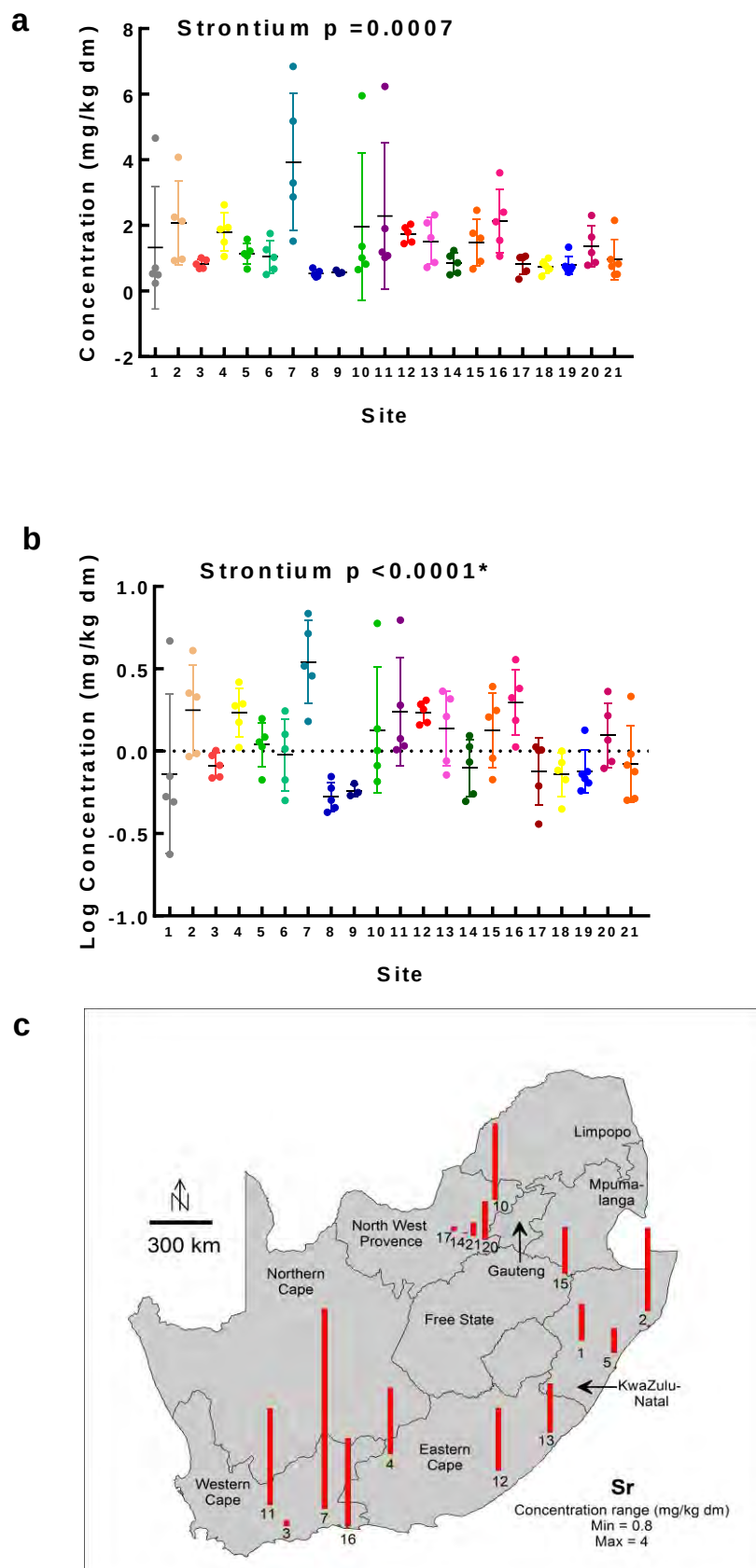


Figure 3.13 Distribution of strontium according to sites.

Figure 3.13a Scatterplot of untransformed strontium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.13b Scatterplot of log-transformed strontium data according to sites. The means and standard deviations are indicated.

All distributions were normal. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 7; 3 vs. 7; 6 vs. 7; 7 vs. 8, 9, 14, 17, 18, 19, 21 and 8 vs. 16.

Figure 3.13c Map of mean strontium concentrations.

Site 7 had the highest mean concentration. Sites 3, 14, and 17 had significantly lower mean concentrations. All sites along the coastline, except Site 3, had relatively high mean concentrations.

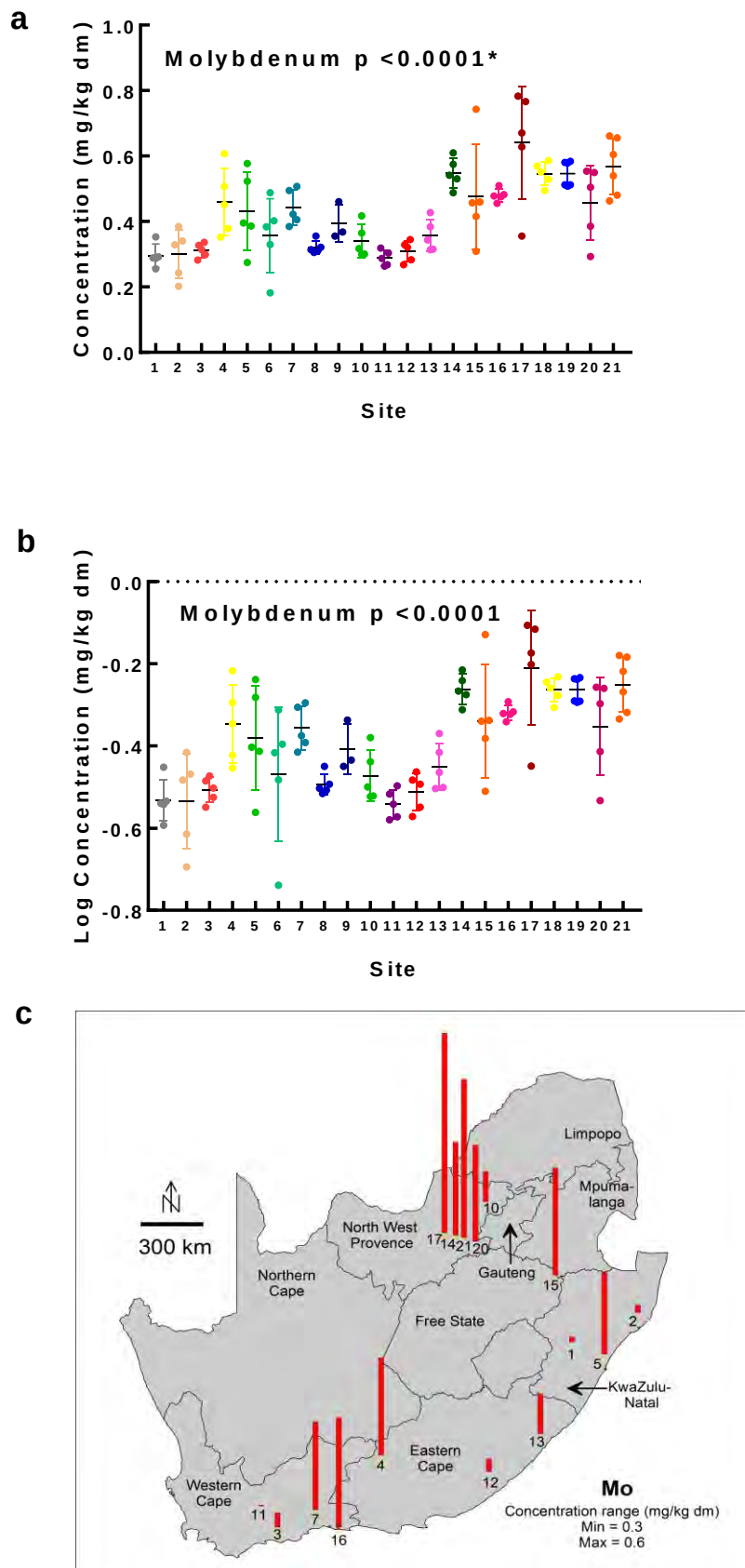


Figure 3.14 Distribution of molybdenum according to sites.

Figure 3.14a Scatterplot of untransformed molybdenum data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 14, 17, 18, 19, 21; 2 vs. 14, 17, 18, 19, 21; 3 vs. 14, 17, 18, 19, 21; 5 vs. 17; 6 vs. 14, 17, 19, 21; 7 vs. 17; 8 vs. 14; 17, 18, 19, 21; 9 vs. 17; 10 vs. 14, 17, 18, 19, 21; 11 vs. 14, 16, 17, 18, 19, 21; 12 vs. 14, 17; 18, 19, 21 and 13 vs. 14, 17, 19, 21.

Figure 3.14b Scatterplot of log-transformed molybdenum data according to sites.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further..

Figure 3.14c Map of mean molybdenum concentrations.

Sites around Potchefstroom (17 and 21) had the highest mean concentration. Sites 1, 2, 3, 11, and 12 were all low.

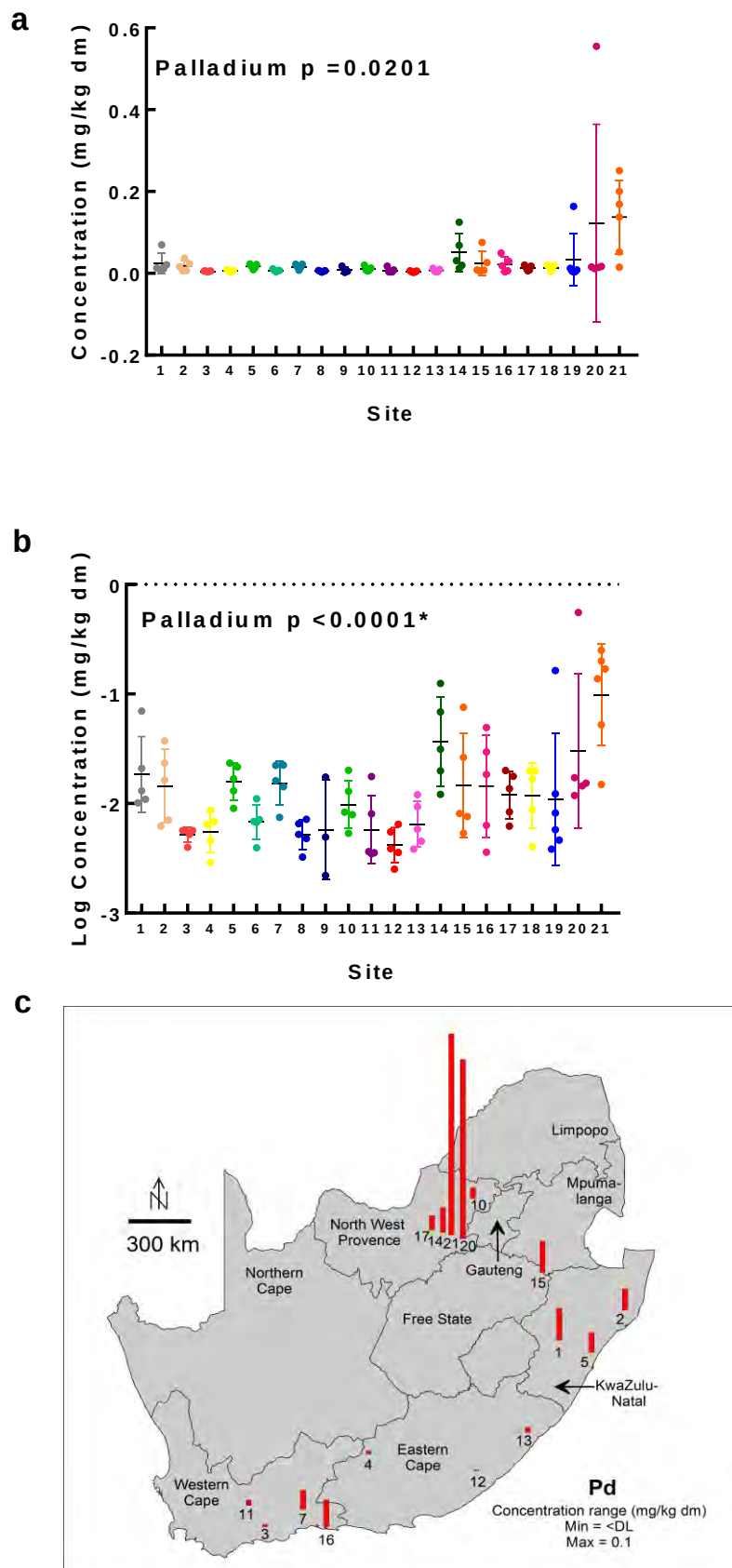


Figure 3.15 Distribution of palladium according to sites.

Figure 3.15a Scatterplot of untransformed palladium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.15b Scatterplot of log-transformed palladium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 3, 19 and 20. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 2; 3 vs. 14; 21; 4 vs. 21; 6 vs. 21; 7 vs. 21; 8 vs. 14, 21; 9 vs. 21; 10 vs. 21; 11 vs. 21; 12 vs. 14, 20, 21; 13 vs. 21; 15 vs. 21; 16 vs. 21; 17 vs. 21; 18 vs. 21 and 19 vs. 21.

Figure 3.15c Map of mean palladium concentrations.

Sites 20 and 21 were noticeably higher than all other sites. All sites in the Eastern Cape had low mean concentrations.

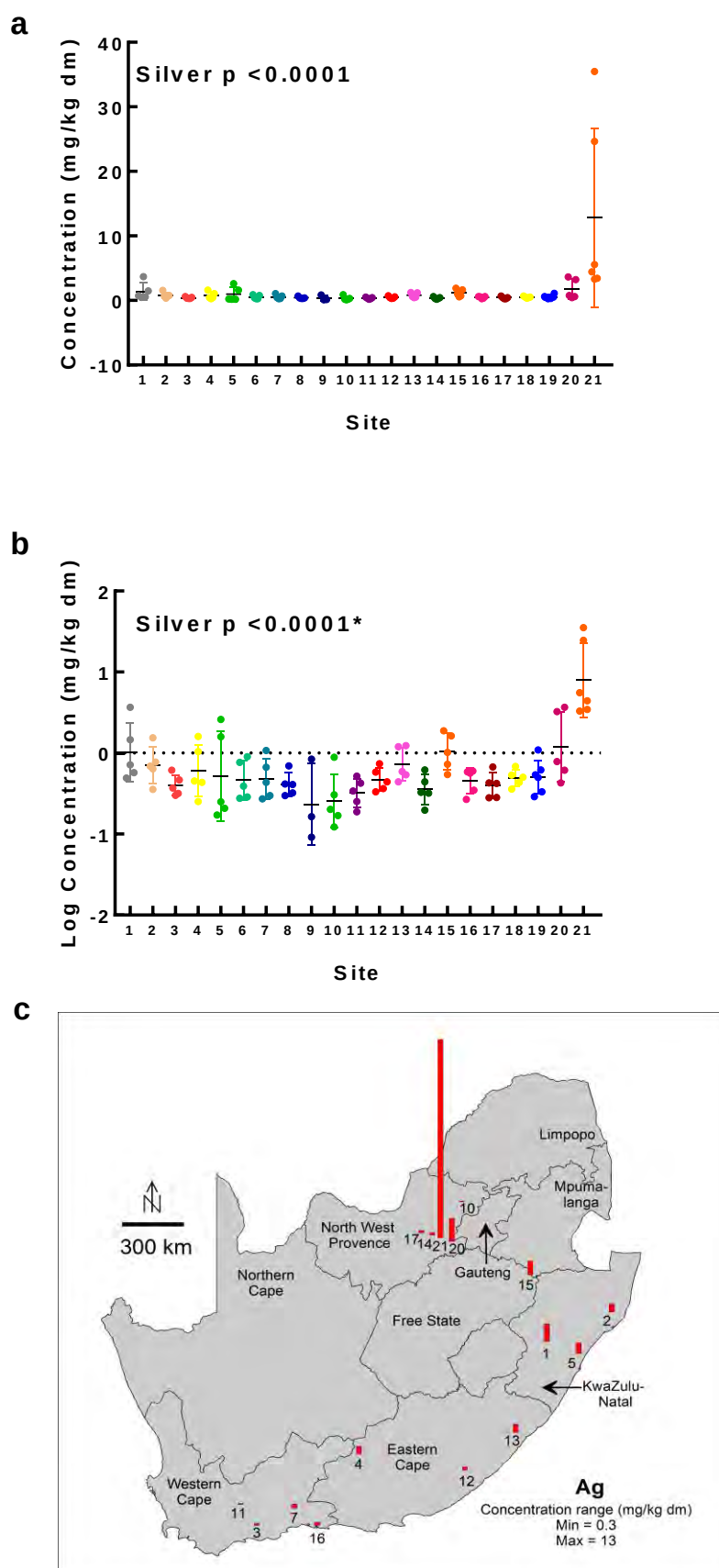


Figure 3.16 Distribution of silver according to sites.

Figure 3.16a Scatterplot of untransformed silver data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not normally distributed. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.16b Scatterplot of log-transformed silver data according to sites. The means and standard deviations are indicated.

All distributions were normal. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 21; 2 vs. 21; 3 vs. 21; 4 vs. 21; 5 vs. 21; 6 vs. 21; 7 vs. 21; 8 vs. 21; 9 vs. 21; 10 vs. 20, 21; 11 vs. 21; 12 vs. 21; 13 vs. 21; 14 vs. 21; 15 vs. 21; 16 vs. 21; 17 vs. 21; 18 vs. 21; 19 vs. 21 and 20 vs. 21.

Figure 3.16c Map of mean silver concentrations.

All sites had low mean concentrations compared to Site 21. All sites in the Western –and Eastern Cape were low. Site 20 had the second highest mean concentration.

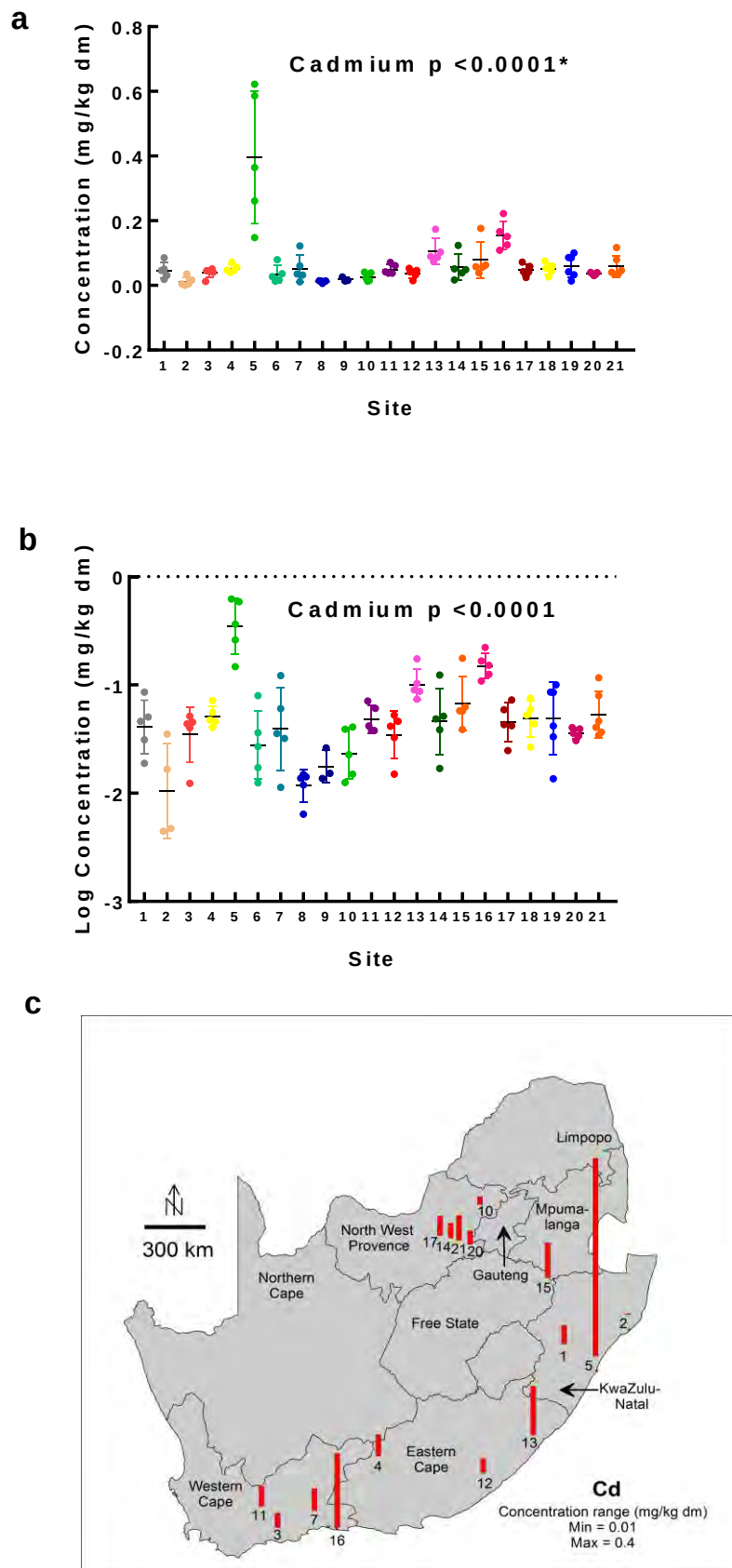


Figure 3.17 Distribution of cadmium according to sites.

Figure 3.17a Scatterplot of untransformed cadmium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 15. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 5, 16; 2 vs. 4, 5, 11, 13, 14, 15, 16, 17, 18, 18, 21; 3 vs. 5, 16; 4 vs. 5, 8; 5 vs. 6, 7, 8, 9, 10, 11, 13, 14, 15, 17, 18, 19, 20; 6 vs. 13, 16; 7 vs. 16; 8 vs. 11, 13, 14, 15, 16, 17, 18, 19, 21; 9 vs. 13, 16; 10 vs. 13, 16; 12 vs. 16 and 16 vs. 20.

Figure 3.17b Scatterplot of log-transformed data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.17c Map of mean cadmium concentrations.

Sites along the coast had higher mean concentrations than sites in the centre. Site 5 had the highest mean concentration followed by sites 13 and 16. Site 2 had the lowest mean concentration.

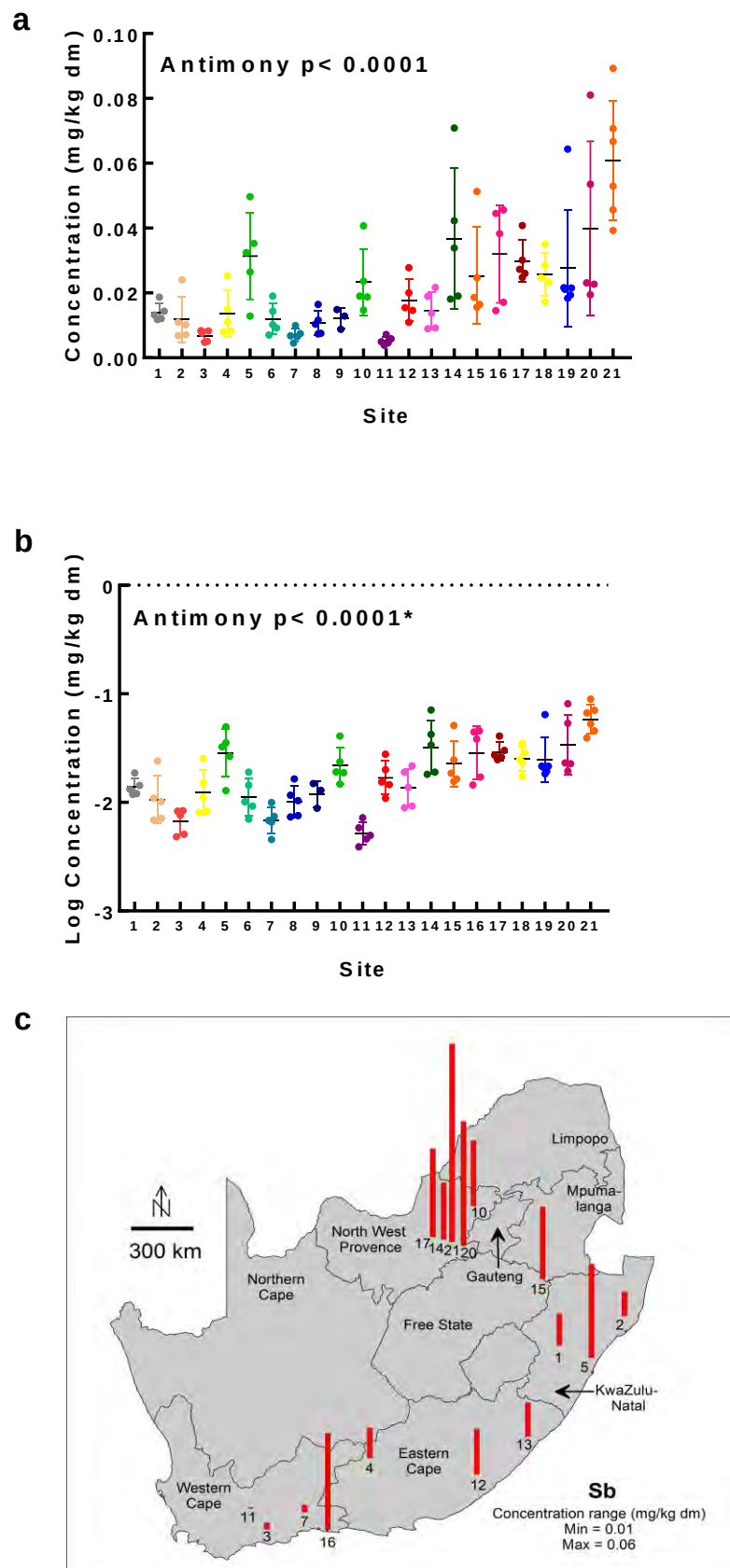


Figure 3.18 Distribution of antimony according to sites.

Figure 3.18a Scatterplot of untransformed antimony data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.18b Scatterplot of log-transformed antimony data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 11, 21; 2 vs. 5, 14, 16, 17, 20, 21; 3 vs. 5, 10, 14, 15, 16, 17, 18, 19, 20, 21; 4 vs. 14, 20, 21; 5 vs. 7, 8, 11; 6 vs. 14, 17, 20, 21; 7 vs. 10, 14, 15, 16, 17, 18, 19, 20, 21; 8 vs. 14, 16, 17, 20; 9 vs. 21; 10 vs. 11, 21; 11 vs. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21; 12 vs. 21; 13 vs. 21 and 15 vs. 21.

Figure 3.18c Map of mean antimony concentrations.

All sites in the North West Province were high. Other sites that were also high are 5, 15, and 16. Sites in the Western Cape were low with the exception of Site 16.

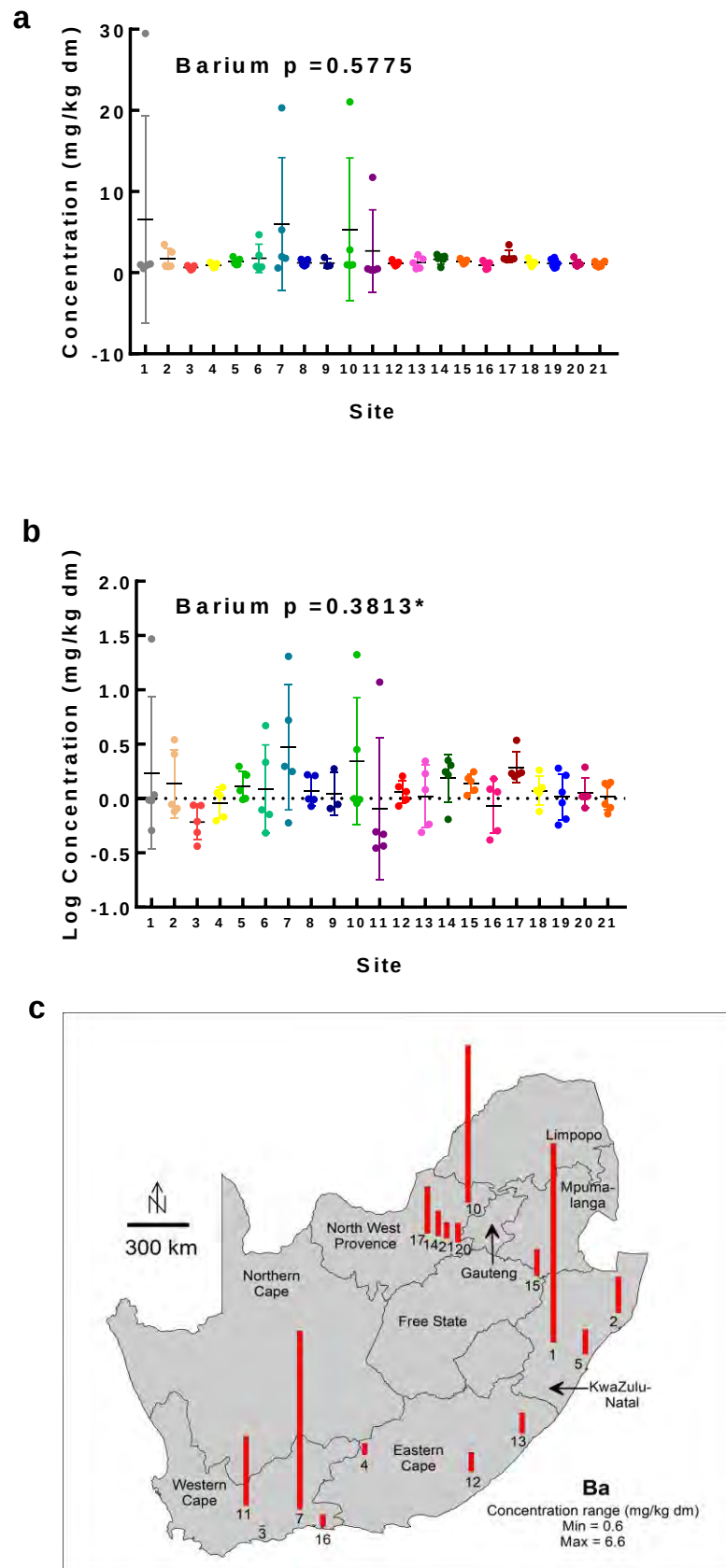


Figure 3.19 Distribution of barium according to sites.

Figure 3.19a Scatterplot of untransformed barium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.19b Scatterplot of log-transformed barium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1, 10, 11, 14, and 17. One-way ANOVA showed no differences between sites. Tukey's multiple comparisons also showed no differences for any comparison between sites.

Figure 3.19c Map of mean barium concentrations.

Sites 1, 7, and 10 were noticeably higher than other sites. Site 3 had the lowest mean concentration. Sites close to the coast were lower than sites more towards the centre. Site 11 also had relatively high mean concentration.

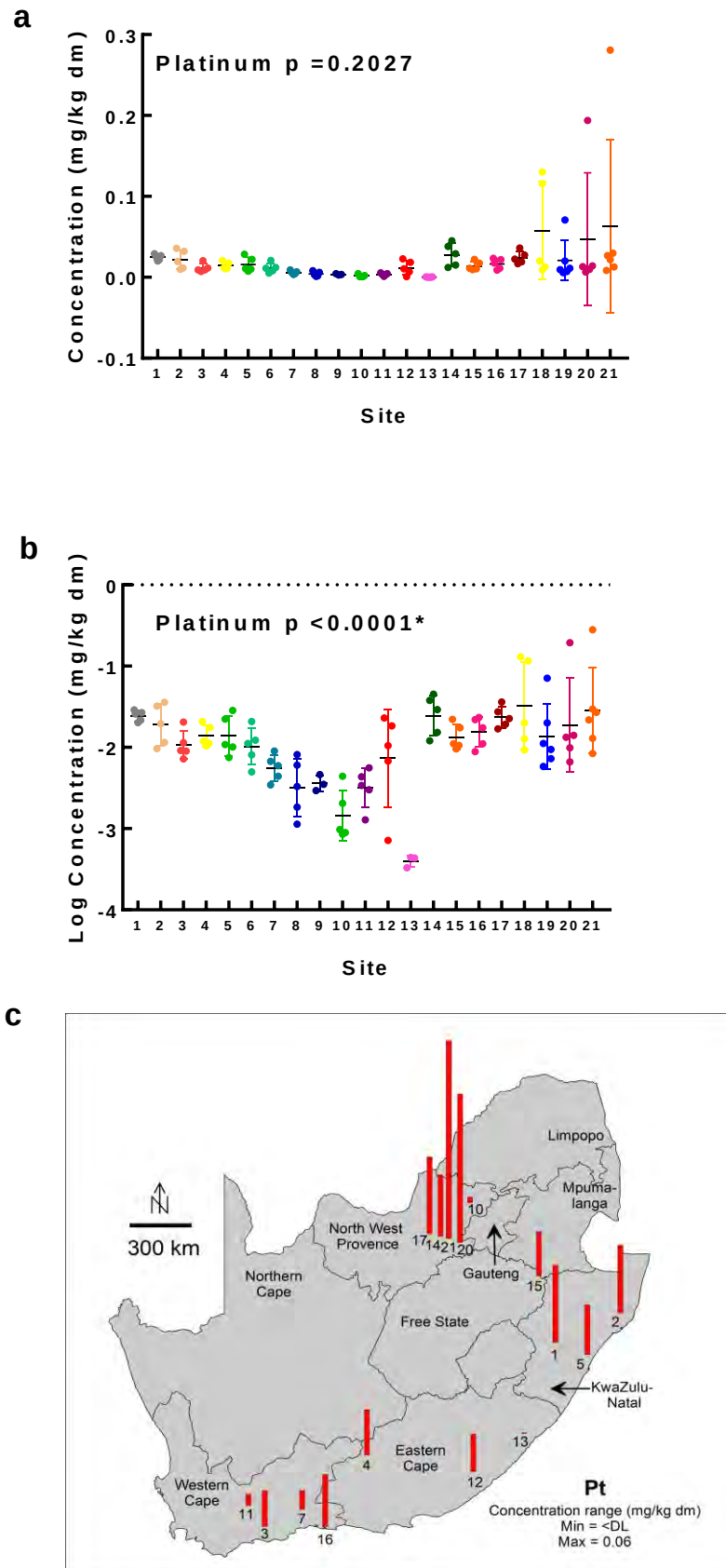


Figure 3.20 Distribution of platinum according to sites.

Figure 3.20a Scatterplot of untransformed platinum data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.20b Scatterplot of log-transformed platinum data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 20. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 8, 10, 11, 13; 2 vs. 8, 10, 11, 13; 3 vs. 10, 13; 4 vs. 10, 13; 5 vs. 10, 13; 6 vs. 10, 13; 7 vs. 13; 8 vs. 13, 14, 17, 18, 20, 21; 9 vs. 18, 19; 10 vs. 14, 15, 16, 17, 18, 19, 20, 21; 11 vs. 13, 14, 17, 18, 20, 21; 12 vs. 13 and 13 vs. 14, 15, 16, 17, 18, 19, 20, 21;

Figure 3.20c Map of mean platinum concentrations.

Sites clustered around Potchefstroom – 1, 2, 14, 17, 20, and 2 – were relatively higher than other sites. Sites 10 and 13 had notably lower mean concentrations.

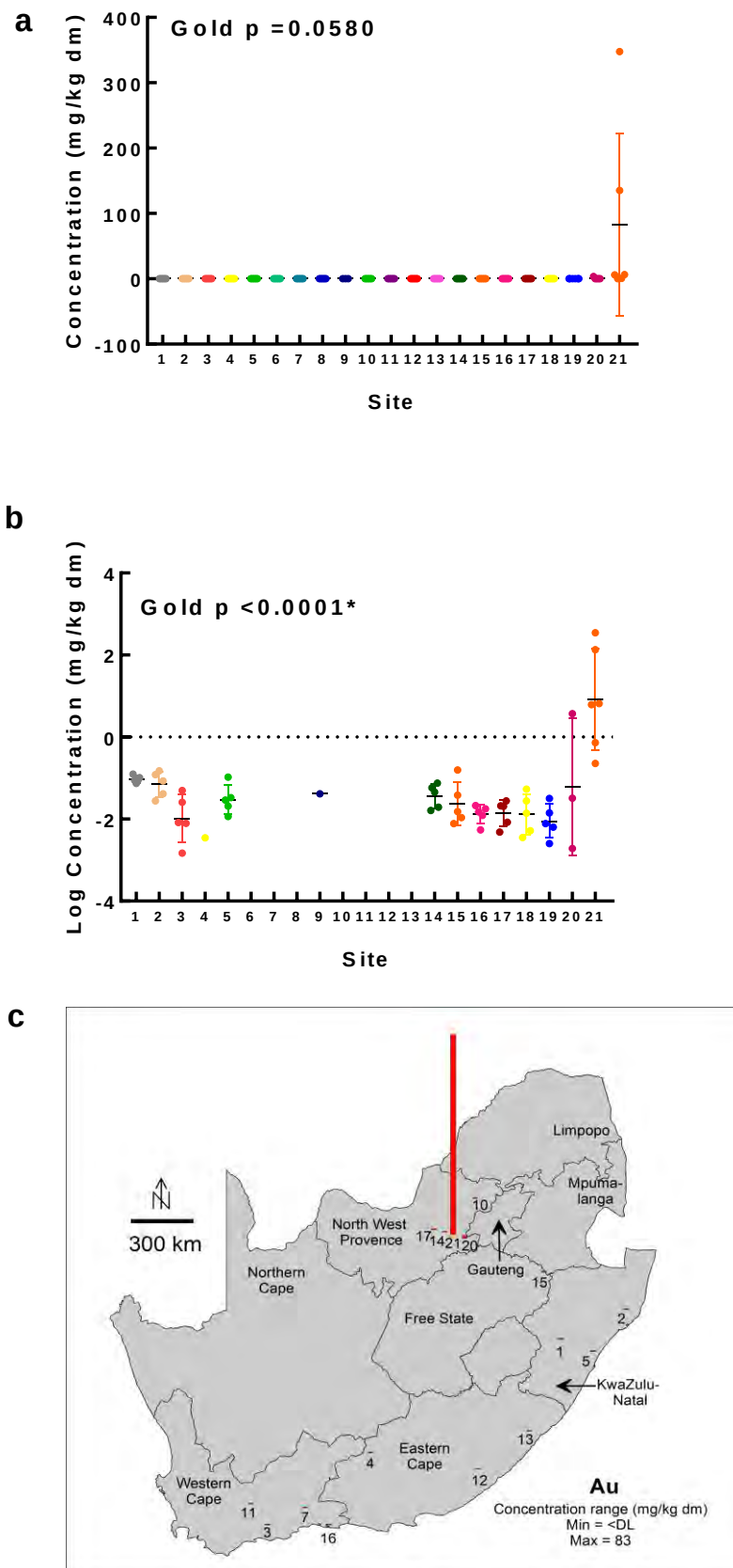


Figure 3.21 Distribution of gold according to sites.

Figure 3.21a Scatterplot of untransformed gold data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.21b Scatterplot of log-transformed gold data according to sites. The means and standard deviations are indicated.

All distributions were normal. However, two sites had too low N values to run the tests and 7 sites had no trace of Au. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 21; 2 vs. 21; 3 vs. 21; 4 vs. 21; 5 vs. 21; 14 vs. 21; 15 vs. 21; 16 vs. 21; 17 vs. 21; 18 vs. 21; 19 vs. 21 and 20 vs. 21.

Figure 3.21c Map of mean gold concentrations.

Site 21 had noticeably the highest mean concentration. All other sites were extremely low in comparison.

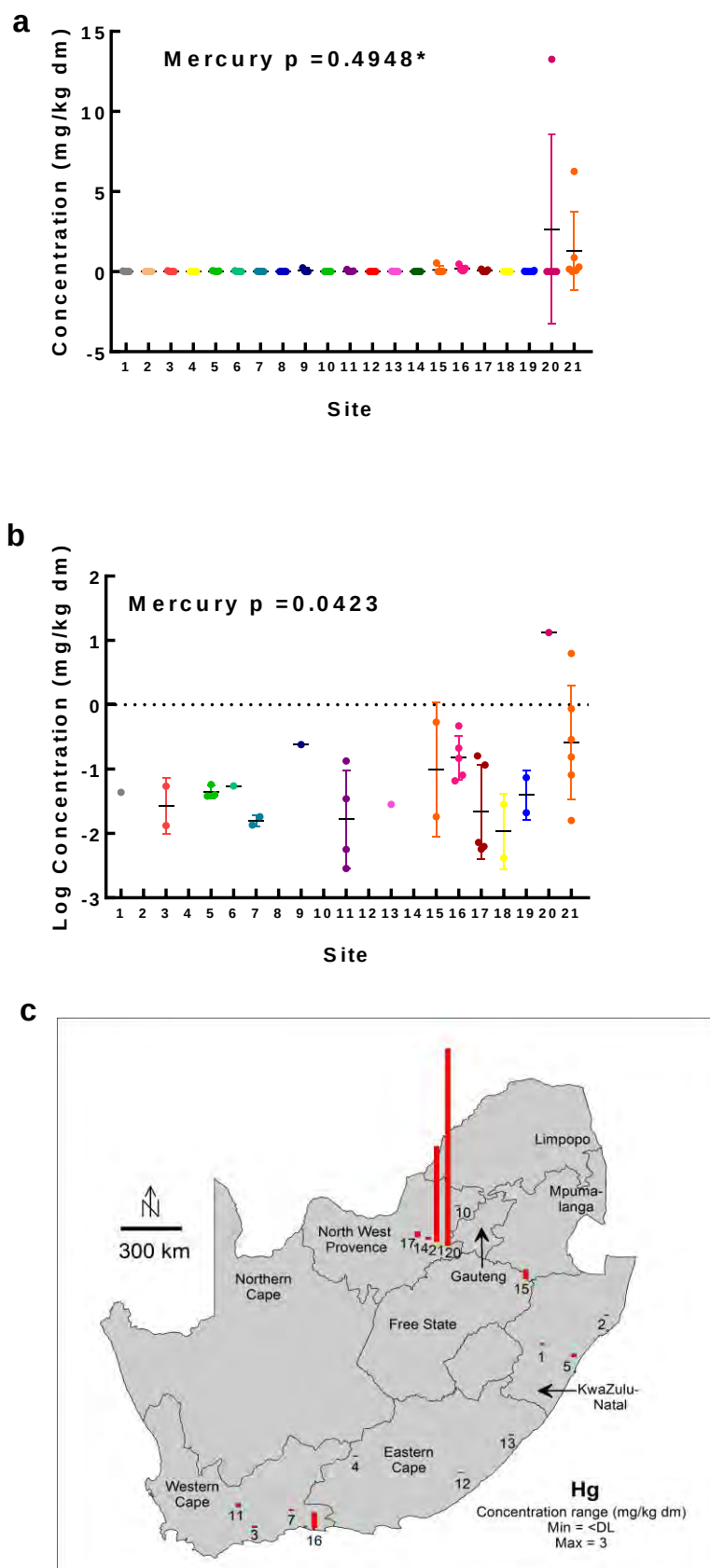


Figure 3.22 Distribution of mercury according to sites.

Figure 3.22a Scatterplot of untransformed mercury data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 5 and 6. One-way ANOVA showed no differences between sites. Tukey's multiple comparisons also showed no differences for any comparison between sites.

Figure 3.22b Scatterplot of log-transformed mercury data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.22c Map of mean mercury concentrations.

Sites around Potchefstroom (20 and 21) were noticeably higher than other sites. Sites 15 and 16 were lower than sites 20 and 21 but relatively higher than all other sites.

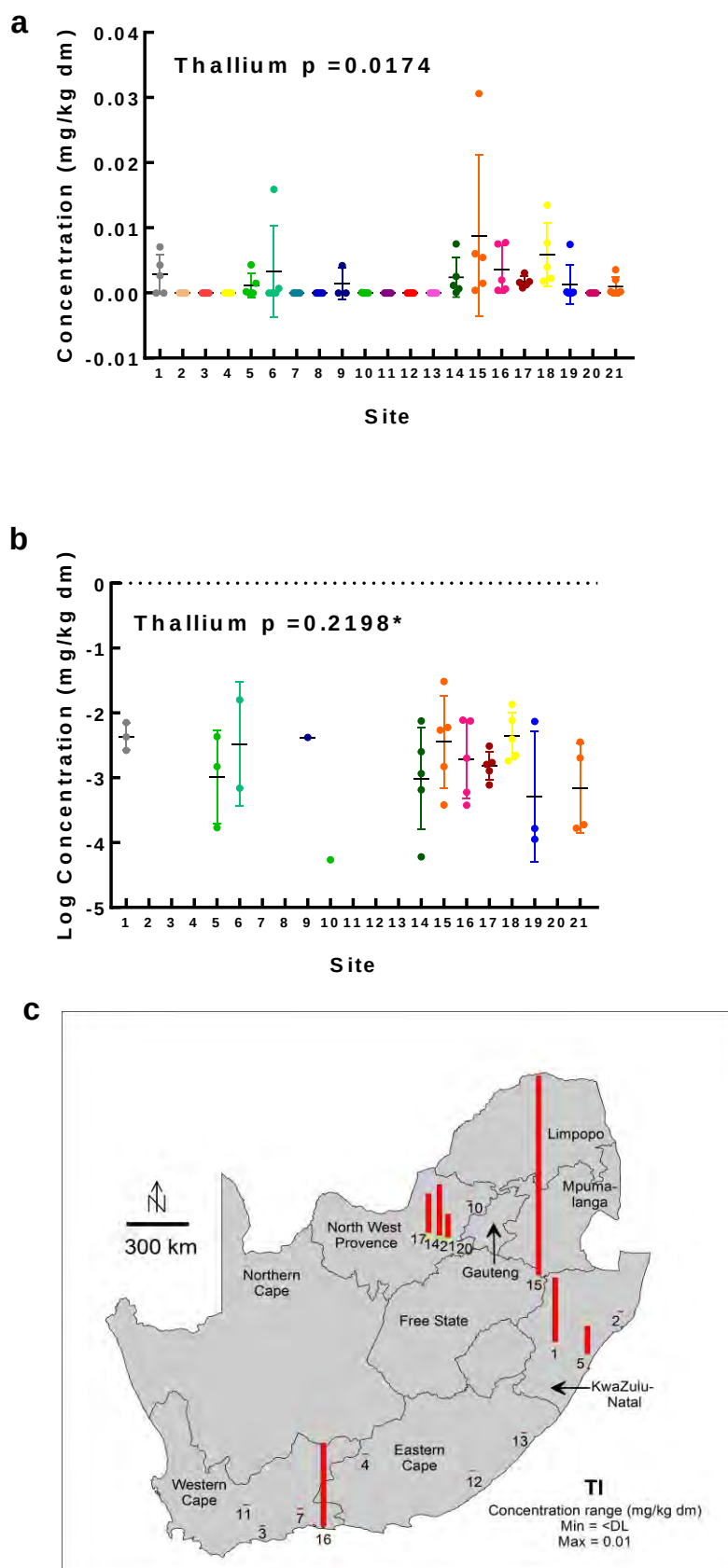


Figure 3.23 Distribution of thallium according to sites.

Figure 3.23a Scatterplot of untransformed thallium data according to sites. The untransformed concentrations were not distributed normally.

Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.23b Scatterplot of log-transformed thallium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1, 5, 14, 15, 16, 17, 18, and 19. N values were too low. One-way ANOVA showed no differences between sites. Tukey's multiple comparisons also showed no differences for any comparison between sites.

Figure 3.23c Map of mean thallium concentrations.

The highest mean concentrations were found at Site 15. Sites 1 and 16 were also relatively high. Sites around Potchefstroom – 5, 14, 17, 20, and 21 – were also noticeably higher than the extremely low concentrations found at all remaining sites.

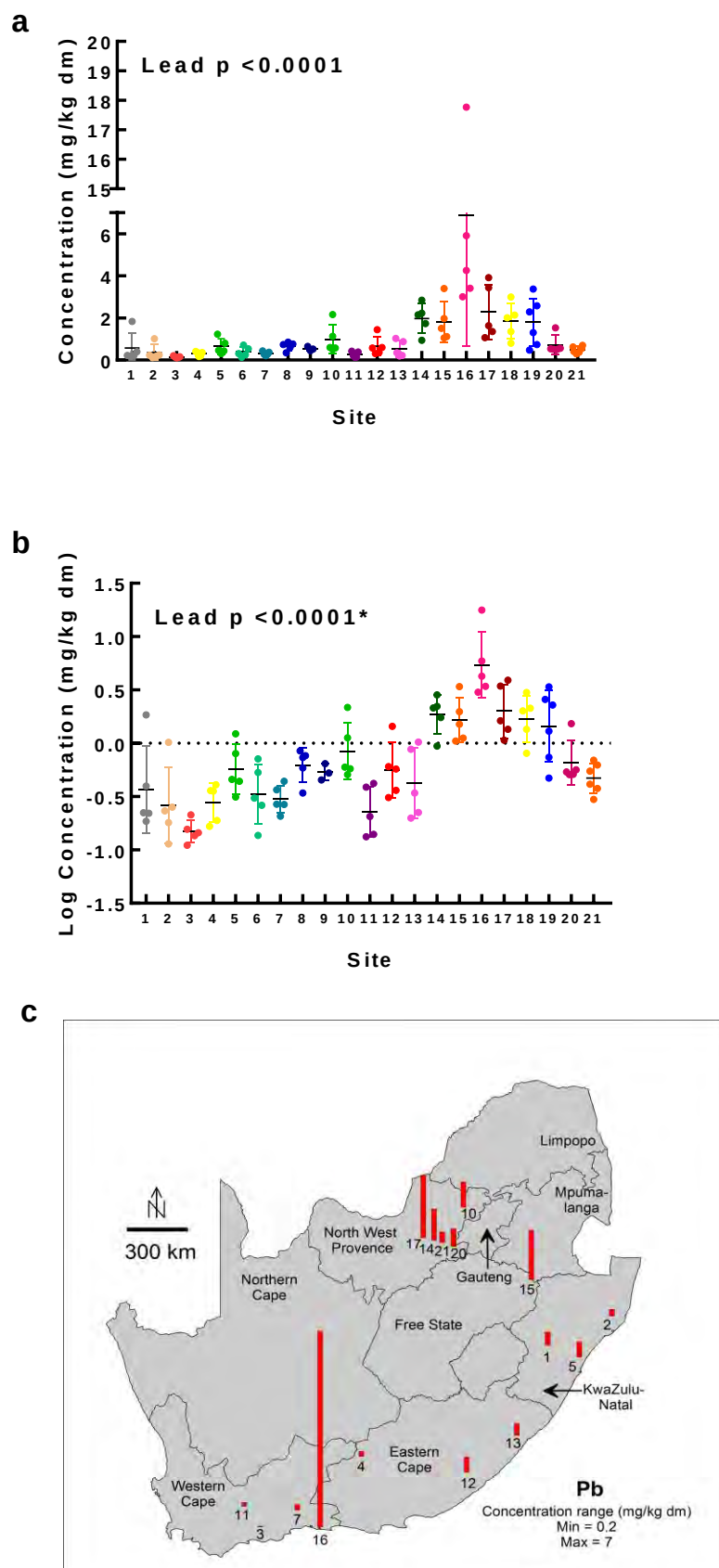


Figure 3.24 Distribution of lead according to sites.

Figure 3.24a Scatterplot of untransformed lead data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.24b Scatterplot of log-transformed lead data according to sites. The means and standard deviations are indicated.

All distributions were normal except for 20. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 14; 15, 16, 17, 18, 19; 2 vs. 14, 15, 16, 17, 18, 19; 3 vs. 5, 8, 10, 14; 3 vs. 5, 8, 10, 14, 15, 16, 17, 18, 19, 20; 4 vs. 14, 15, 16, 17, 18, 19; 5 vs. 16; 6 vs. 14, 15, 16, 17, 18, 19; 7 vs. 14, 15, 16, 17, 18, 19; 8 vs. 16; 9 vs. 16; 10 vs. 16; 11 vs. 14, 15, 16, 17, 18, 19; 12 vs. 16; 13 vs. 14, 15, 16, 17, 18; 14 vs. 21; 16 vs. 19, 20, 21 and 17 vs. 21.

Figure 3.24c Map of mean lead concentrations.

The highest concentrations were at Site 16. Sites 15 and 17 were also relatively high. All coastal sites were low, except for Site 16. Site 3 had the lowest mean concentration.

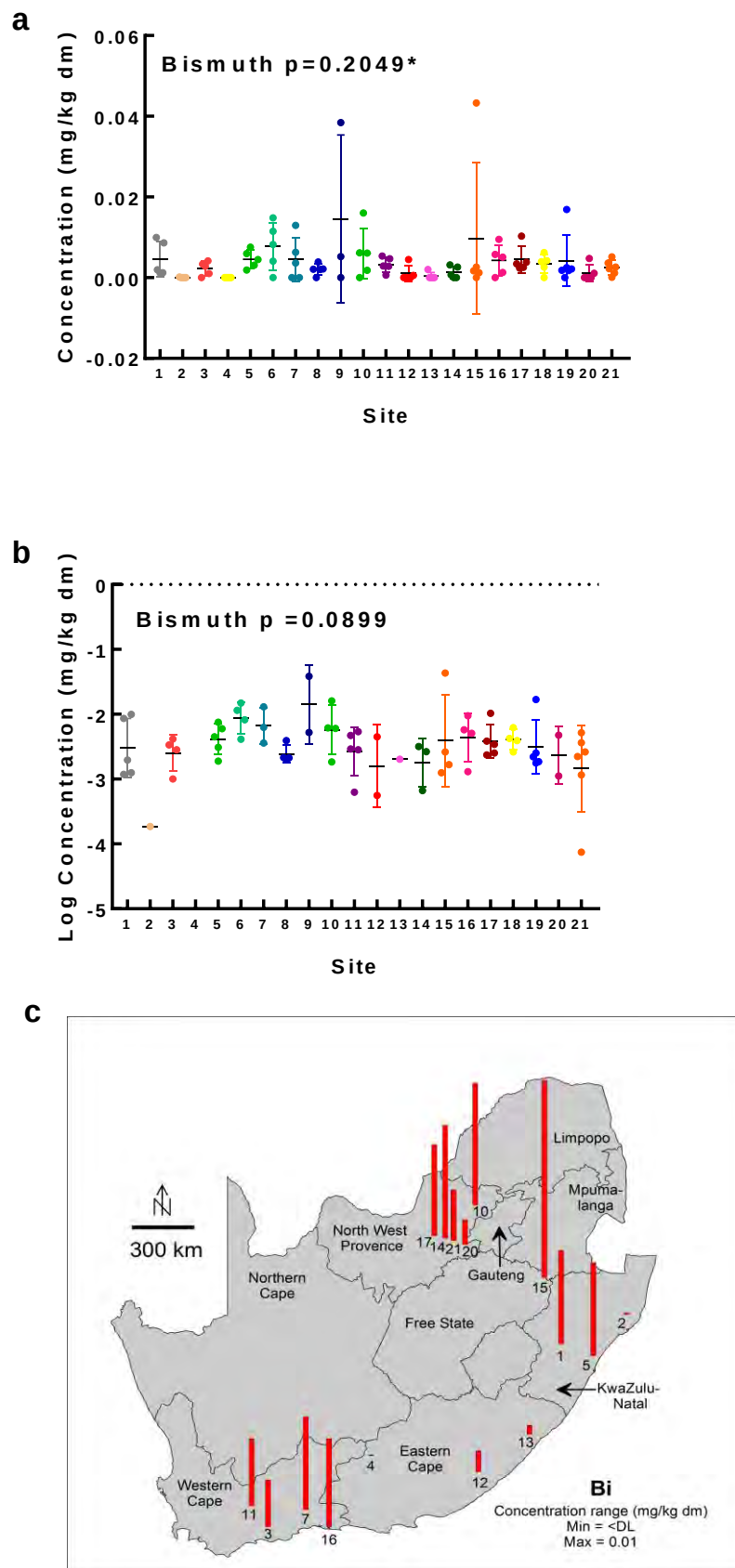


Figure 3.25 Distribution of bismuth according to sites.

Figure 3.25a Scatterplot of untransformed bismuth data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 2, 4, 12, 13, 15, 17, 19, and 20. One-way ANOVA showed no differences between sites. Tukey's multiple comparisons also showed no differences for any comparison between sites.

Figure 3.25b Scatterplot of log-transformed bismuth data according to sites.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.25c Map of mean bismuth concentrations.

All sites had relatively high mean concentrations except for sites located in the Eastern Cape as well as Site 2. Site 15 had the highest mean concentration.

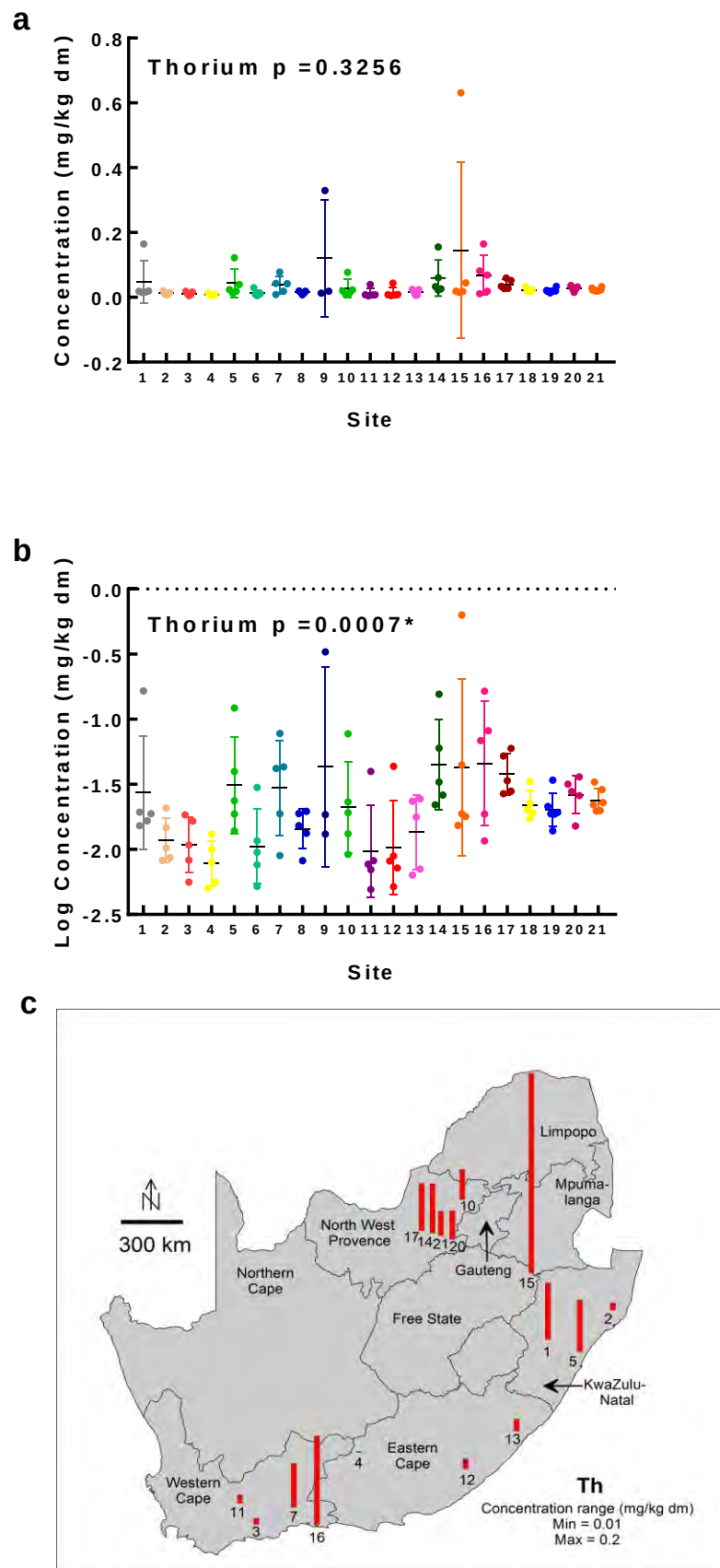


Figure 3.26 Distribution of thorium according to sites.

Figure 3.26a Scatterplot of untransformed thorium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.26b Scatterplot of log-transformed thorium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1, 10 and 15. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons showed no differences for any comparison between sites.

Figure 3.26c Map of mean thorium concentrations.

Sites clustered around Potchefstroom – 10, 14, 17, 20 and 21 – were all relatively high. The highest mean concentration was at Site 15. Other sites that also had high mean concentrations were 1, 5, 7, and 16. All sites in the Eastern Cape were low.

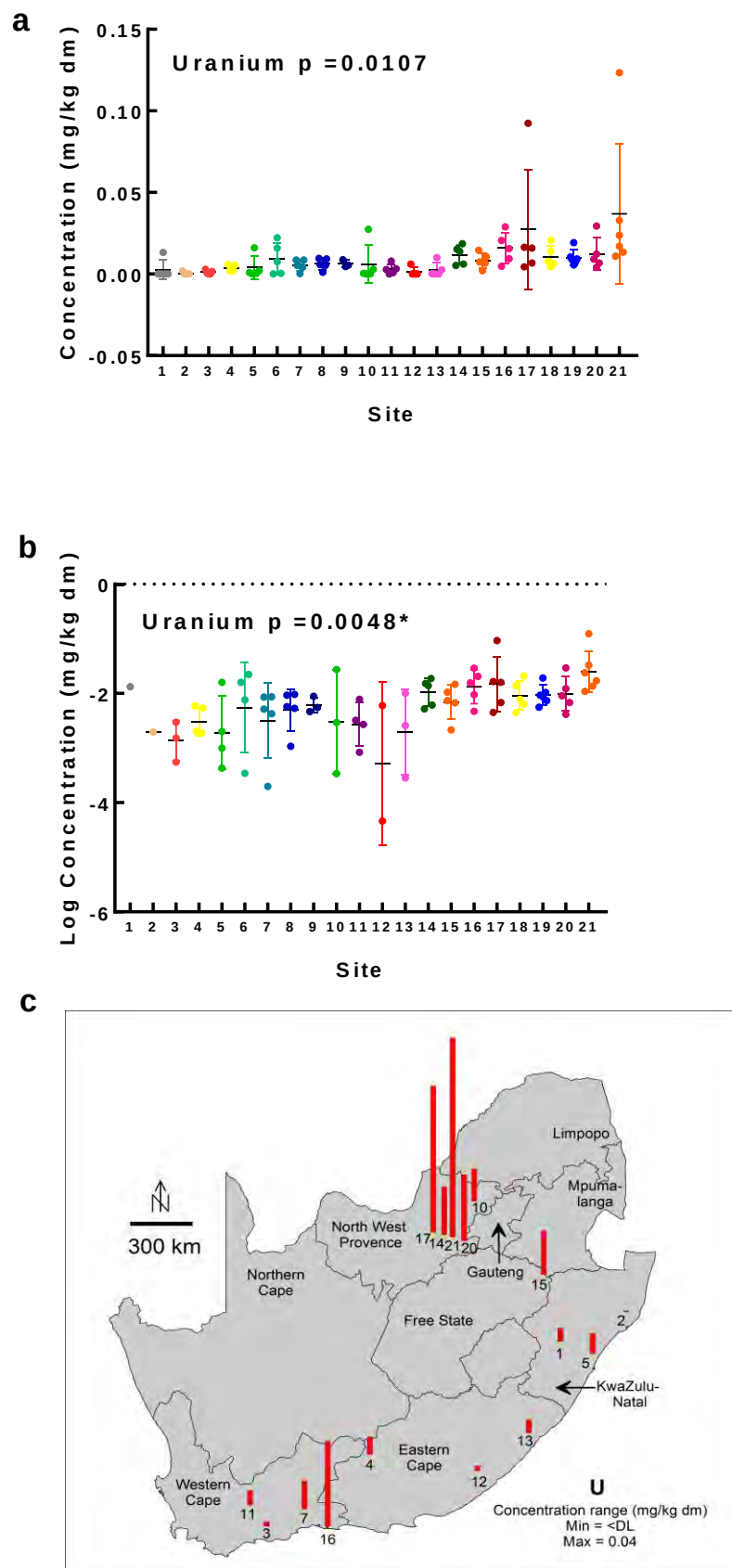


Figure 3.27 Distribution of uranium according to sites.

Figure 3.27a Scatterplot of untransformed uranium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.27b Scatterplot of log-transformed uranium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 1, 2, 4, 6, and 12. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 12 vs. 21.

Figure 3.27c Map of mean uranium concentrations.

Sites 17 and 21 were noticeably higher. Other sites that also had a high mean concentration were 14, 15, 16, and 20. All other sites were low in comparison.

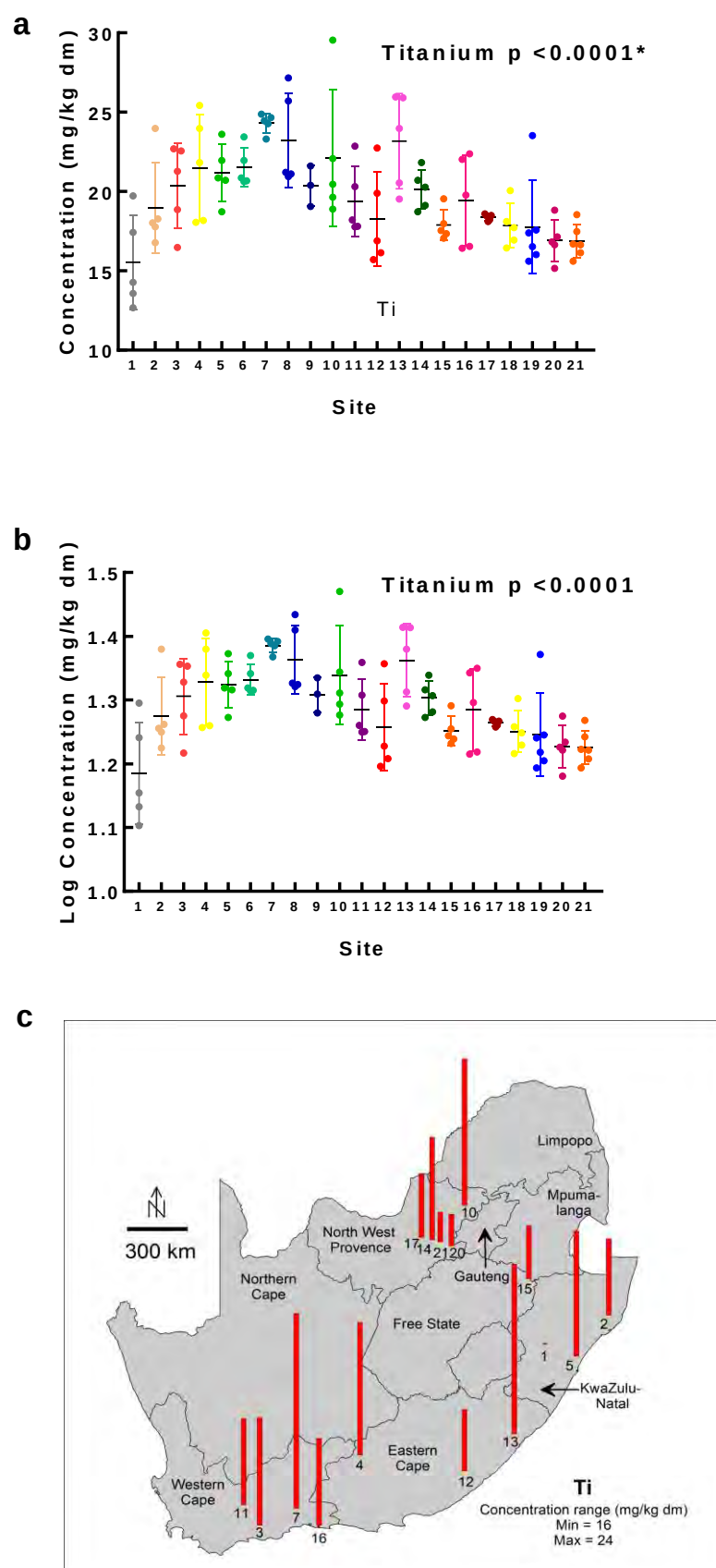


Figure 3.28 Distribution of titanium according to sites.

Figure 3.28a Scatterplot of untransformed titanium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 2 and 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 4, 5, 6, 7, 8, 10, 13; 7 vs. 12, 15, 17, 18, 19, 20, 21; 8 vs. 19, 20, 21 and 13 vs. 19, 20, 21.

Figure 3.28b Scatterplot of log-transformed titanium data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.28c Map of mean titanium concentrations.

All sites had noticeably high mean concentrations with the exception of Site 1. Site 1 had extremely low mean concentrations in comparison. Sites 7 and 13 had the highest mean concentration.

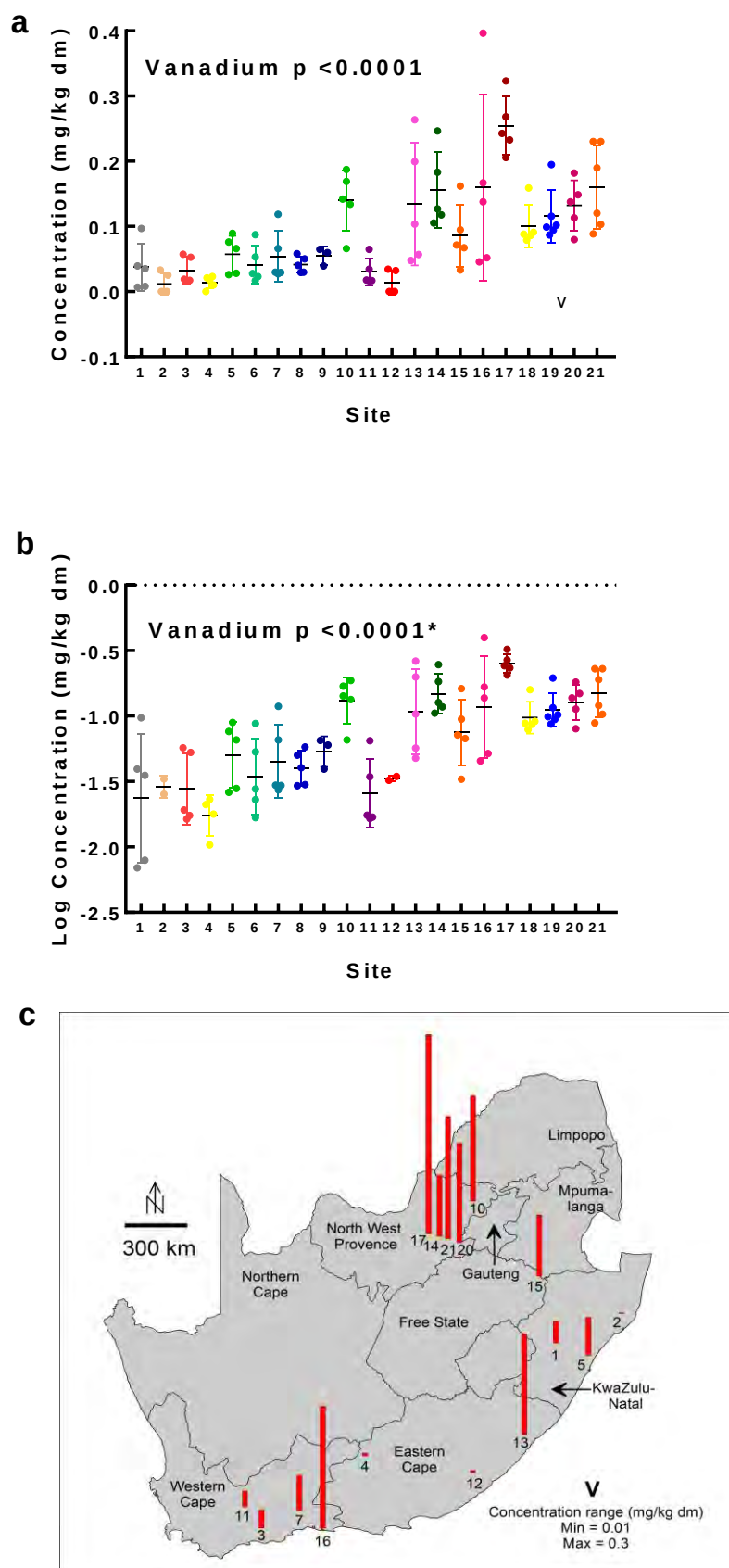


Figure 3.29 Distribution of vanadium according to sites.

Figure 3.29a Scatterplot of untransformed vanadium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.29b Scatterplot of log-transformed vanadium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 2, 3, 12, 18, and 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 10, 13, 14, 16, 17, 18, 19, 20, 21; 2 vs. 17; 3 vs. 10, 13, 14, 16, 17, 19, 20, 21; 4 vs. 10, 13, 14, 15, 16, 17, 18, 19, 20, 21; 5 vs. 17; 6 vs. 10, 14, 17, 21; 7 vs. 17; 8 vs. 14, 17, 21; 9 vs. 17; 10 vs. 11; 11 vs. 13, 14, 16, 17, 18, 19, 20, 21 and 12 vs. 17.

Figure 3.29c Map of mean vanadium concentrations.

Sites clustered around Potchefstroom – 10, 14, 17, 20, and 21 – all had noticeably high mean concentrations. Other sites that also had high mean concentrations were sites 13 and 16. Sites 2, 4, and 12 had extremely low mean concentrations.

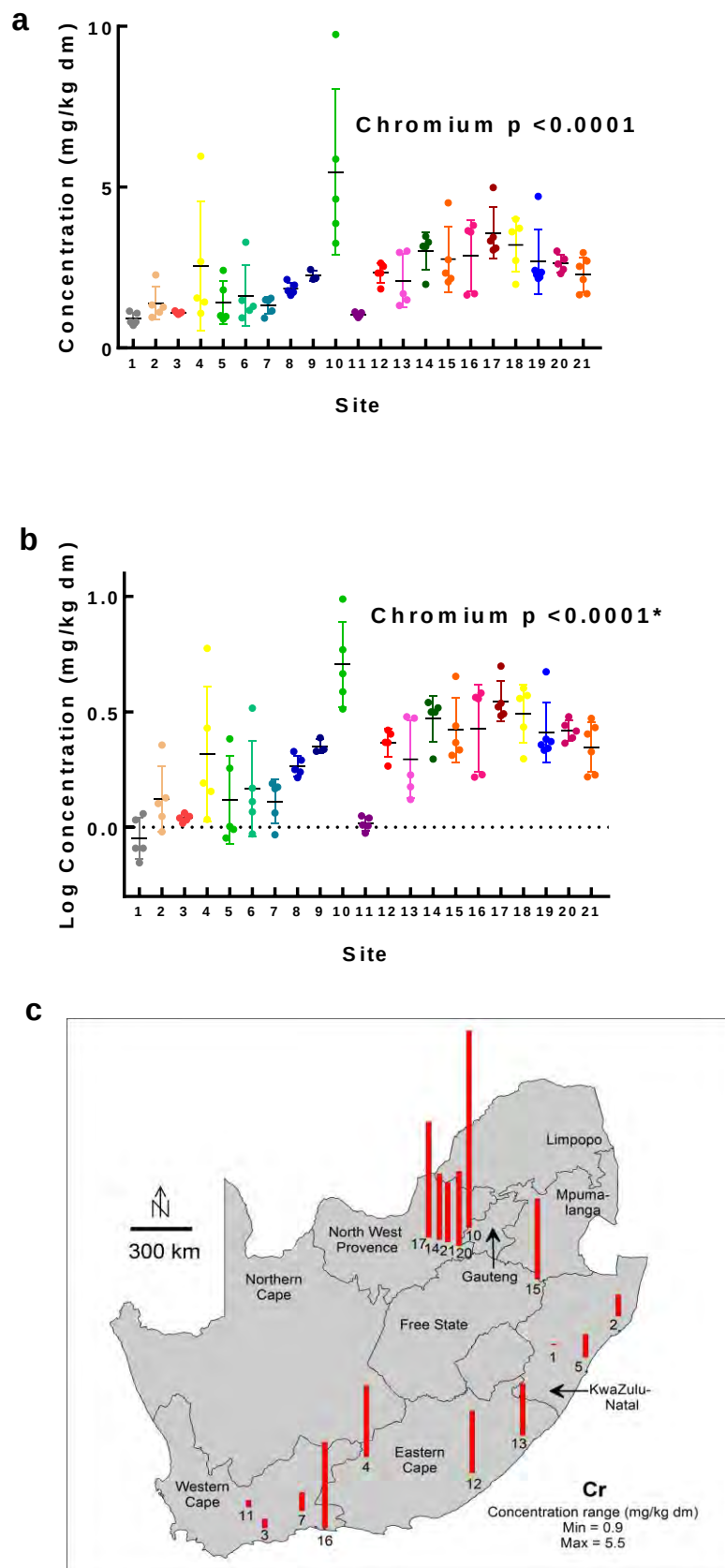


Figure 3.30 Distribution of chromium according to sites.

Figure 3.30a Scatterplot of untransformed chromium data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.30b Scatterplot of log-transformed chromium data according to sites. The means and standard deviations are indicated.

All distributions were normal except for sites 14, 16, 17, and 19. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 4, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21; 2 vs. 10, 14, 17, 18; 3 vs. 10, 12, 14, 15, 16, 17, 18, 19, 20; 4 vs. 10; 5 vs. 10, 14, 17, 18; 6 vs. 10, 17, 18; 7 vs. 10, 14, 17, 18; 8 vs. 10; 10 vs. 11, 12, 13, 21 and 11 vs. 12, 14, 15, 16, 17, 18, 19, 20, 21.

Figure 3.30c Map of mean chromium concentrations.

Sites clustered around Potchefstroom (10, 14, 17, 20, and 21 had elevated concentrations of chromium). Sites that had notably lower mean concentrations are all three from KwaZulu-Natal and sites from Western Cape, with the exception of 16. Sites 4, 12, 13, 15 and 16 were also relatively high.

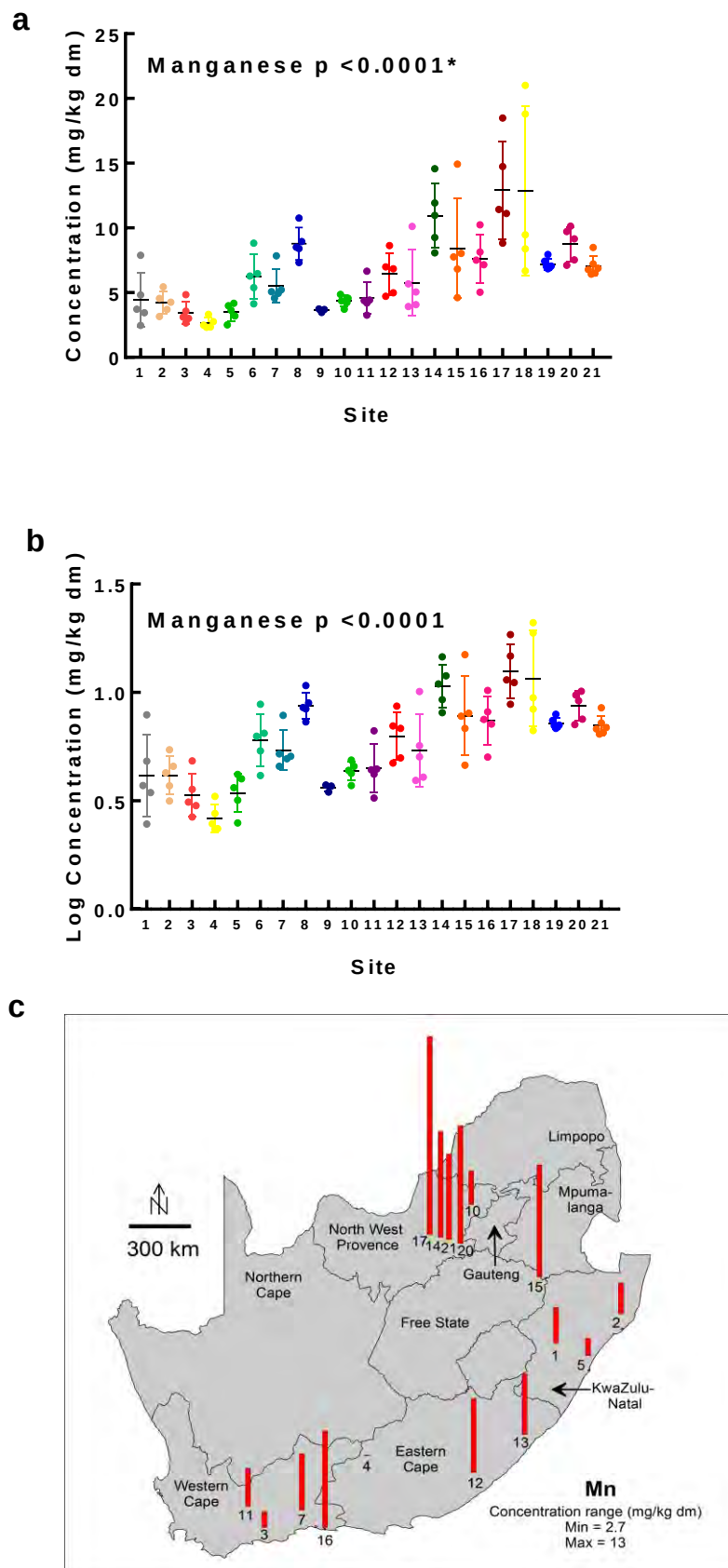


Figure 3.31 Distribution of manganese according to sites.

Figure 3.31a Scatterplot of untransformed manganese data according to sites. The means and standard deviations are indicated.

All distributions were normal except for Site 7. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 14, 17, 18; 2 vs. 14, 17, 18; 3 vs. 8, 14, 17, 18, 20; 4 vs. 8, 14, 15, 17, 18, 20; 5 vs. 14, 17, 18; 6 vs. 17, 18; 7 vs. 14, 17, 18; 9 vs. 14, 17, 18; 10 vs. 14, 17, 18; 11 vs. 14, 17, 18; 12 vs. 17, 18; 13 vs. 17, 18; 16 vs. 17; 17 vs. 19, 21 and 18 vs. 19, 21.

Figure 3.31b Scatterplot of log-transformed manganese data according to sites. The means and standard deviations are indicated.

Because the untransformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.31c Map of mean manganese concentrations.

All sites had relatively high mean concentrations. Site 4 had the lowest mean concentration. Other sites that had low mean concentrations were 1, 2, 3, 5, and 10. The highest mean concentration was at Site 17.

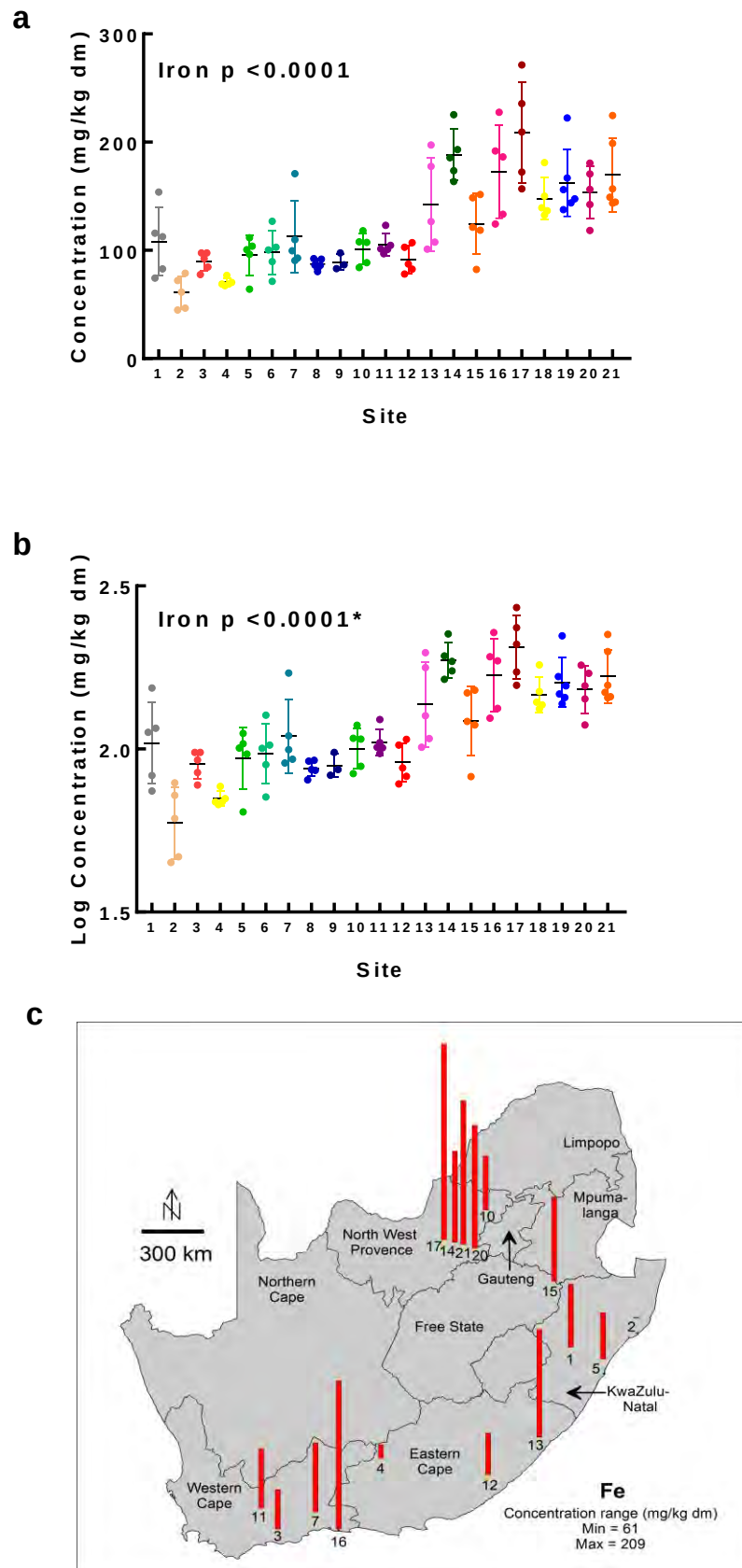


Figure 3.32 Distribution of iron according to sites.

Figure 3.32a Scatterplot of untransformed iron data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.32b Scatterplot of log-transformed iron data according to sites. The means and standard deviations are indicated.

All distributions were normal. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2, 14, 16, 17, 21; 2 vs. 5, 6, 7, 10, 11, 13, 14, 14, 15, 16, 17, 18, 19, 20, 21; 3 vs. 14, 16, 17, 18, 19, 20, 21; 4 vs. 13, 14, 15, 16, 17, 18, 19, 20, 21; 5 vs. 14, 16, 17, 18, 19, 20, 21; 6 vs. 14, 16, 17, 19, 20, 21; 7 vs. 14, 17; 8 vs. 13, 14, 16, 17, 18, 19, 20, 21; 9 vs. 14, 9 vs. 16, 17, 19, 20, 21; 10 vs. 14, 16, 17, 19, 21; 10 vs. 16; 17, 19, 21; 11 vs. 14, 17, 21; 12 vs. 14, 16, 17, 18, 19, 20, 21 and 15 vs. 17.

Figure 3.32c Map of mean iron concentrations.

All sites had high mean concentrations except Site 2 and 4. The highest mean concentration was at Site 17 and the lowest Site 2.

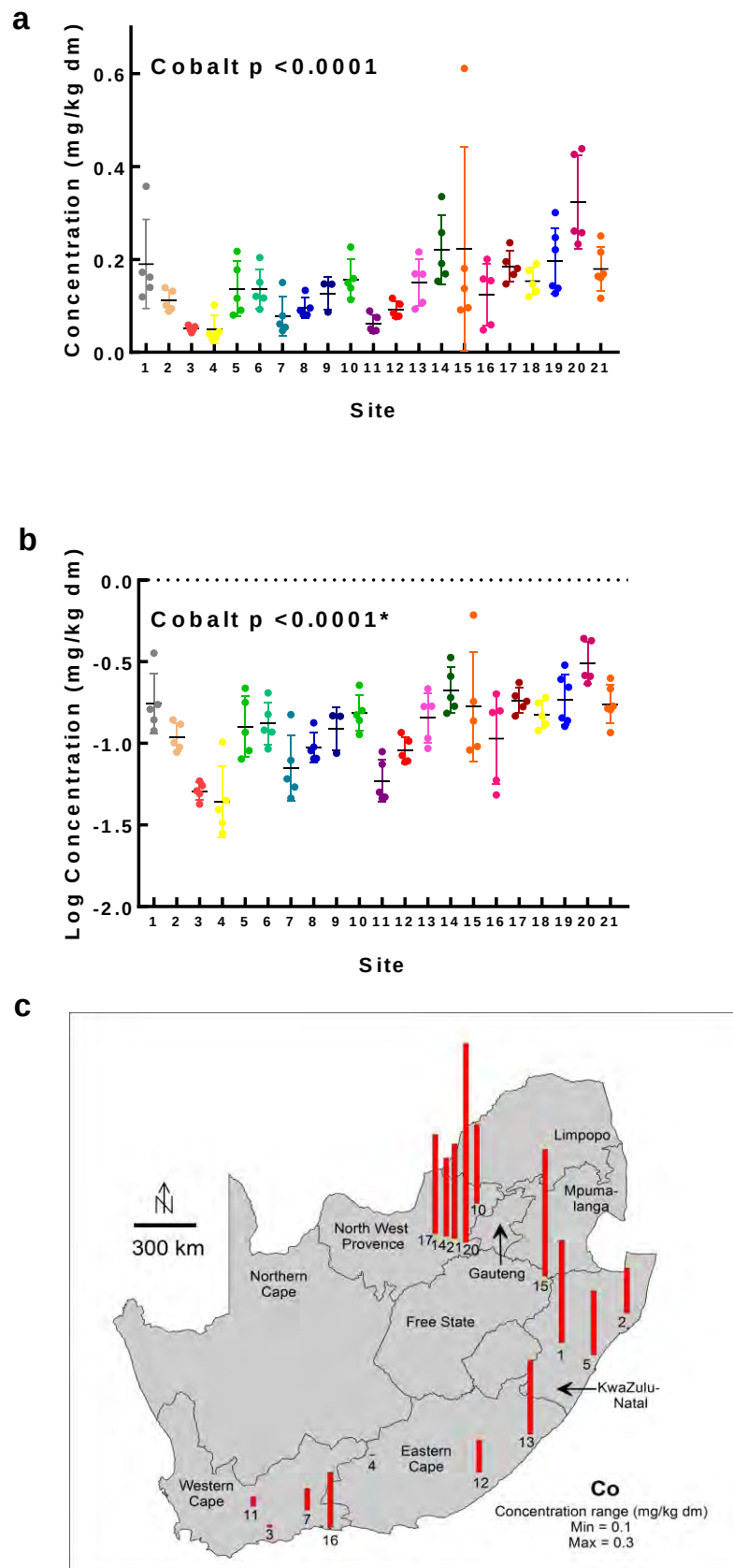


Figure 3.33 Distribution of cobalt according to sites.

Figure 3.33a Scatterplot of untransformed cobalt data according to sites. The means and standard deviations are indicated.

The untransformed concentrations were not distributed normally. Because the log-transformed concentrations were normally distributed, this graph will not be discussed further.

Figure 3.33b Scatterplot of log-transformed cobalt data according to sites. The means and standard deviations are indicated.

All distributions were normal. One-way ANOVA showed significant differences between sites ($p < 0.0001$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 3, 4, 7, 11; 2 vs. 4, 20; 3 vs. 5, 6, 10, 13, 14, 15, 17, 18, 19, 20, 21; 4 vs. 5, 6, 9, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21; 5 vs. 20; 7 vs. 14, 17, 19, 20, 21; 8 vs. 20; 10 vs. 11; 11 vs. 13, 14, 15, 17, 18, 19, 20, 21; 12 vs. 20; 16 vs. 20.

Figure 3.33c Map of mean cobalt concentrations.

All sites had measurable mean concentrations except sites 3 and 4 that were relatively low. Sites in the North West Province, Mpumalanga, and KwaZulu-Natal were all high. The Eastern Cape and Western Cape sites had low as well as high mean concentrations.

3.3 Nonmetric multidimensional scaling site results

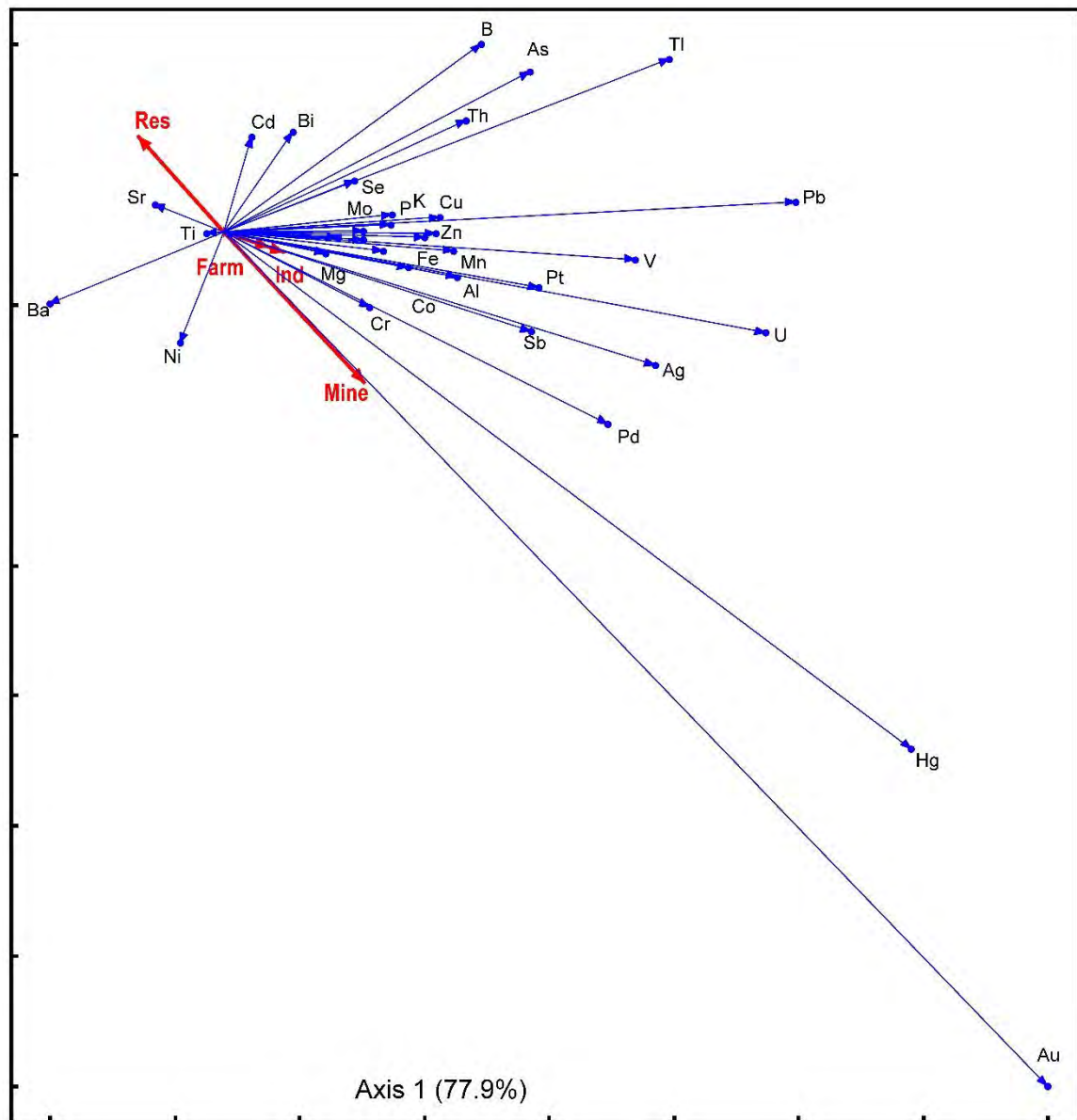


Figure 3.34 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%. Only the elements are shown in this plot to allow easier interpretation of the relative changes in elemental contributions. The environmental factors are shown as red vectors.

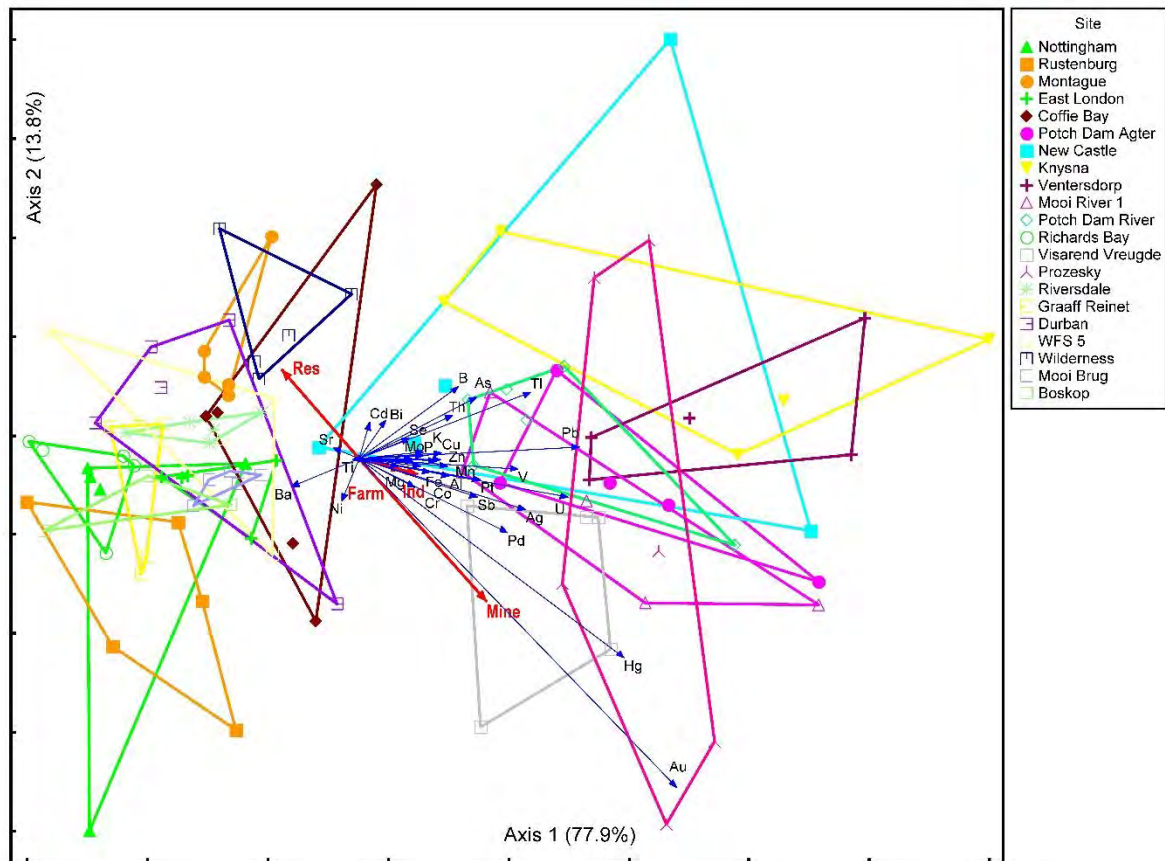


Figure 3.35 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%. The environmental factors are shown as red vectors. Convex hulls for the 21 sites are shown, together with the elements.

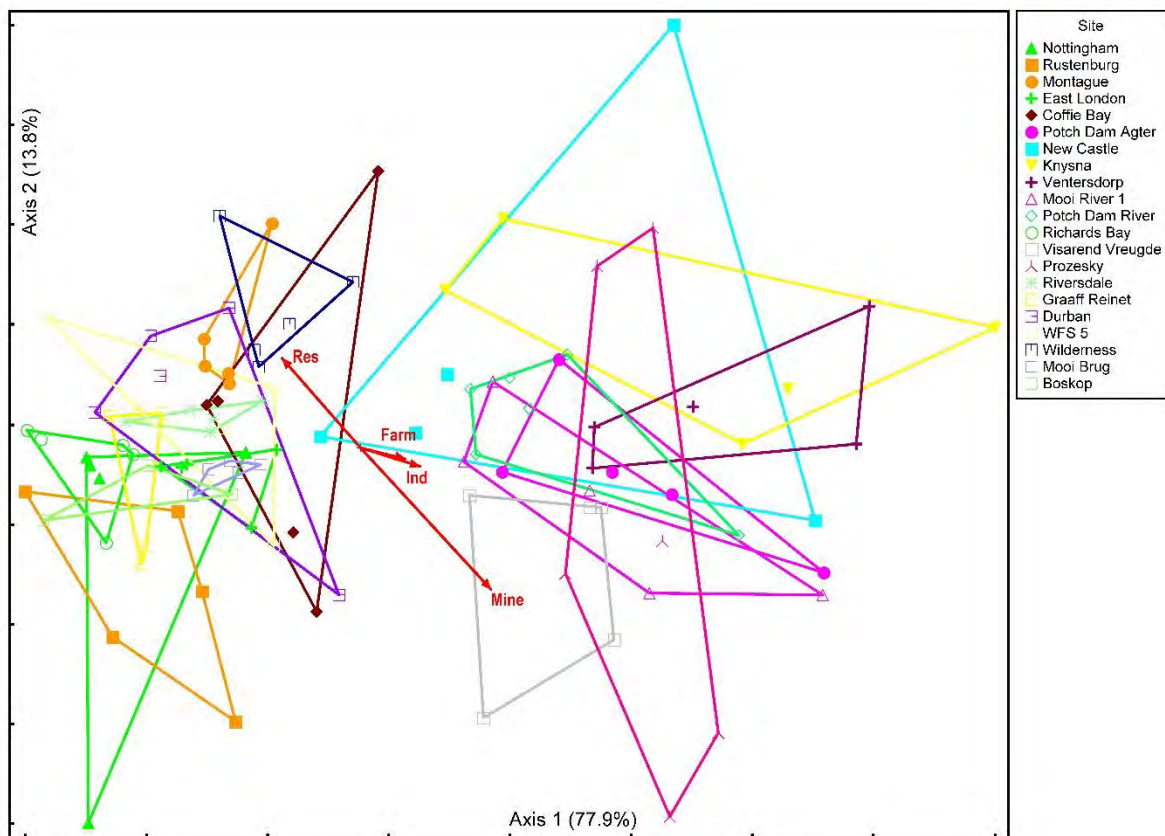


Figure 3.36 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%. The elements are not shown to allow easier interpretation of the relative positions of the 21 sites, shown here as convex hulls. The environmental factors are shown as red vectors.

Only two dimensions were needed for NMS ordination. Final stress was 13.6, and the final instability was 0.0000. According to Clarke's rule of thumb (McCune and Grace, 2002) a final stress between 10 and 20 presents a usable picture for interpretation. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%. The 21 sites are indicated by convex hulls in Figure 3.35. Elements such as Au and Hg ordinated with sites associated with mining activity, whereas Sr and Cd ordinated with sites associated with residential areas. Farming and Industry were not associated with any element. The convex hulls of the 21 sites, with only the environmental factors (in red) are shown in Figure 3.36.

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3.4 Size and species results

Scatterplots of all metallic elements that have been analysed.

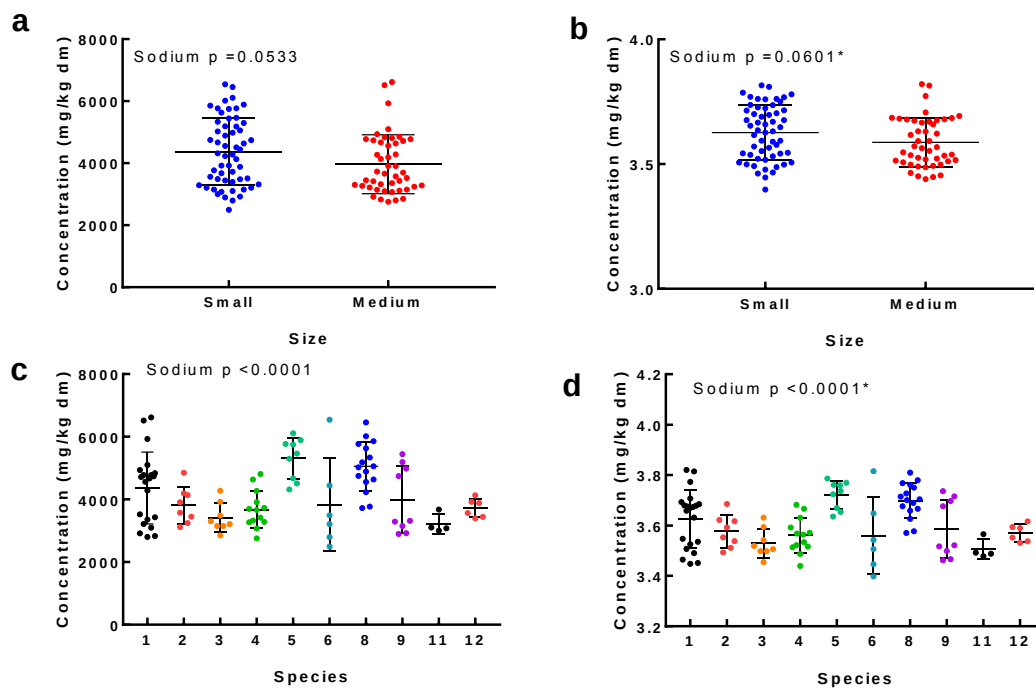


Figure 3.37 Scatterplots of sodium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

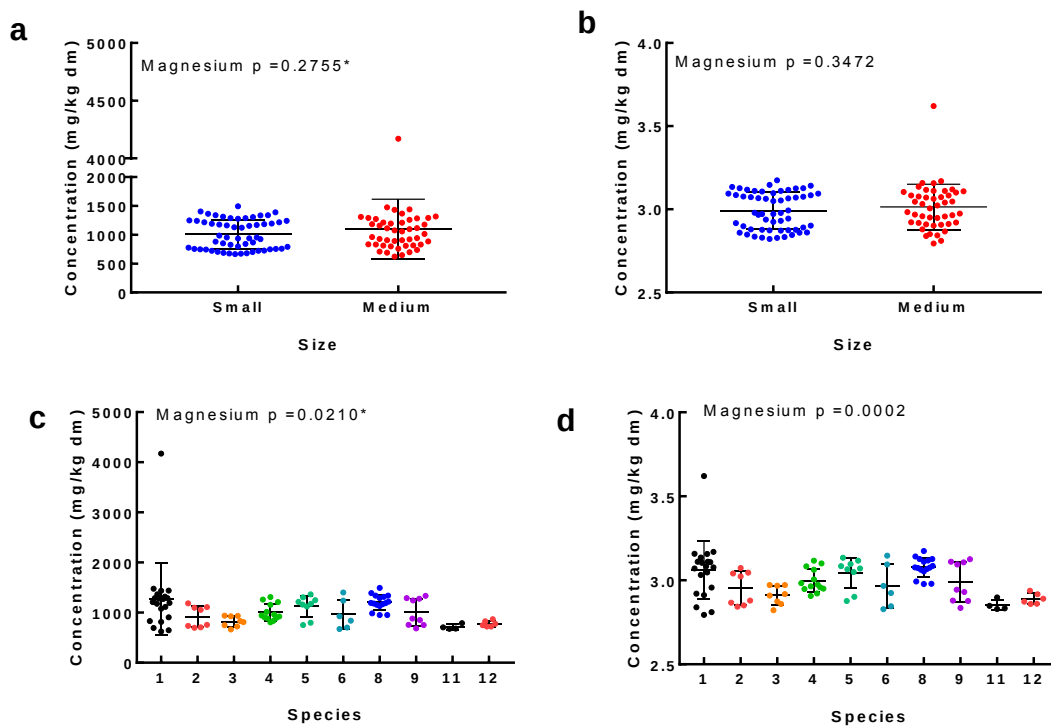


Figure 3.38 Scatterplots of magnesium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.37a Scatterplot of untransformed sodium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of both small and medium species were not normally distributed, this graph will not be discussed further.

Figure 3.37b Scatterplot of log-transformed sodium data according to size. The means and standard deviations are indicated.

Only the concentrations for the medium sized group was normally distributed. Unpaired two-way t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.37c Scatterplot of untransformed sodium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.37d Scatterplot of log-transformed sodium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 9. Unpaired, one-way ANOVA showed significant differences between species ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$).

Figure 3.38a Scatterplot of untransformed magnesium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.38b Scatterplot of log-transformed magnesium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.38c Scatterplot of untransformed magnesium concentrations according to species.

All concentrations are normal except 19. One-way ANOVA showed no significant differences between species between sites ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($p > 0.05$).

Figure 3.38d Scatterplot of log-transformed magnesium data. The untransformed concentrations were not normally distributed.

Because the transformed concentrations were normally distributed, this graph will not be discussed further.

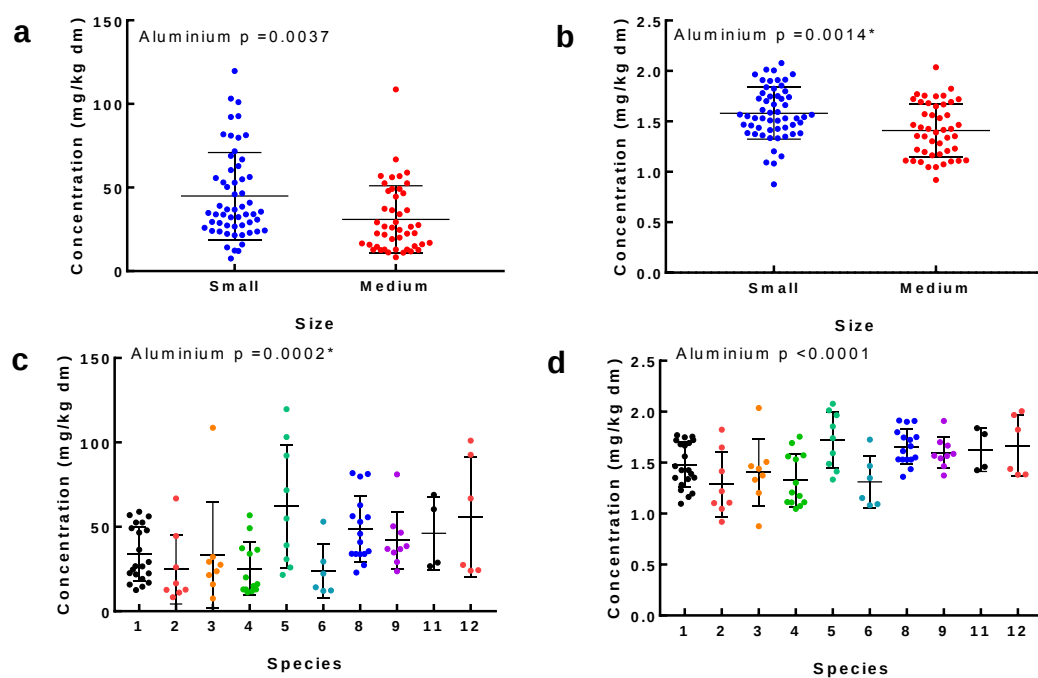


Figure 3.39 Scatterplots of aluminium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

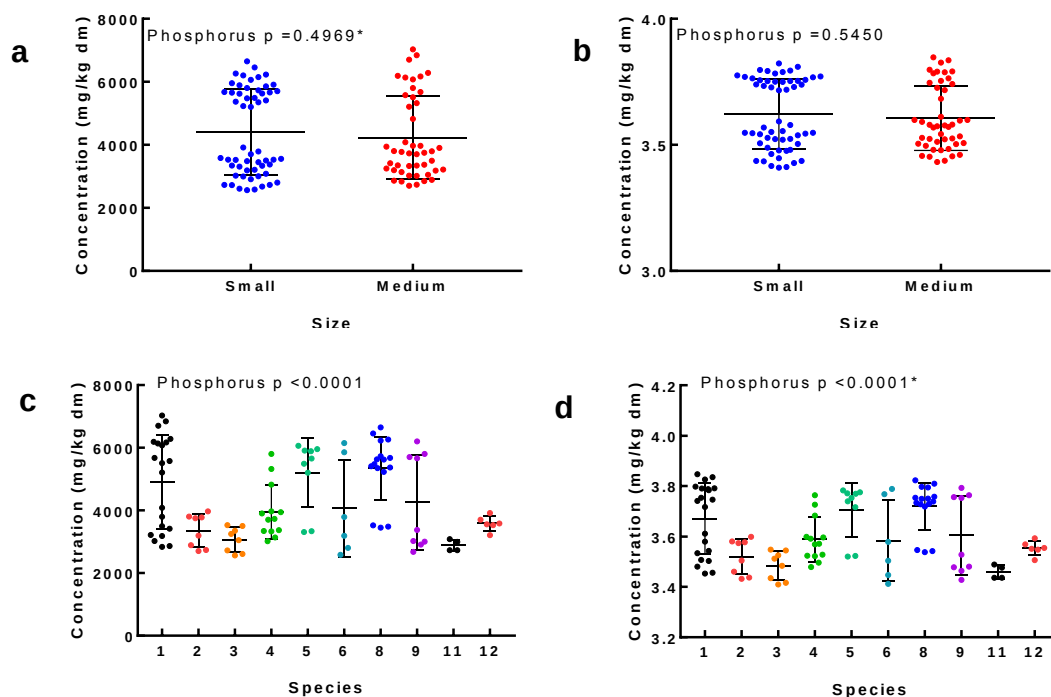


Figure 3.40 Scatterplots of phosphorus concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.39a Scatterplot of untransformed aluminium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.39b Scatterplot of log-transformed aluminium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.39c Scatterplot of untransformed aluminium concentrations according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1, 2, 3, 4 and 9. Unpaired, one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 6 and 4 vs. 5.

Figure 3.39d Scatterplot of log-transformed aluminium data according to species. The means and standard deviations are indicated.

Because the log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.40a Scatterplot of untransformed phosphorus data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.40b Scatterplot of transformed phosphorus data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.40c Scatterplot of untransformed phosphorus data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed except 1, 5, 8, and 9. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2, 3, 11; 2 vs. 5, 8; 3 vs. 5; 5 vs. 11 and 8 vs. 11

Figure 3.40d Scatterplot of log-transformed phosphorus data according to species. The means and standard deviations are indicated.

Because the log-transformed concentrations were not normally distributed, this graph will not be discussed further.

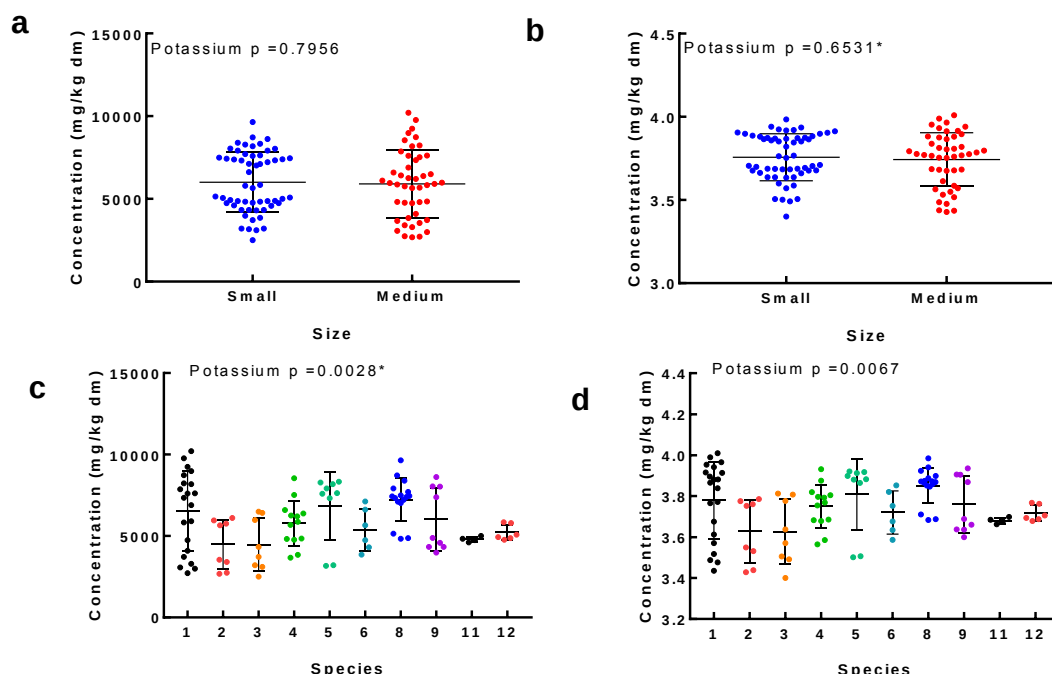


Figure 3.41 Scatterplots of potassium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

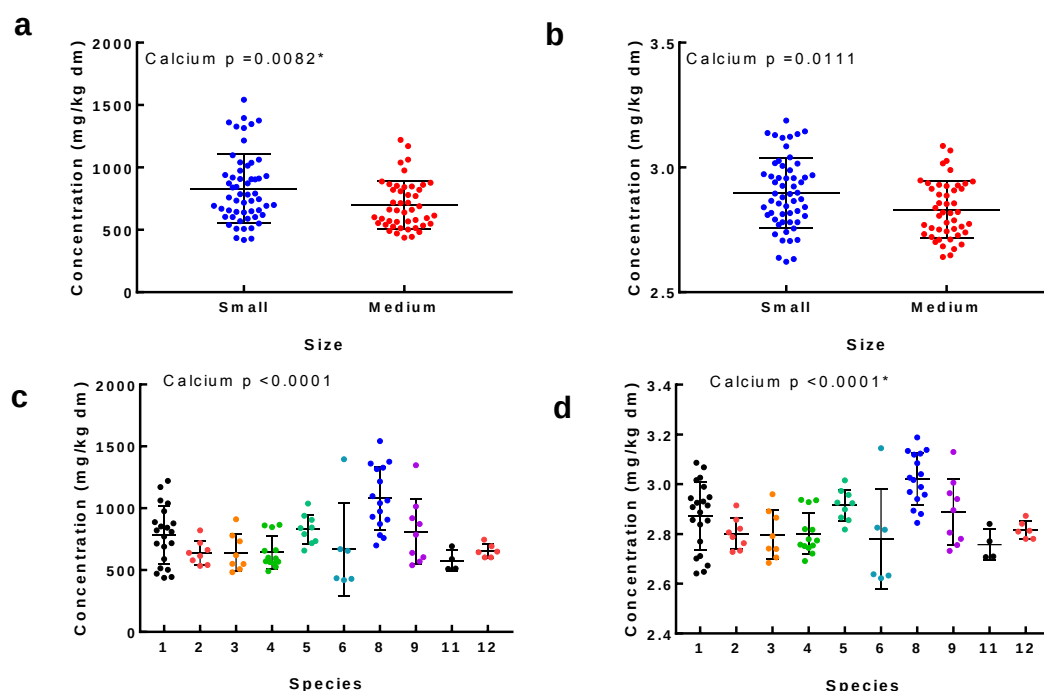


Figure 3.42 Scatterplots of calcium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.41a Scatterplot of untransformed potassium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed; this graph will not be discussed further.

Figure 3.41b Scatterplot of log-transformed potassium data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.41c Scatterplot of untransformed potassium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2, 5, and 9. Unpaired, one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 8 and 3 vs. 8.

Figure 3.41d Scatterplot of log-transformed potassium data according to species. The means and standard deviations are indicated.

Because the log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.42a Scatterplot of untransformed calcium data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.42a Scatterplot of log-transformed calcium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were normally distributed; this graph will not be discussed further.

Figure 3.42c Scatterplot of untransformed calcium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 8; 2 vs. 8; 3 vs. 8; 4 vs. 8; 6 vs. 8 and 8 vs. 11, 12.

Figure 3.42d Scatterplot of log-transformed calcium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

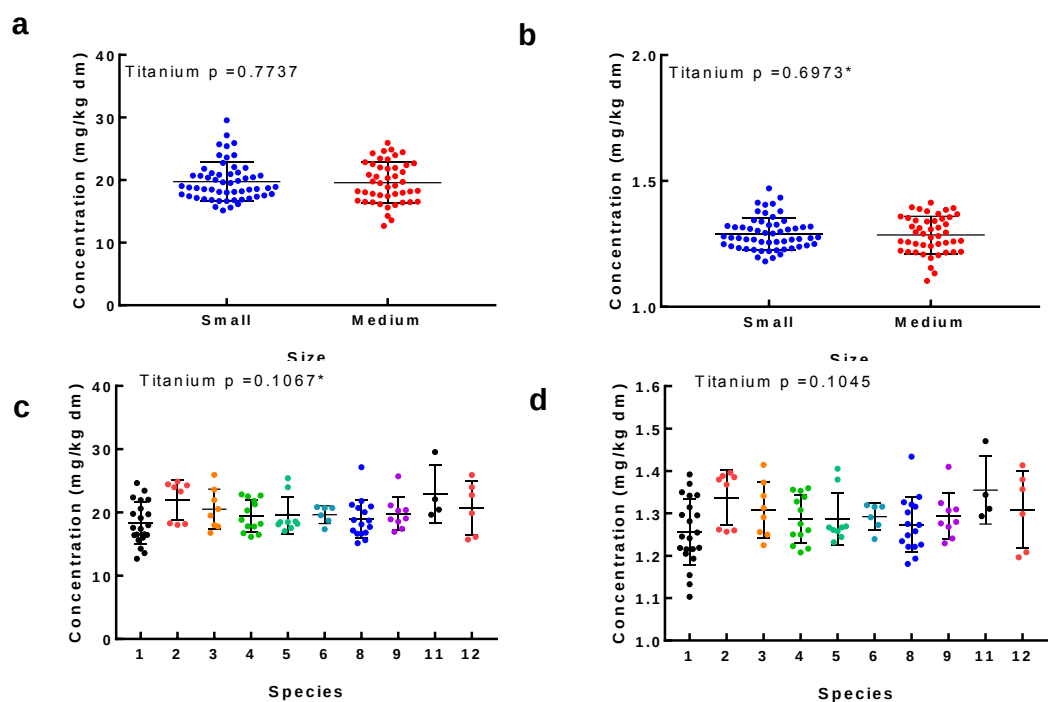


Figure 3.43 Scatterplots of titanium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

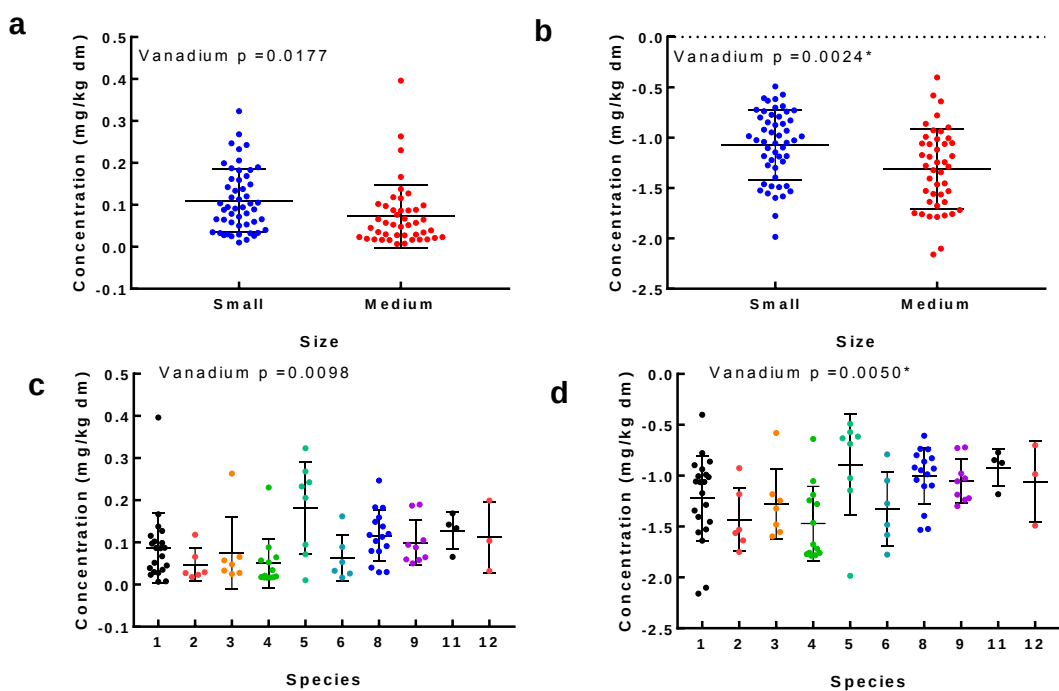


Figure 3.44 Scatterplots of vanadium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.43a Scatterplot of untransformed titanium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed; this graph will not be discussed further.

Figure 3.43b Scatterplot of log-transformed titanium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.43c Scatterplot of untransformed titanium concentrations according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2 and 5. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($p > 0.05$).

Figure 3.43d Scatterplot of log-transformed titanium data according to species. The means and standard deviations are indicated.

Because the log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.44a Scatterplot of untransformed vanadium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the small sized group was normally distributed; this graph will not be discussed further.

Figure 3.44b Scatterplot of log-transformed vanadium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.44c Scatterplot of untransformed vanadium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.44d Scatterplot of log-transformed vanadium concentrations according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4 and 5. One-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 4 vs. 5, 8.

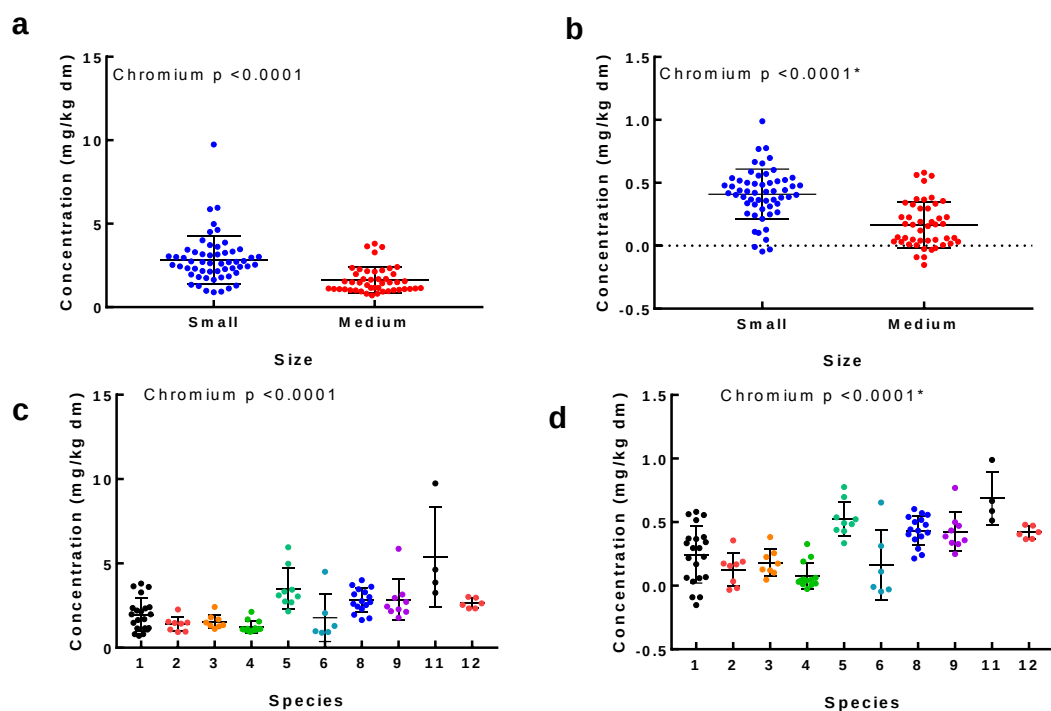


Figure 3.45 Scatterplots of chromium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

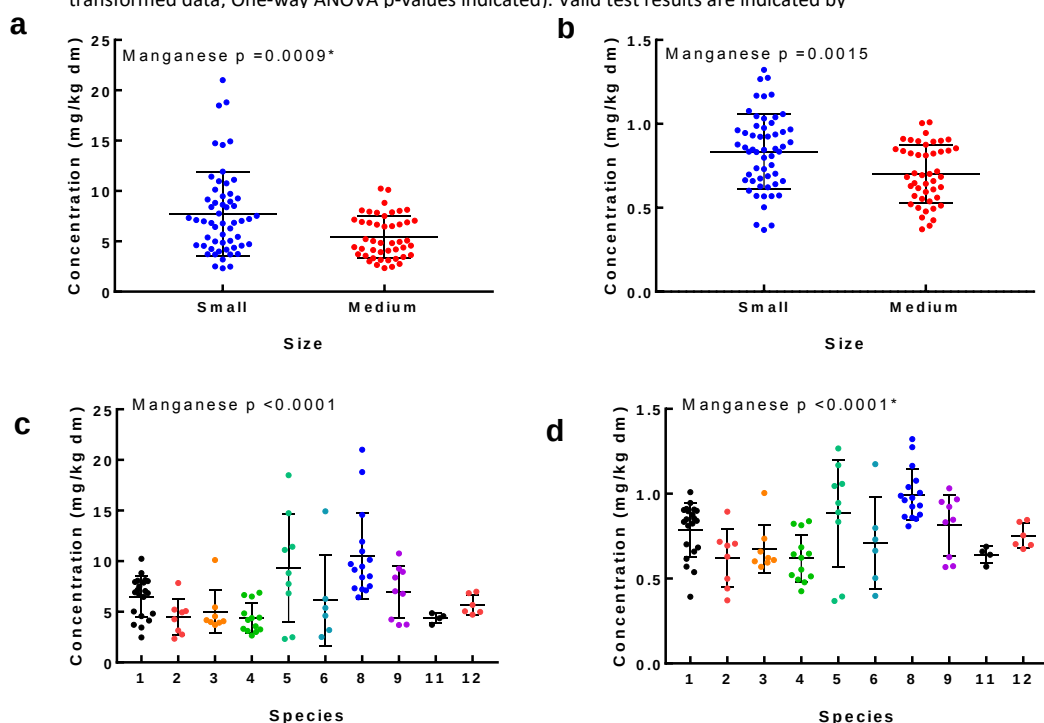


Figure 3.46 Scatterplots of manganese concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.45a Scatterplot of untransformed chromium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.45b Scatterplot of log-transformed chromium data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.45c Scatterplot of untransformed chromium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.45d Scatterplot of log-transformed chromium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 5, 8, 11; 2 vs. 5, 8, 9, 11, 12; 3 vs. 5, 8, 11, 12; 5 vs. 6 and 6 vs. 8, 11.

Figure 3.46a Scatterplot of untransformed manganese data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.46b Scatterplot of log-transformed manganese data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed; this graph will not be discussed further.

Figure 3.46c Scatterplot of untransformed manganese data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.46d Scatterplot of log-transformed manganese data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1 and 3. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 8; 2 vs. 8; 3 vs. 8; 4 vs. 5, 8; 6 vs. 8 and 8 vs. 11.

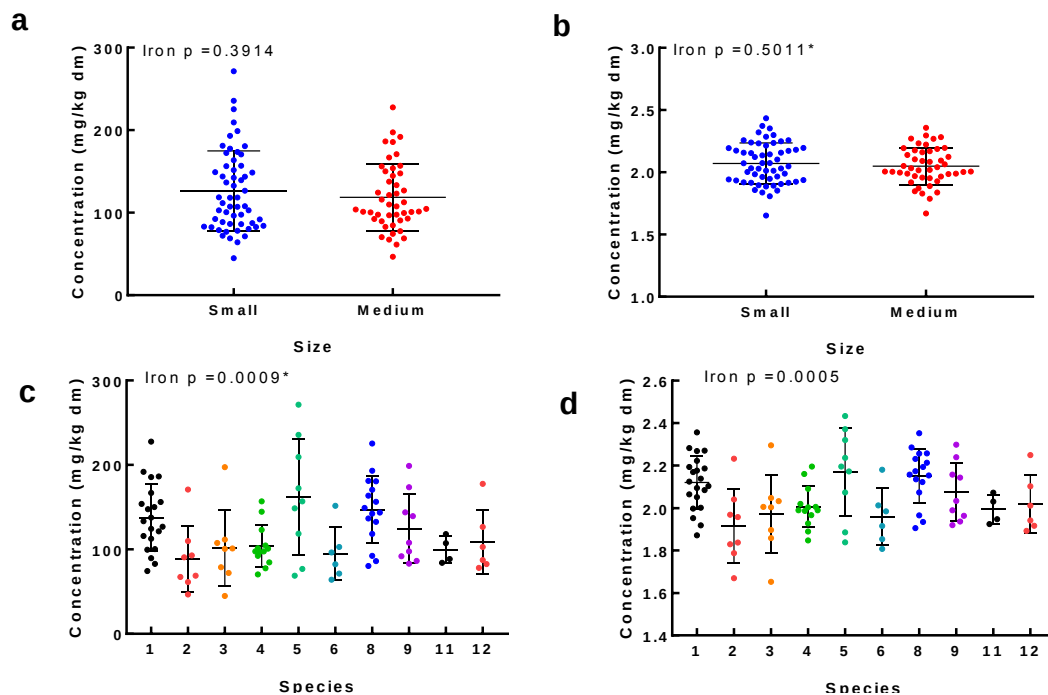


Figure 3.47 Scatterplots of iron concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

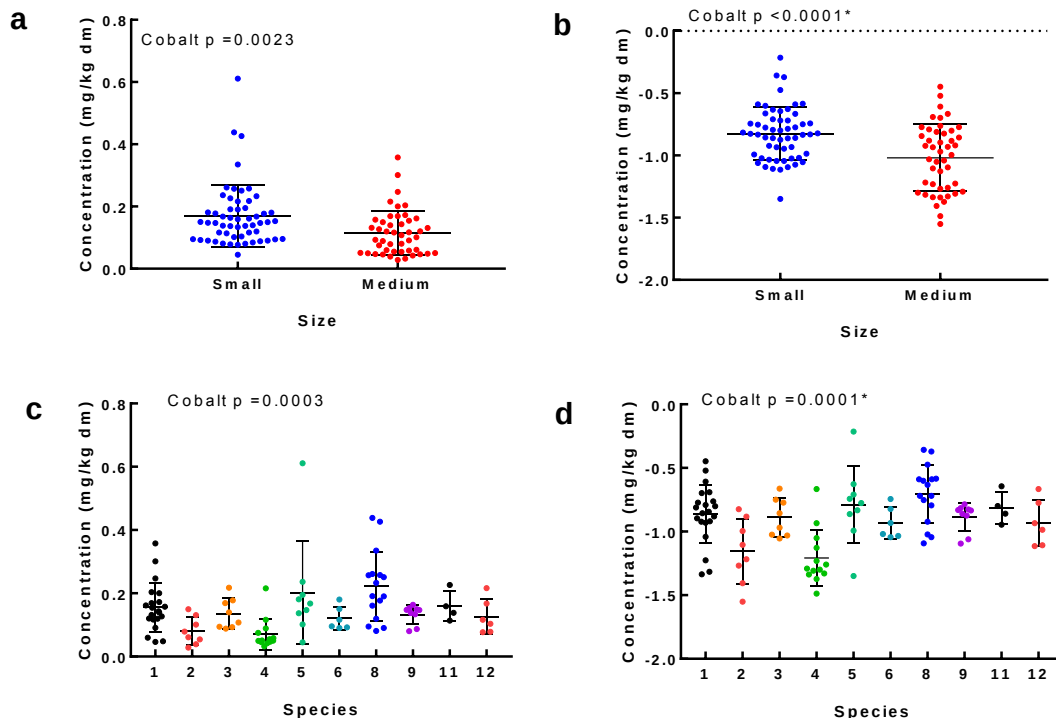


Figure 3.48 Scatterplots of cobalt concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.47a Scatterplot of untransformed iron data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed; this graph will not be discussed further.

Figure 3.47b Scatterplot of log-transformed iron concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.47c Scatterplot of untransformed iron concentrations according to species. The means and standard deviations are indicated.

All concentrations were normally distributed. One-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 5, 8 and 4 vs. 5.

Figure 3.47d Scatterplot of untransformed iron data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.48a Scatterplot of untransformed cobalt data according to size. The means and standard deviations are indicated.

The untransformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.48b Scatterplot of log-transformed cobalt data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.48c Scatterplot of untransformed cobalt data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.48d Scatterplot of log-transformed cobalt data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2, 4; 2 vs. 5, 8; 3 vs. 4 and 4 vs. 5, 8, 9.

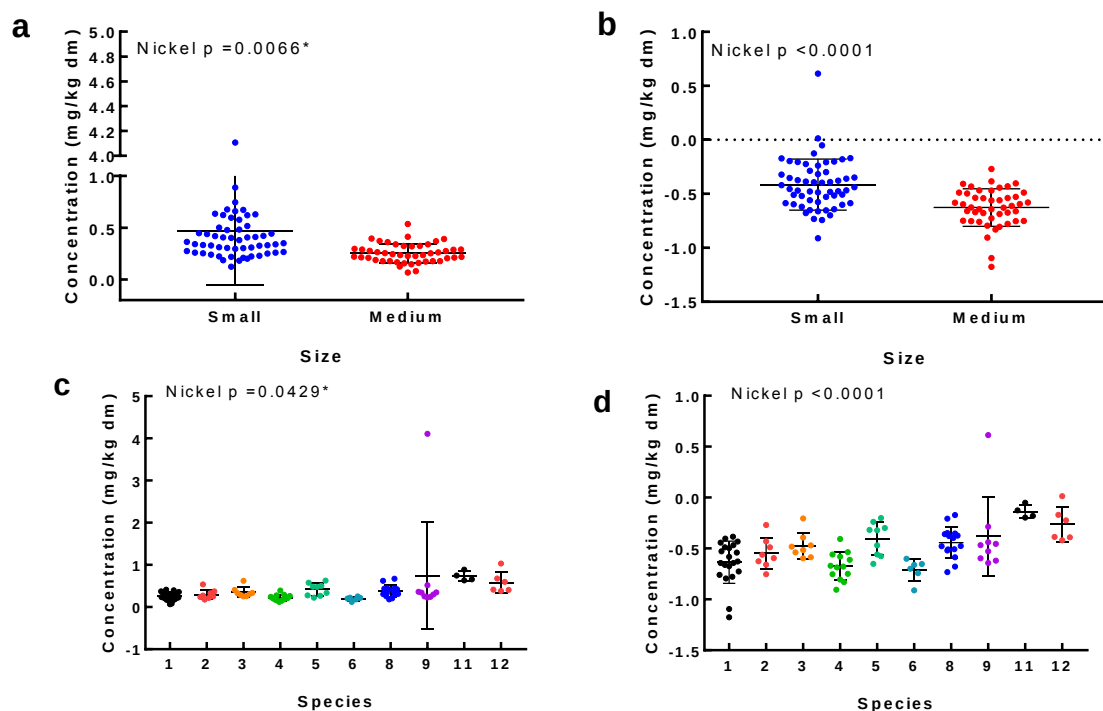


Figure 3.49 Scatterplots of nickel concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

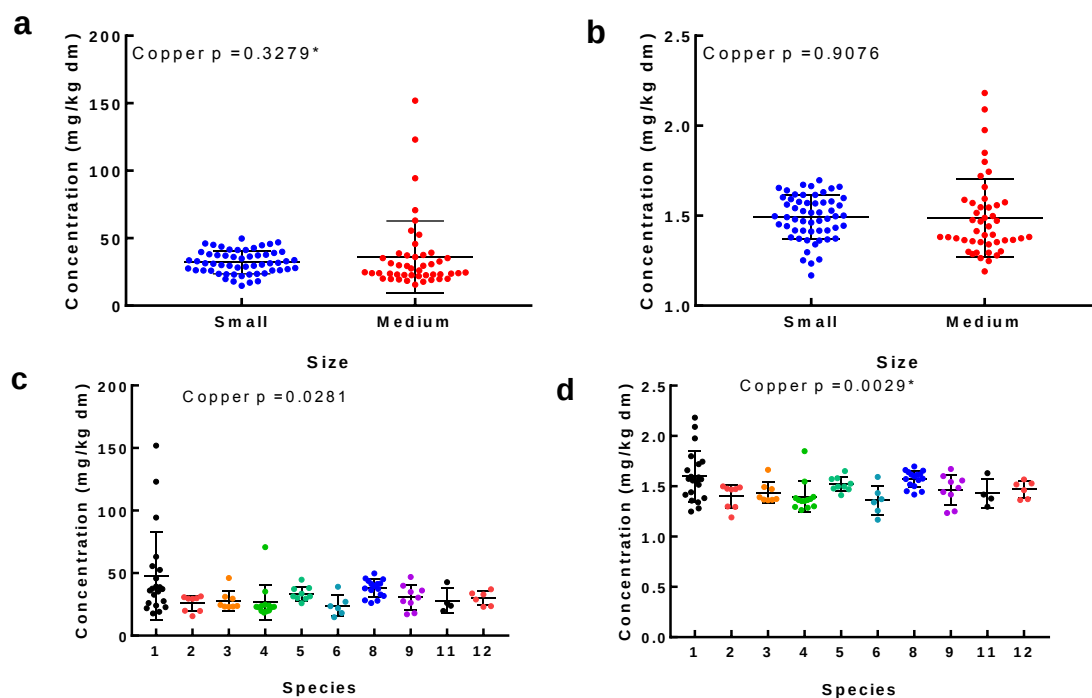


Figure 3.50 Scatterplots of copper concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.49a Scatterplot of untransformed nickel data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.49b Scatterplot of log-transformed nickel data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.49c Scatterplot of untransformed nickel data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 3 and 9. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons showed no significant differences between species ($p > 0.05$).

Figure 3.49d Scatterplot of log-transformed nickel data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.50a Scatterplot of untransformed copper concentrations according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the small sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.50b Scatterplot of log-transformed copper data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed; this graph will not be discussed further.

Figure 3.50c Scatterplot of untransformed copper data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.50d Scatterplot of log-transformed copper data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2, 3 and 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 4, 6.

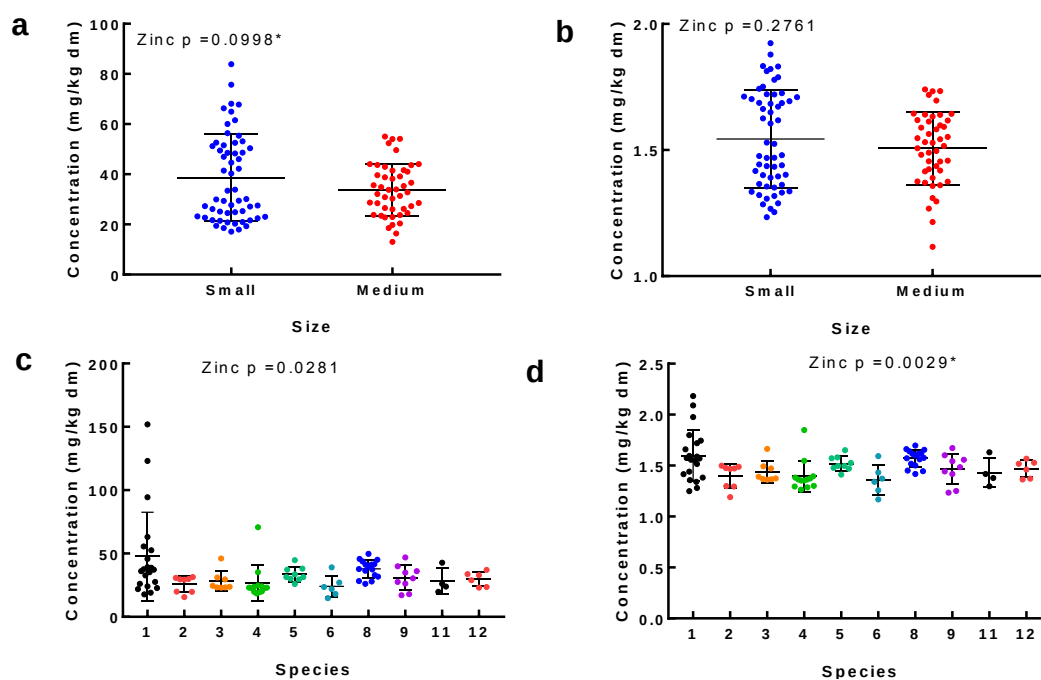


Figure 3.51 Scatterplots of zinc concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

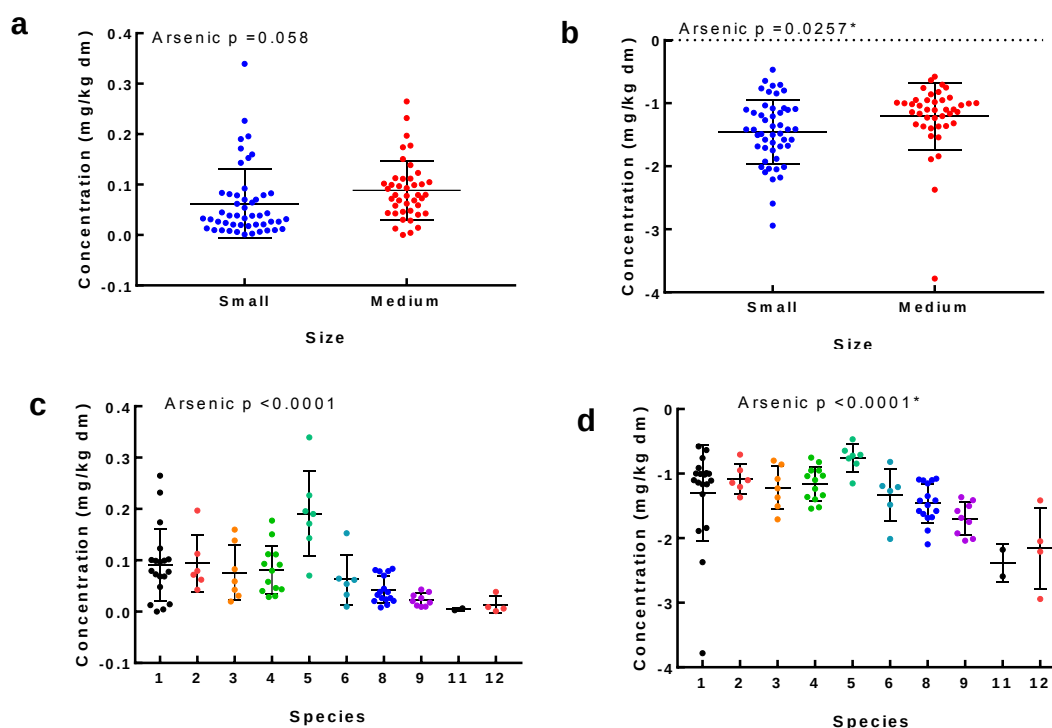


Figure 3.52 Scatterplots of arsenic concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.51a Scatterplot of untransformed zinc data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.51b Scatterplot of log-transformed zinc data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the medium sized group was normally distributed; this graph will not be discussed further.

Figure 3.51c Scatterplot of untransformed zinc data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.51d Scatterplot of transformed zinc data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2, 3 and 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 4 and 6.

Figure 3.52a Scatterplot of untransformed arsenic data according to size. The means and standard deviations are indicated.

The concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.52b Scatterplot of log-transformed arsenic data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.52c Scatterplot of untransformed arsenic data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.52d Scatterplot of log-transformed arsenic data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1. Species 11 had too low N values. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 12; 2 vs. 11, 12; 3 vs. 12; 4 vs. 11, 12 and 5 vs. 8, 9, 11, 12.

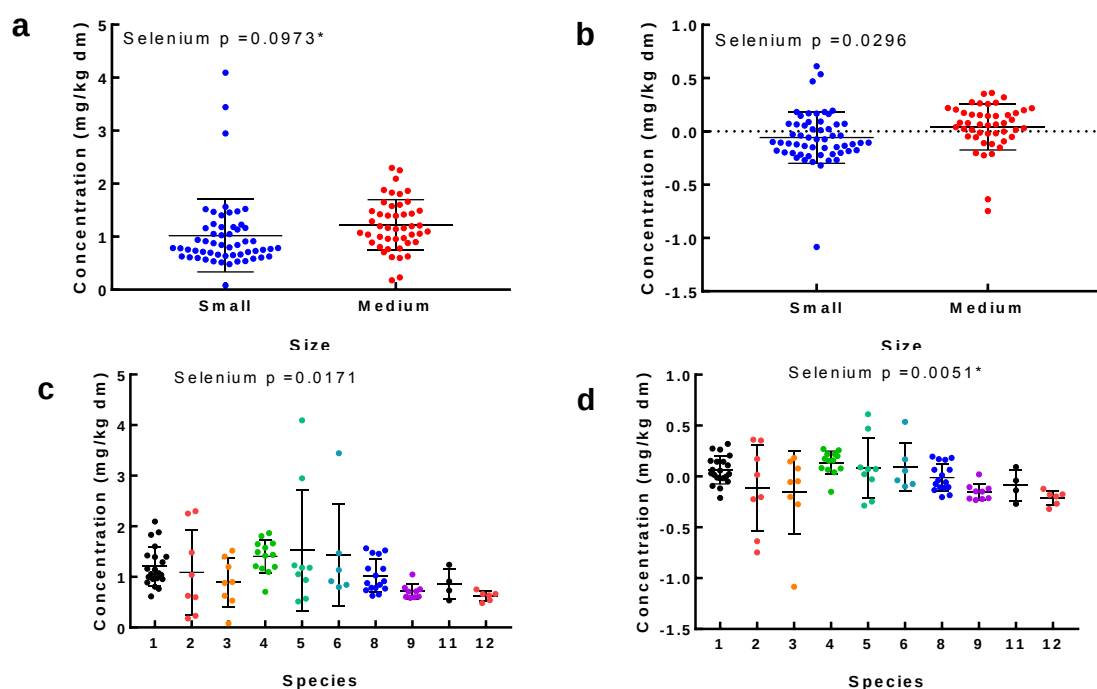


Figure 3.53 Scatterplots of selenium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

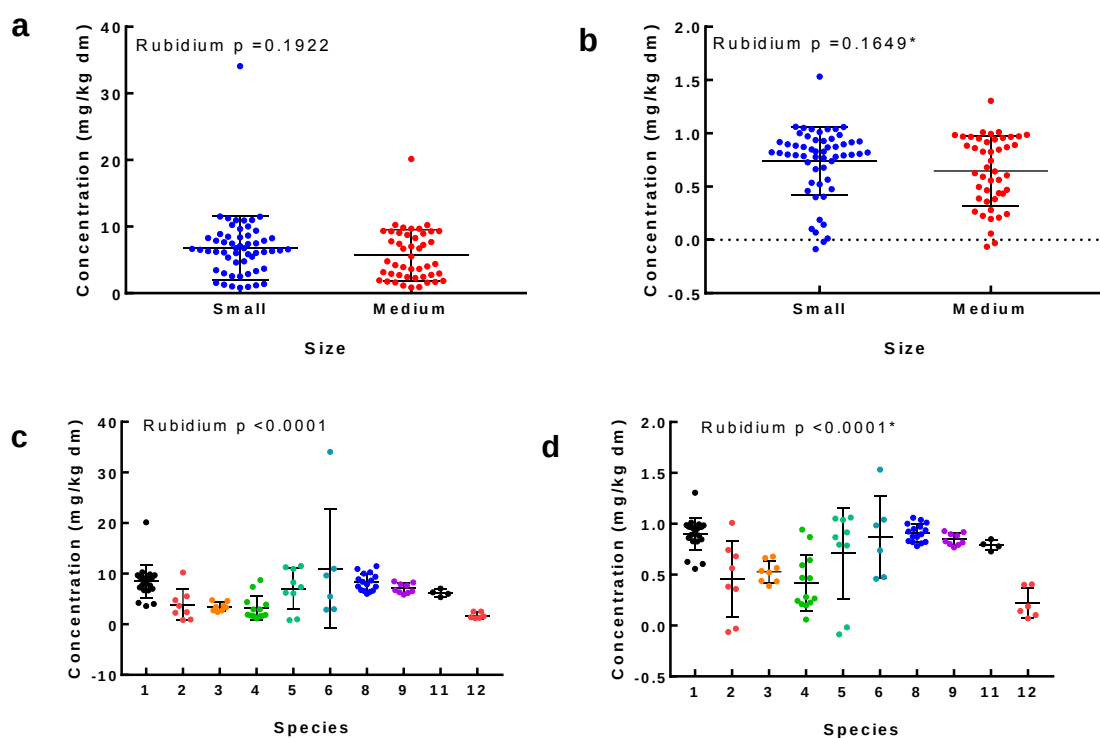


Figure 3.54 Scatterplots of rubidium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.53a Scatterplot of untransformed selenium concentrations according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.53b Scatterplot of log-transformed selenium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.53c Scatterplot of untransformed selenium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.53d Scatterplot of log-transformed selenium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 3. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons showed no significant differences between species ($p > 0.05$).

Figure 3.54a Scatterplot of untransformed rubidium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.54b Scatterplot of log-transformed rubidium concentrations according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.54c Scatterplot of untransformed rubidium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.54d Scatterplot of log-transformed rubidium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1 and 5. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2, 3, 4, 12; 2 vs. 8, 9; 3 vs. 8; 4 vs. 6, 8, 9; 5 vs. 12; 6 vs. 12; 8 vs. 12; 9 vs. 12 and 11 vs. 12.

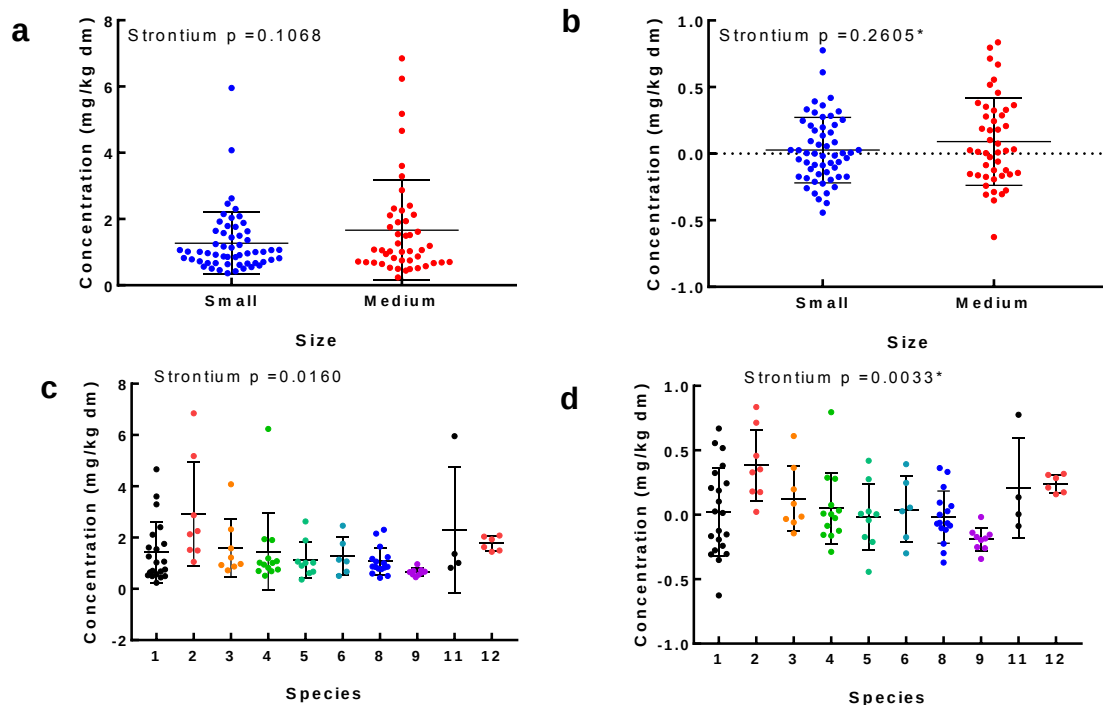


Figure 3.55 Scatterplots of strontium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

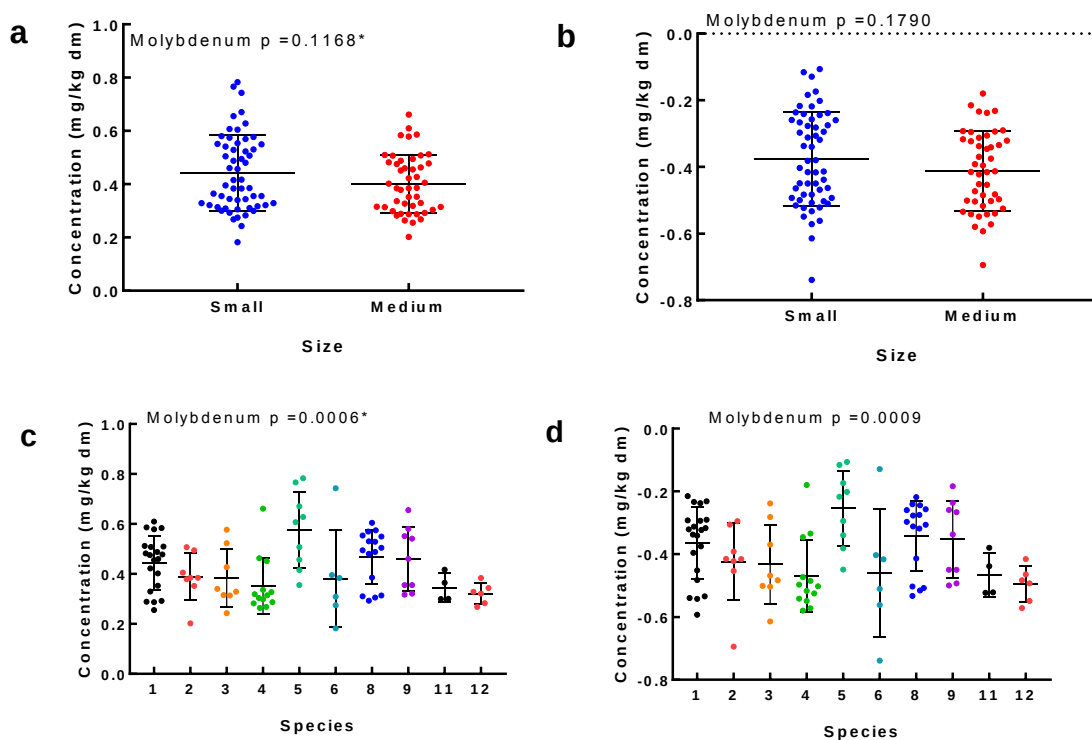


Figure 3.56 Scatterplots of molybdenum concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.55a Scatterplot of untransformed strontium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.55b Scatterplot of log-transformed strontium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.55c Scatterplot of untransformed strontium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.55d Scatterplot of log-transformed strontium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2 and 2 vs. 8, 9.

Figure 3.56a Scatterplot of untransformed molybdenum concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.56b Scatterplot of log-transformed molybdenum data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were normally distributed; this graph will not be discussed further.

Figure 3.56c Scatterplot of untransformed molybdenum data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4 and 8. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 2 vs. 5; 3 vs. 5; 4 vs. 5 and 5 vs. 11, 12.

Figure 3.56d Scatterplot of log-transformed molybdenum data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

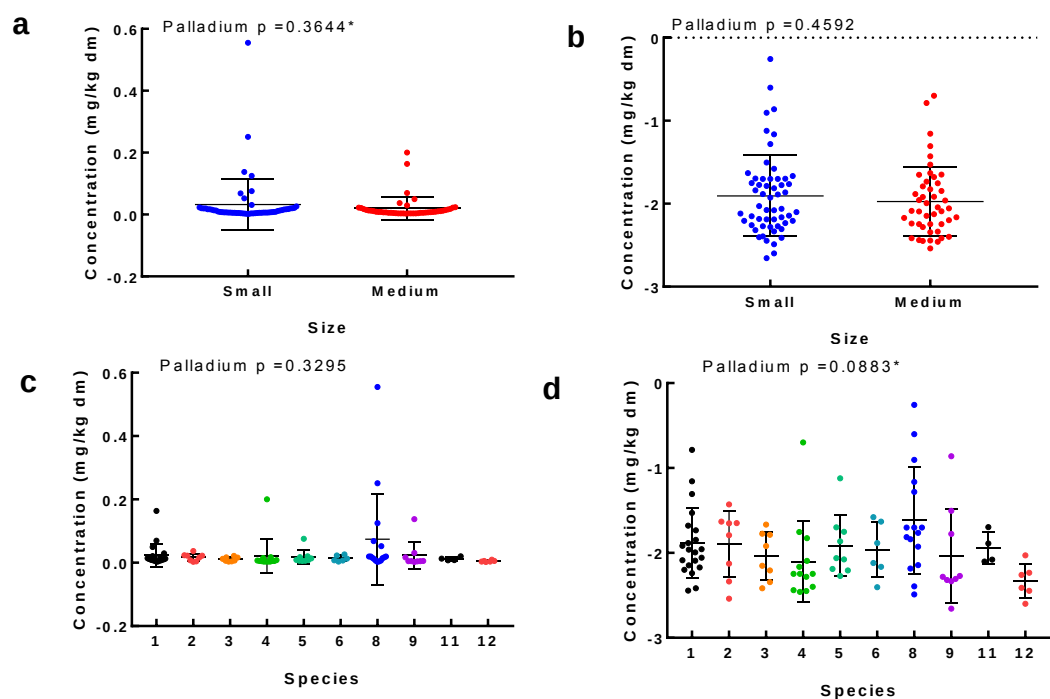


Figure 3.57 Scatterplots of palladium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

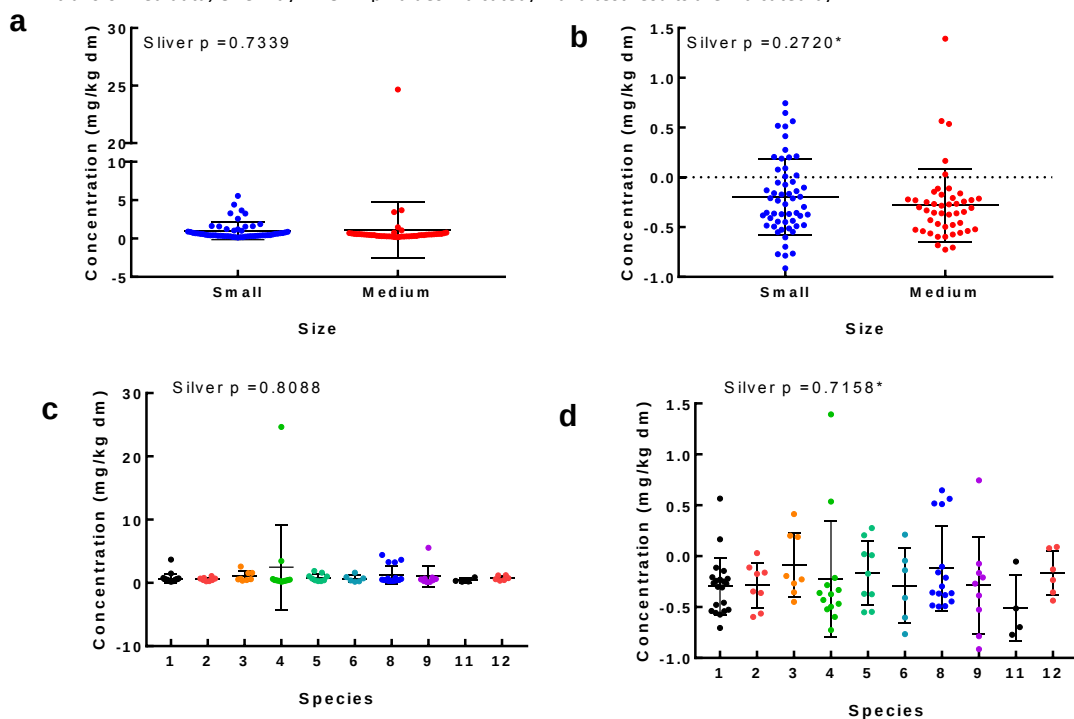


Figure 3.58 Scatterplots of silver concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.57a Scatterplot of untransformed palladium data according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.57b Scatterplot of log-transformed palladium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.57c Scatterplot of untransformed palladium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.57d Scatterplot of log-transformed palladium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1 and 4. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons showed significant differences ($p < 0.05$) between: 8 vs. 12.

Figure 3.58a Scatterplot of untransformed silver data according to size. The means and standard deviations are indicated.

The untransformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.58b Scatterplot of log-transformed silver data according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.58c Scatterplot of untransformed silver data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.58d Scatterplot of log-transformed silver data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1, 4 and 8. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($p > 0.05$).

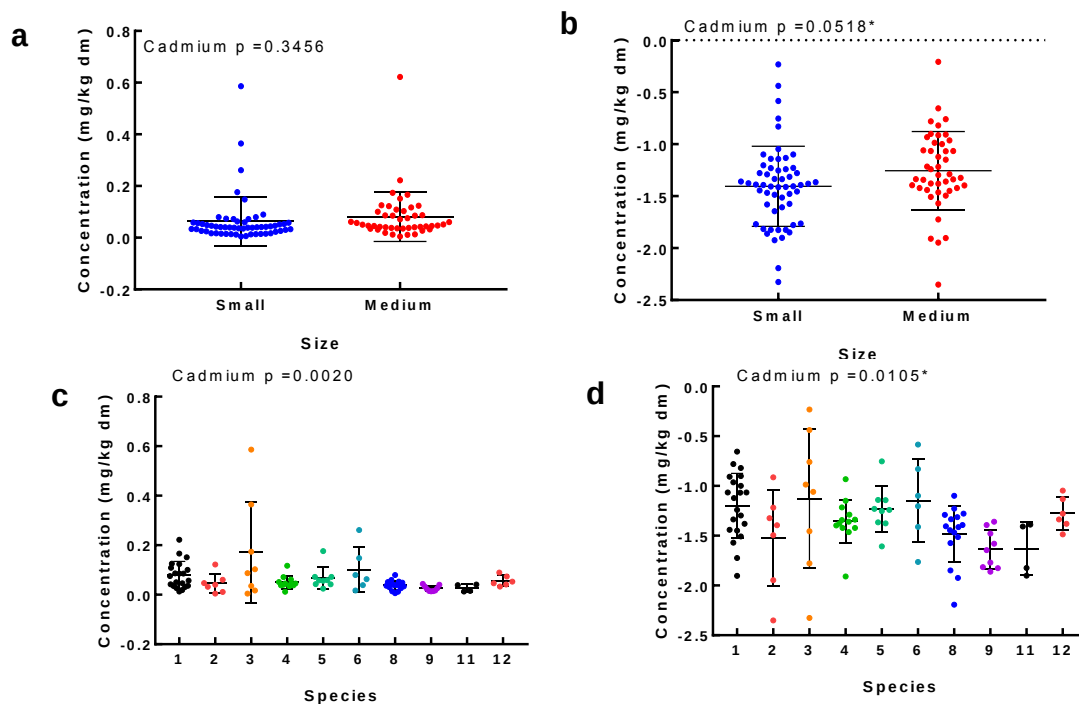


Figure 3.59 Scatterplots of cadmium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

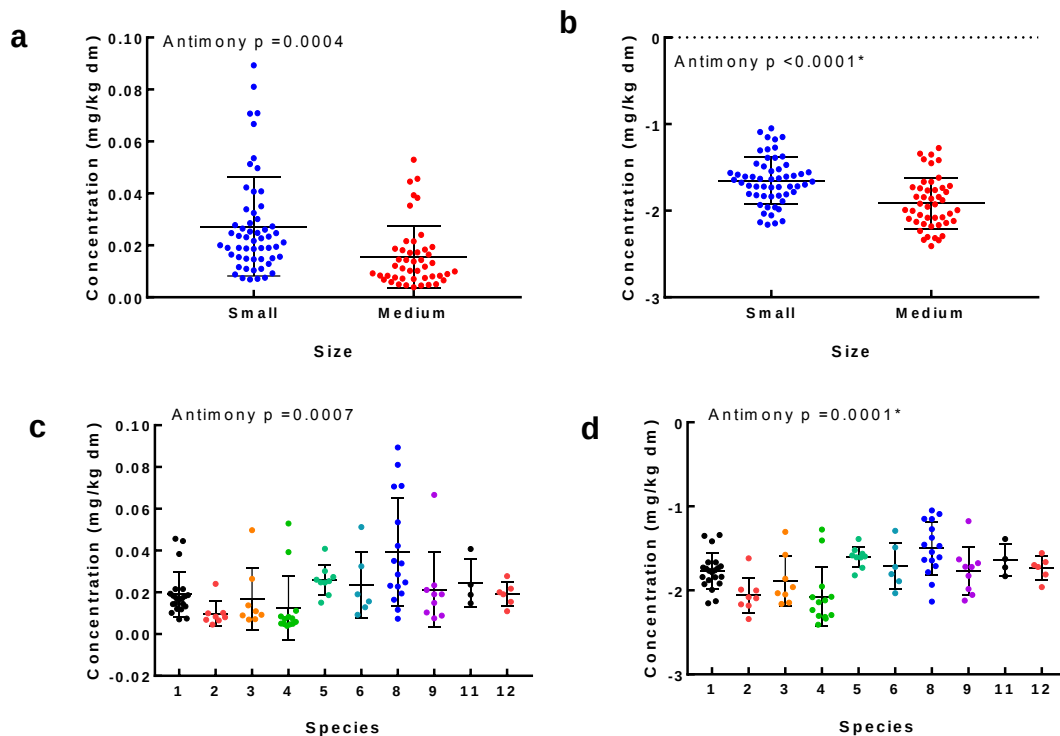


Figure 3.60 Scatterplots of antimony concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.59a Scatterplot of untransformed cadmium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.59b Scatterplot of log-transformed cadmium concentrations according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.59c Scatterplot of untransformed cadmium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.59d Scatterplot of log-transformed cadmium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 8. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons showed no significant differences ($p > 0.05$).

Figure 3.60a Scatterplot of untransformed antimony data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.60b Scatterplot of log-transformed antimony concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$).

Figure 3.60c Scatterplot of untransformed antimony data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.60d Scatterplot of log-transformed antimony data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 4. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 4; 2 vs. 5, 8; 3 vs. 8 and 4 vs. 5, 8)

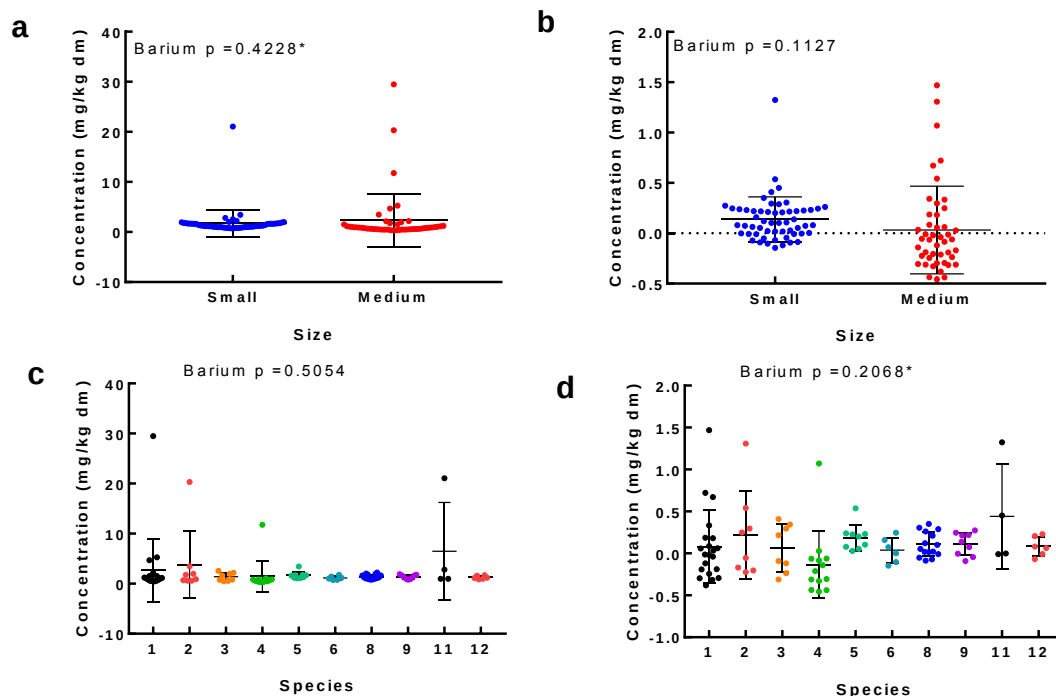


Figure 3.61 Scatterplots of barium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

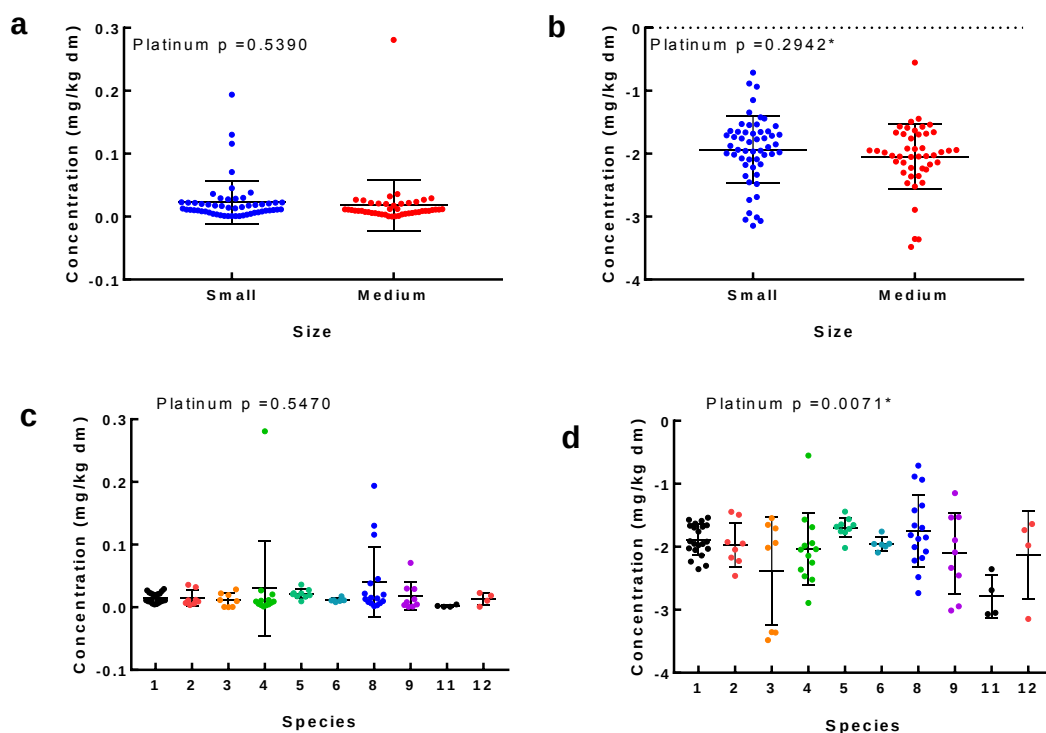


Figure 3.62 Scatterplots of platinum concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.61a Scatterplot of untransformed barium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.61b Scatterplot of log-transformed barium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.61c Scatterplot of untransformed barium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.61d Scatterplot of log-transformed barium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1 and 4. Unpaired one-way ANOVA showed no significant differences between species ($P > 0.05$). Tukey's multiple comparisons also showed no significant differences ($p > 0.05$).

Figure 3.62a Scatterplot of untransformed platinum data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.62b Scatterplot of log-transformed platinum concentrations according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.62c Scatterplot of untransformed platinum data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.62d Scatterplot of log-transformed platinum data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 3. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 11; 5 vs. 11 and 8 vs. 11.

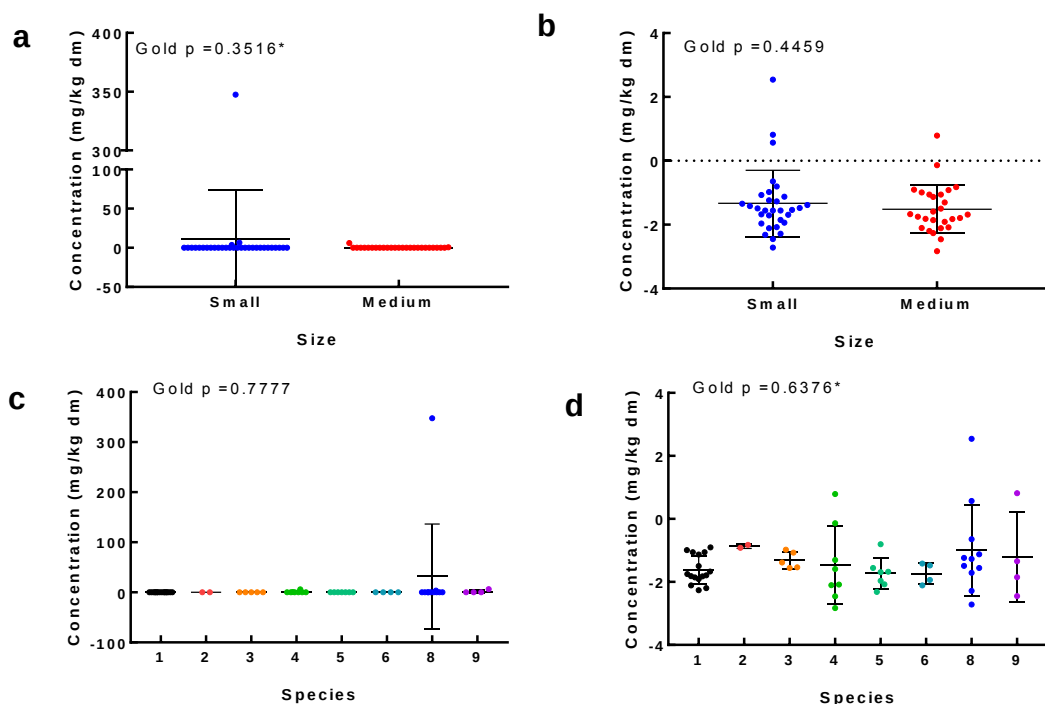


Figure 3.63 Scatterplots of gold concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

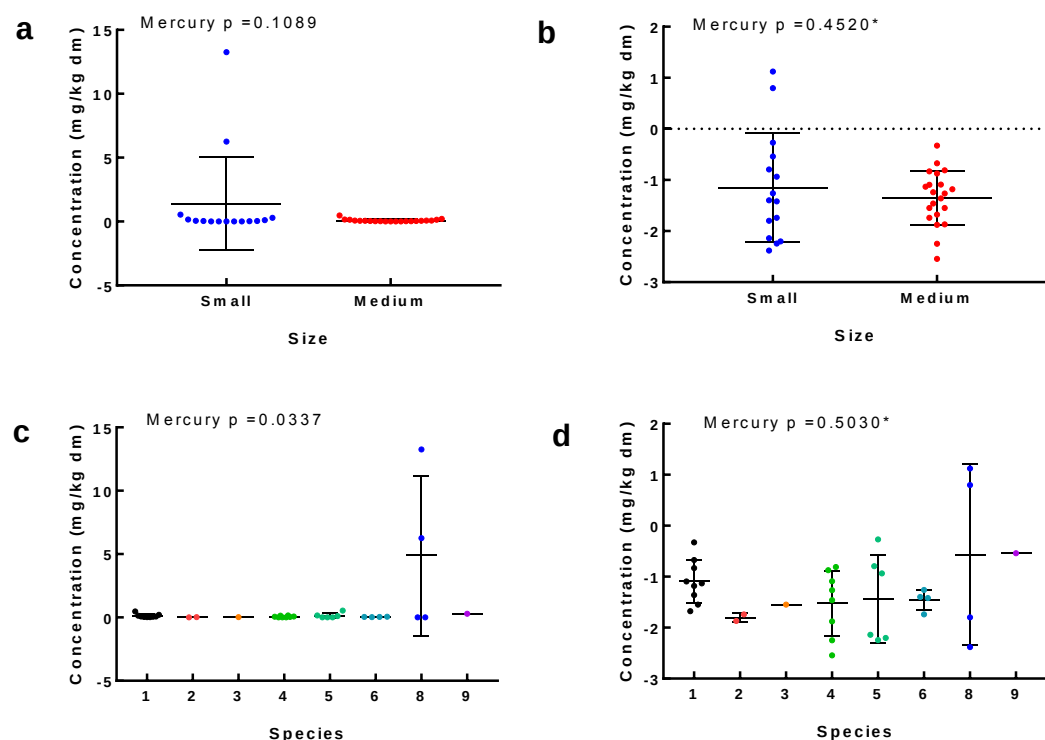


Figure 3.64 Scatterplots of mercury concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.63a Scatterplot of untransformed gold concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.63b Scatterplot of log-transformed gold data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.63c Scatterplot of untransformed gold data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.63d Scatterplot of log-transformed gold data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 8. Species 2 had too low N values. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($0 > 0.05$).

Figure 3.64a Scatterplot of untransformed mercury data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.64b Scatterplot of log-transformed mercury concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.64c Scatterplot of untransformed mercury data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.64d Scatterplot of log-transformed mercury data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2, 3, and 9 had too low N values. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences (0.05).

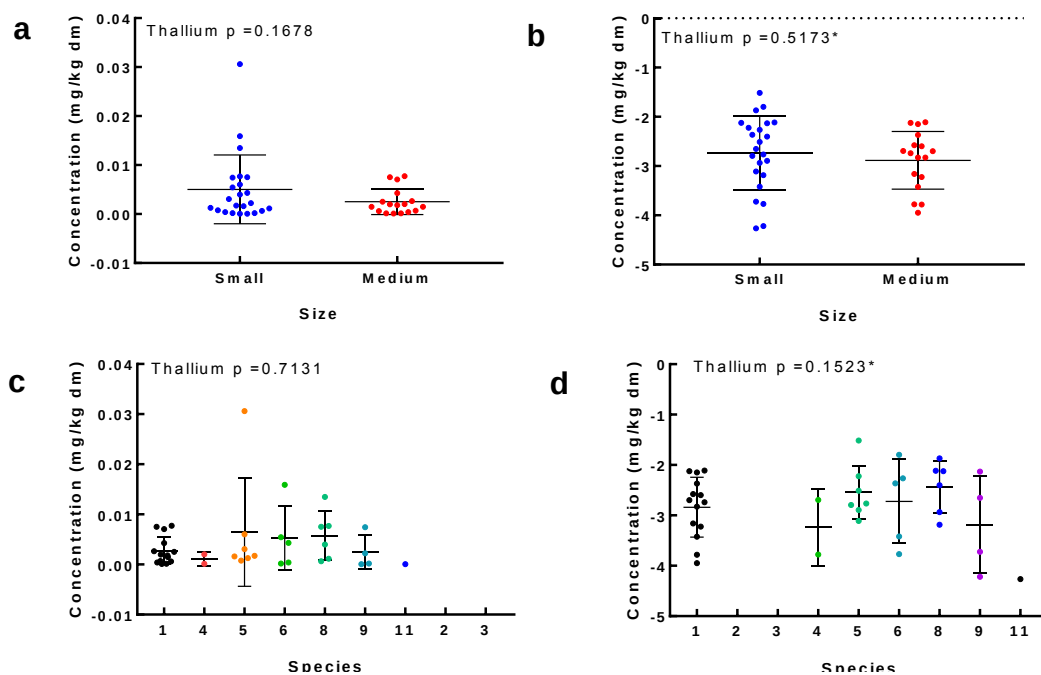


Figure 3.65 Scatterplots of thallium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

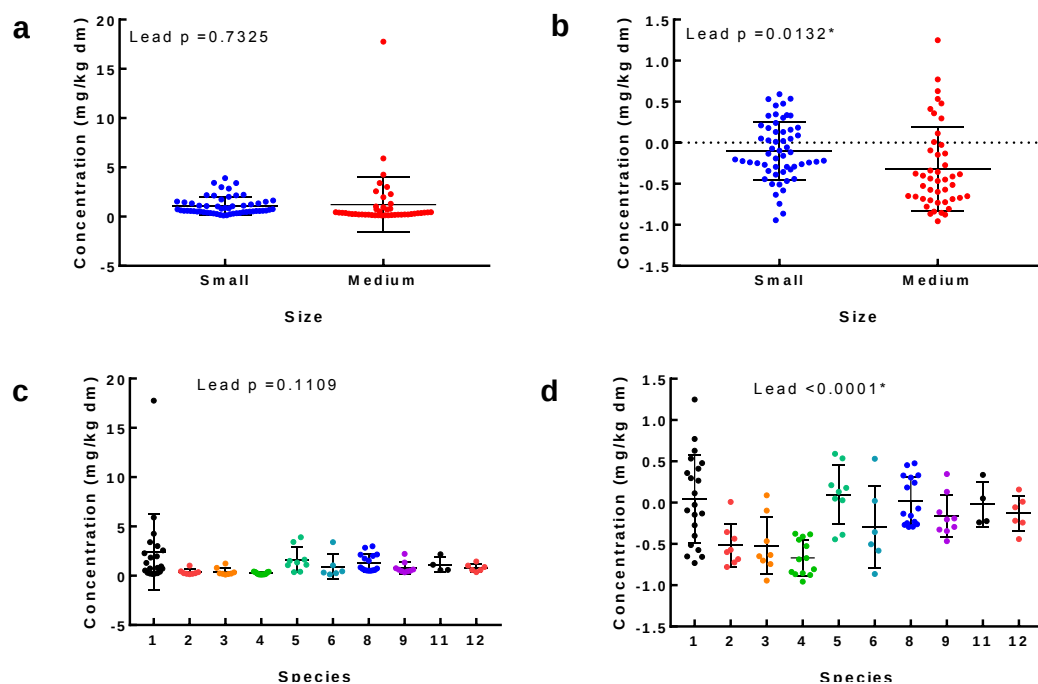


Figure 3.66 Scatterplots of lead concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.65a Scatterplot of untransformed thallium data according to size. The means and standard deviations are indicated.

The untransformed concentrations of only the medium sized group was normally distributed; this graph will not be discussed further.

Figure 3.65b Scatterplot of log-transformed thallium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.65c Scatterplot of untransformed thallium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.65d Scatterplot of log-transformed thallium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 2, 3, 4, 11 had too low N values. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($p > 0.05$).

Figure 3.66a Scatterplot of untransformed lead data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.66b Scatterplot of log-transformed lead concentrations according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the small sized group was normally distributed. The two-way, unpaired, t-test showed significant differences ($p < 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.66c Scatterplot of untransformed lead data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.66d Scatterplot of log-transformed lead data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 8. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 2, 3, 4; 2 vs. 5, 8; 3 vs. 5, 8 and 4 vs. 5, 8.

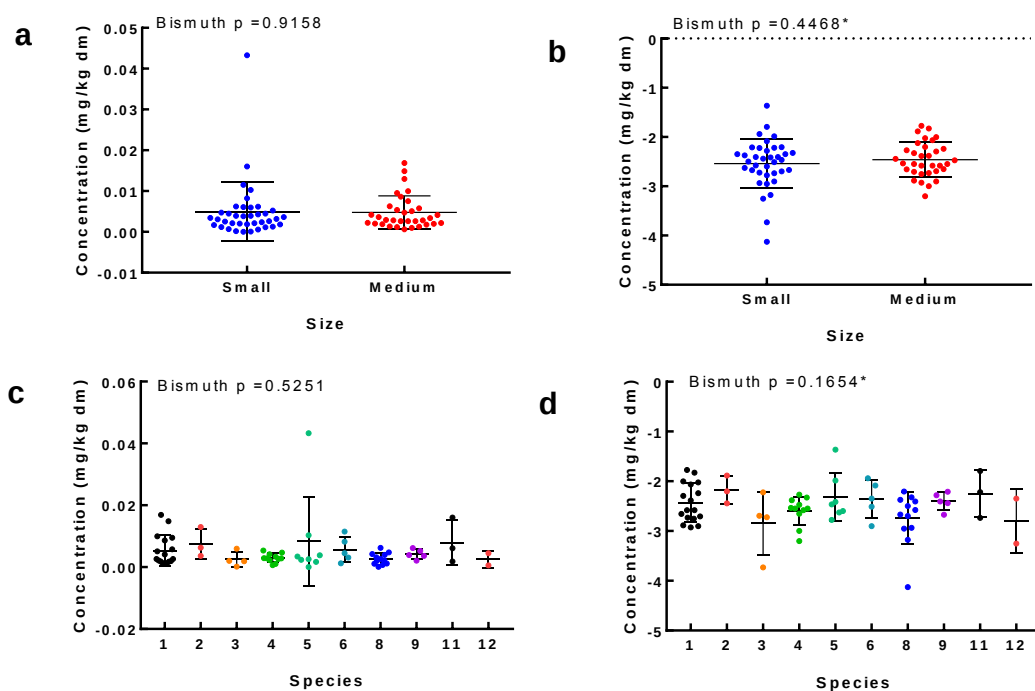


Figure 3.67 Scatterplots of bismuth concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

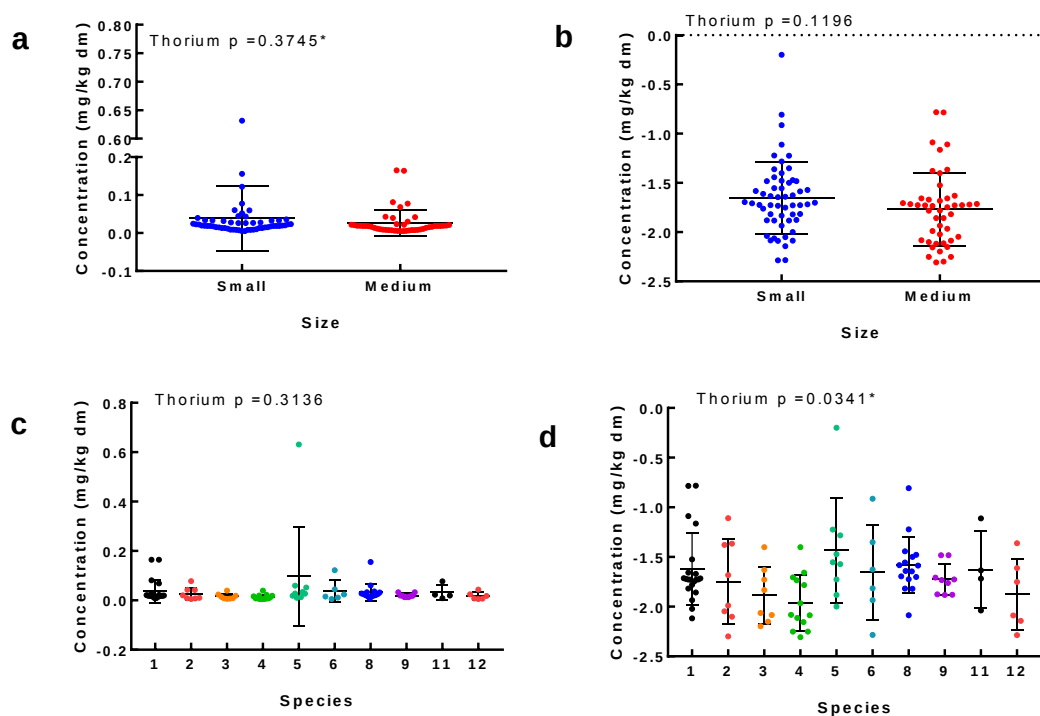


Figure 3.68 Scatterplots of thorium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.67a Scatterplot of untransformed bismuth data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.67b Scatterplot of log-transformed bismuth concentrations according to size. The means and standard deviations are indicated.

The log-transformed concentrations of only the medium sized group was normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$). Care should be taken with interpretation as only one size group had normally distributed data.

Figure 3.67c Scatterplot of untransformed bismuth data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.67d Scatterplot of log-transformed bismuth data according to species. . The means and standard deviations are indicated.

All concentrations were normally distributed, except 8. Species 12 had too low N values. Unpaired one-way ANOVA showed no significant differences between species ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($p > 0.05$).

Figure 3.68a Scatterplot of untransformed thorium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.68b Scatterplot of log-transformed thorium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.68c Scatterplot of untransformed thorium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.68d Scatterplot of log-transformed thorium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 1. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 4 vs. 5.

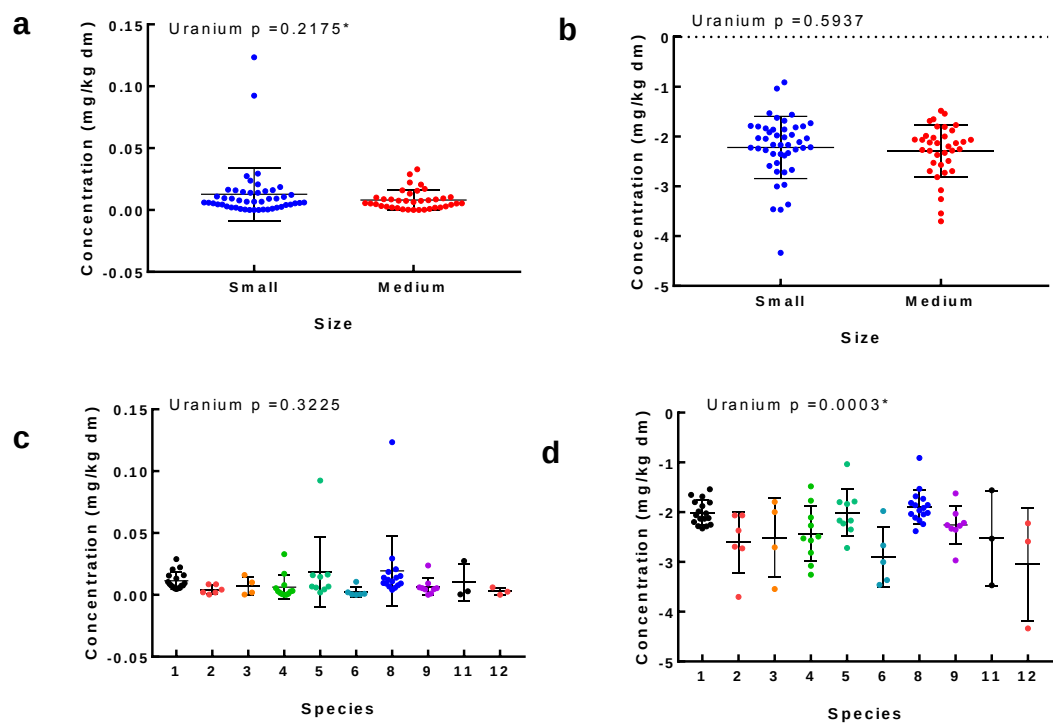


Figure 3.69 Scatterplots of uranium concentrations (means and standard deviations indicated) according to size categories (a, untransformed data; b log-transformed data; t-test p-values indicated), and species (c, untransformed data, d log-transformed data; One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.69a Scatterplot of untransformed uranium concentrations according to size. The means and standard deviations are indicated.

Both concentrations for the medium and small sized groups were not normally distributed. The two-way, unpaired, t-test showed no significant differences ($p > 0.05$).

Figure 3.69b Scatterplot of log-transformed uranium data according to size. The means and standard deviations are indicated.

The transformed concentrations of both small and medium species were not normally distributed; this graph will not be discussed further.

Figure 3.69c Scatterplot of untransformed uranium data according to species. The means and standard deviations are indicated.

Because the untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.69d Scatterplot of log-transformed uranium data according to species. The means and standard deviations are indicated.

All concentrations were normally distributed, except 8. Unpaired one-way ANOVA showed significant differences between species ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($p < 0.05$) between: 1 vs. 6, 12; 6 vs. 8 and 8 vs. 12.

3.5 Multivariate analysis

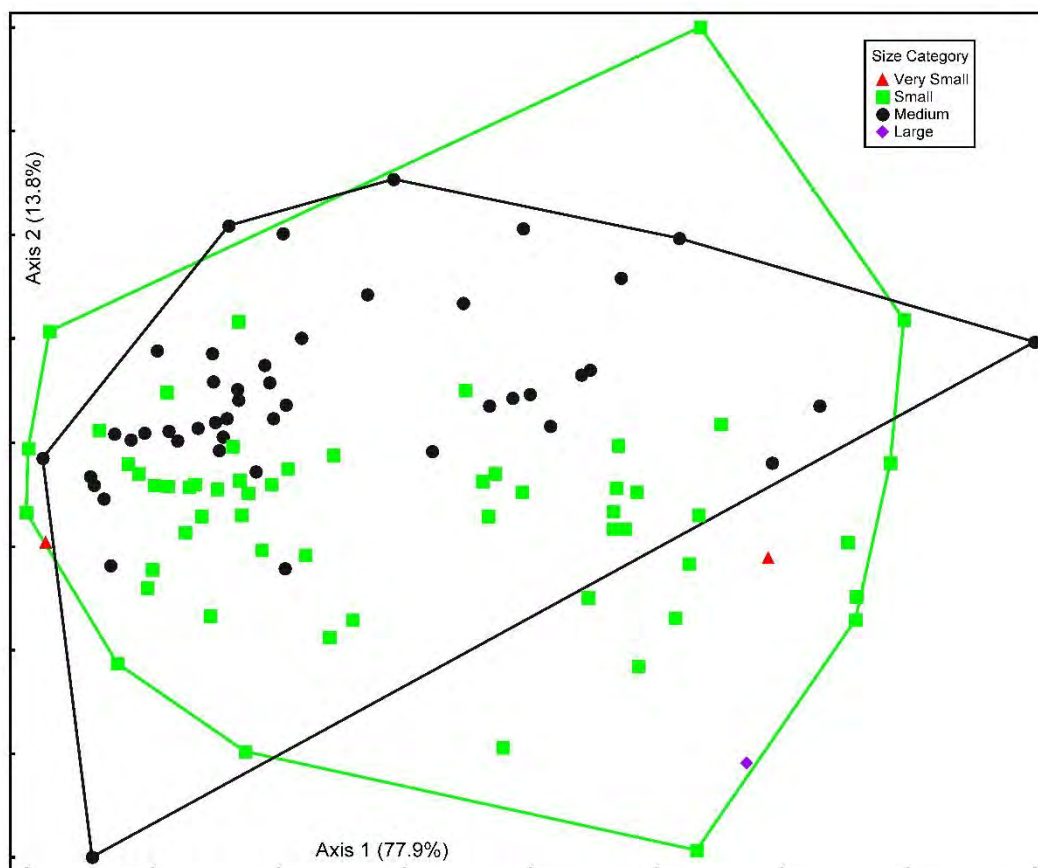


Figure 3.70 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%.

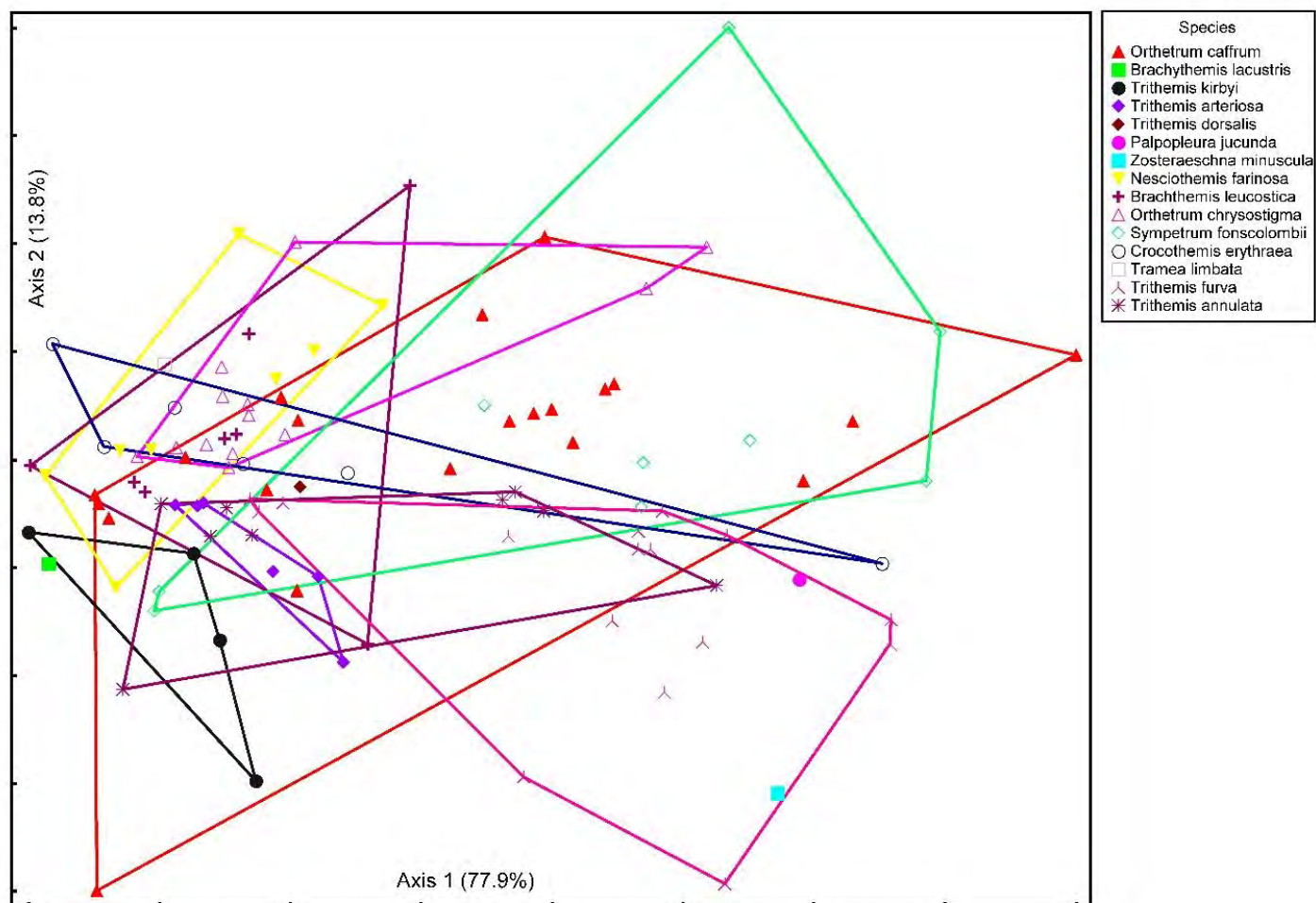


Figure 3.71 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%.

Convex hulls for two of the the four size categories are shown in Figure 3.70. Very small and large categories are indicated by two red triangles, and a single purple diamond. The convex hulls for the 15 species are shown in Figure 3.71. Most of the species ordinated to the left with individuals stretching convex hulls to the right. Due to technical limitations, the scientific names could not be printed in italics.

3.6 Habitat results

Scatterplots of all metallic elements that have been analysed.

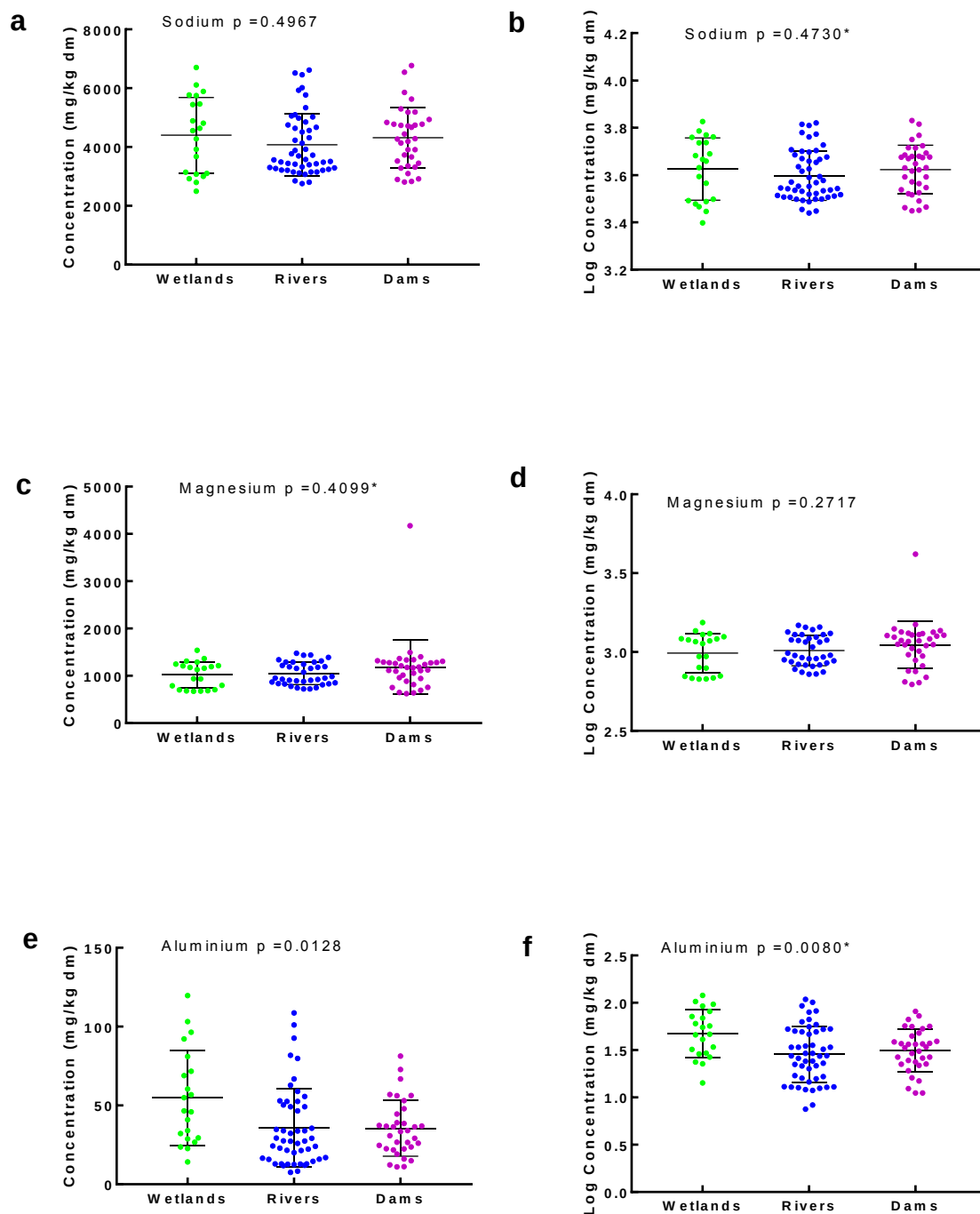


Figure 3.72 Scatterplots of sodium, magnesium, and aluminium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.72a Scatterplot of untransformed sodium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.72.b Scatterplot of log-transformed sodium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in sodium concentrations in the sampled Odonata.

Figure 3.72c Scatterplot of untransformed magnesium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed, except rivers and dams. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in magnesium concentrations in the sampled Odonata.

Figure 3.72d Scatterplot of log-transformed magnesium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.72e Scatterplot of untransformed aluminium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.72f Scatterplot of log-transformed aluminium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams. Therefore, habitat type did play a significant role in aluminium concentrations in the sampled Odonata.

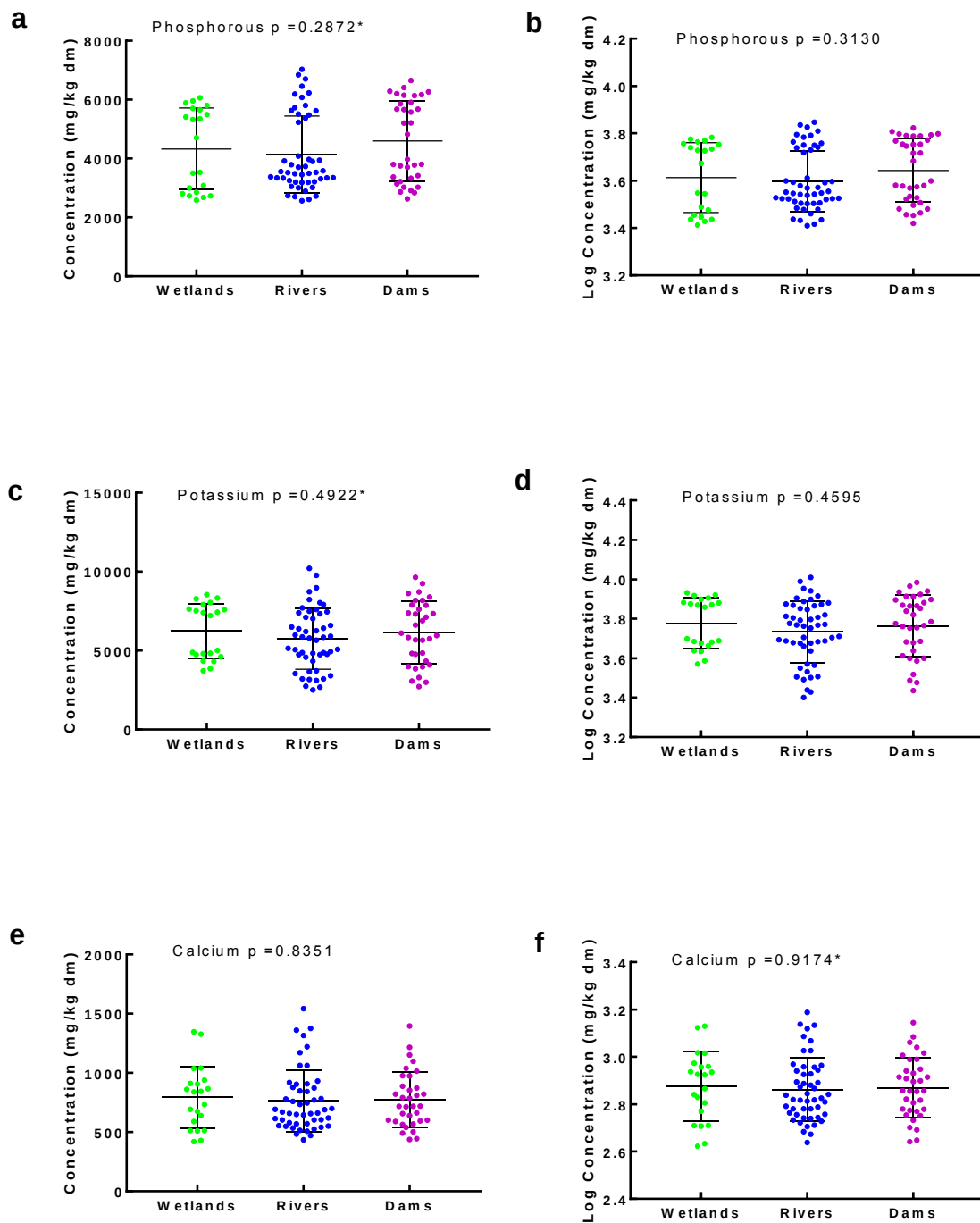


Figure 3.73 Scatterplots of phosphorus, potassium, and calcium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.73a Scatterplot of untransformed phosphorus data according to habitat type. The mean and standard deviation are indicated.

All concentrations were not normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in phosphorus concentrations in the sampled Odonata.

Figure 3.73b Scatterplot of log-transformed phosphorus data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.73c Scatterplot of untransformed potassium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in potassium concentrations in the sampled Odonata.

Figure 3.73d Scatterplot of log-transformed potassium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.73e Scatterplot of untransformed calcium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.73f Scatterplot of log-transformed calcium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in calcium concentrations in the sampled Odonata.

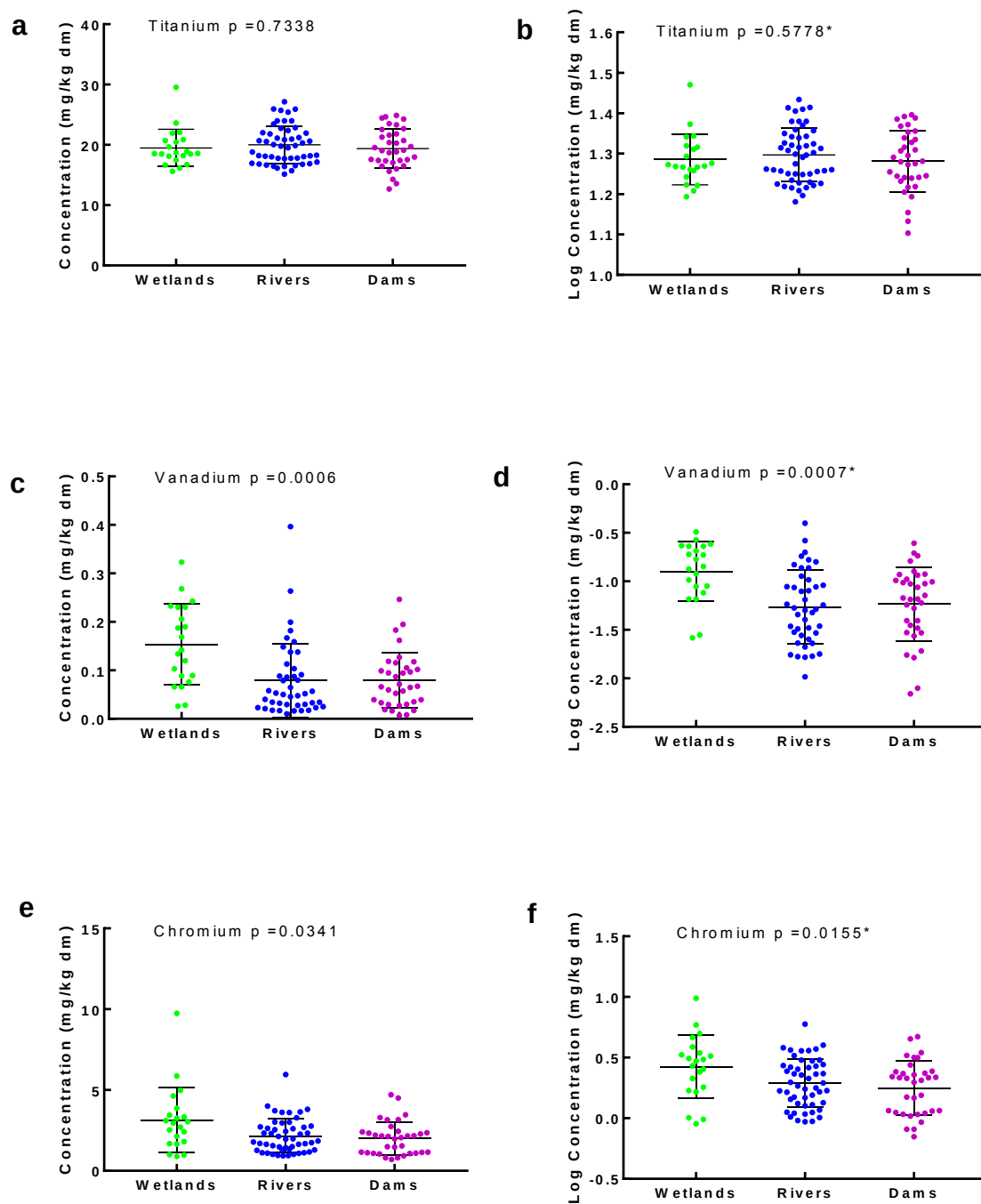


Figure 3.74 Scatterplots of titanium, vanadium, and chromium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.74a Scatterplot of untransformed titanium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in titanium concentrations in the sampled Odonata.

Figure 3.74b Scatterplot of log-transformed titanium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.74c Scatterplot of untransformed vanadium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.74d Scatterplot of log-transformed vanadium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams. Therefore, habitat type did play a significant role in vanadium concentrations in the sampled Odonata between Wetlands, Rivers and Dams.

Figure 3.74e Scatterplot of untransformed chromium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.74f Scatterplot of log-transformed chromium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons also showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams. Therefore, habitat type did play a significant role in chromium concentrations in the sampled Odonata between Wetlands, Rivers and Dams.

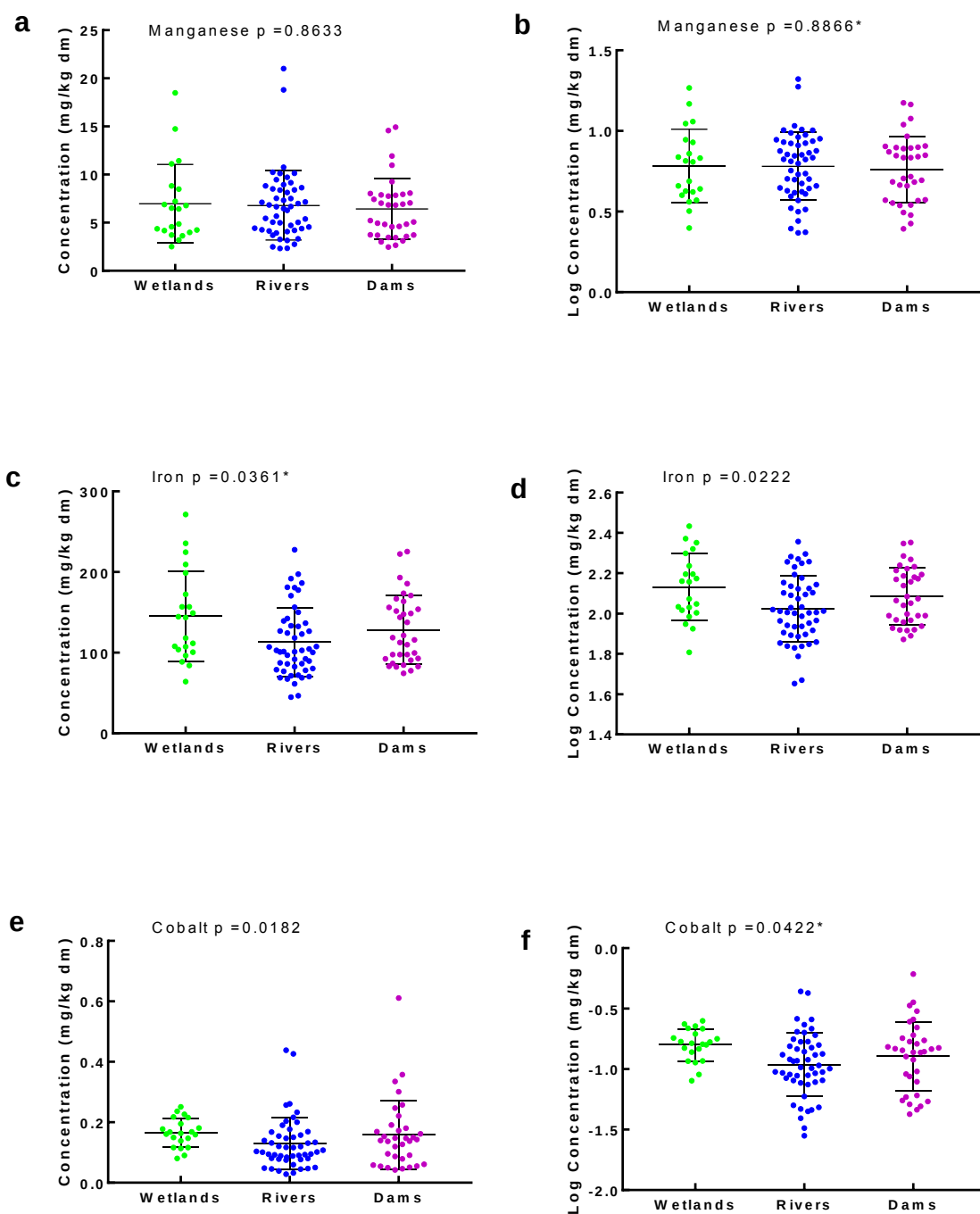


Figure 3.75 Scatterplots of manganese, iron, and cobalt concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.75a Scatterplot of untransformed manganese data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.75b Scatterplot of log-transformed manganese data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons also showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in manganese concentrations in the sampled Odonata.

Figure 3.75c Scatterplot of untransformed iron data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except rivers and dams. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers. Therefore, habitat type did play a significant role in iron concentrations in the sampled Odonata between Wetlands and Rivers.

Figure 3.75d Scatterplot of log-transformed iron data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.75e Scatterplot of untransformed cobalt data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.75f Scatterplot of log-transformed cobalt data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers. Therefore, habitat type did play a significant role in cobalt concentrations in the sampled Odonata between Wetlands and Rivers.

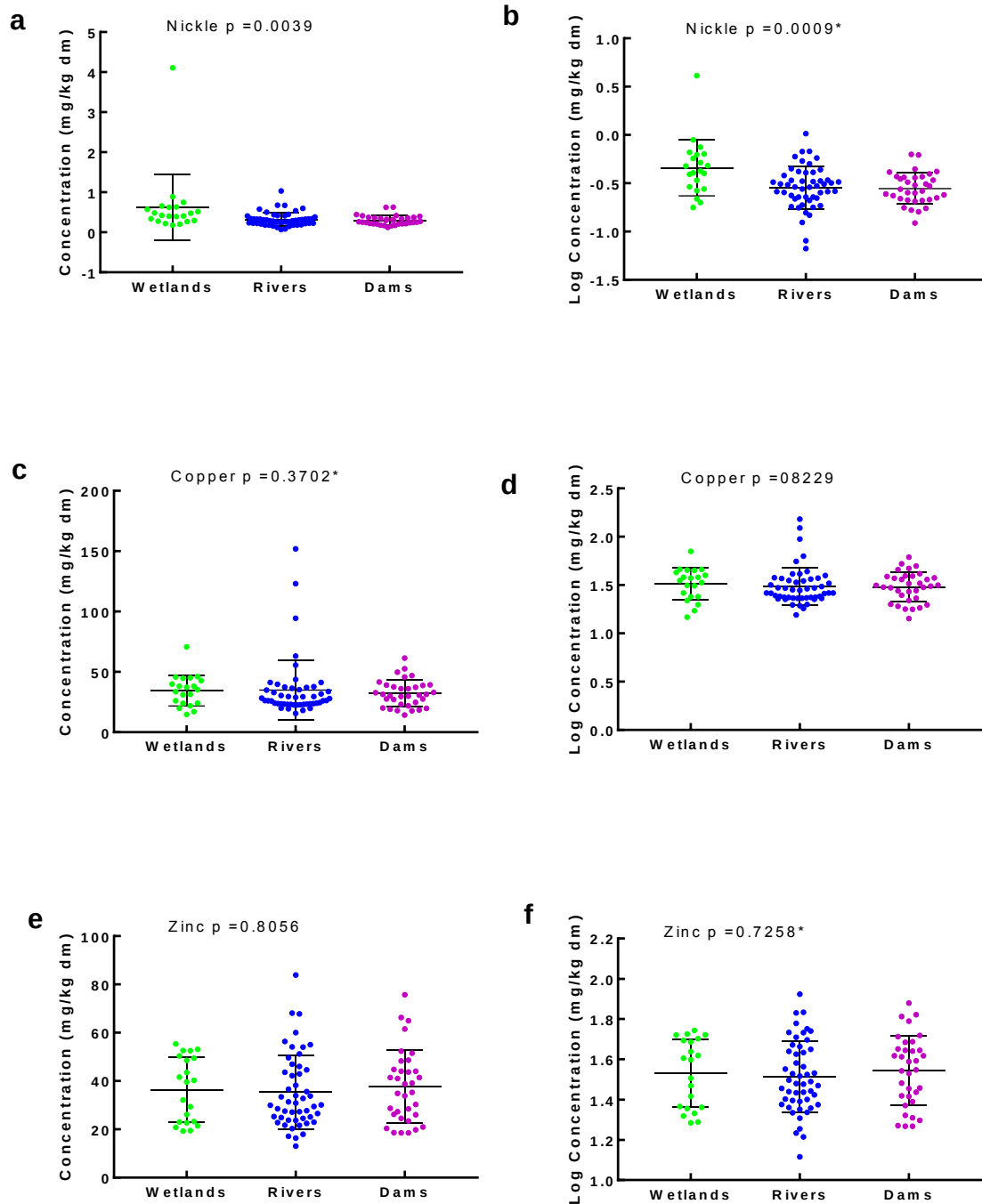


Figure 3.76 Scatterplots of nickel, copper, and zinc concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.76a Scatterplot of untransformed nickel data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.76b Scatterplot of log-transformed nickel data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams. Therefore, habitat type did play a significant role in nickel concentrations in the sampled Odonata between Wetlands, Rivers and Dams.

Figure 3.76c Scatterplot of untransformed copper data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in copper concentrations in the sampled Odonata.

Figure 3.76d Scatterplot of log-transformed copper data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.76e Scatterplot of untransformed zinc data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.76f Scatterplot of log-transformed zinc data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in zinc concentrations in the sampled Odonata.

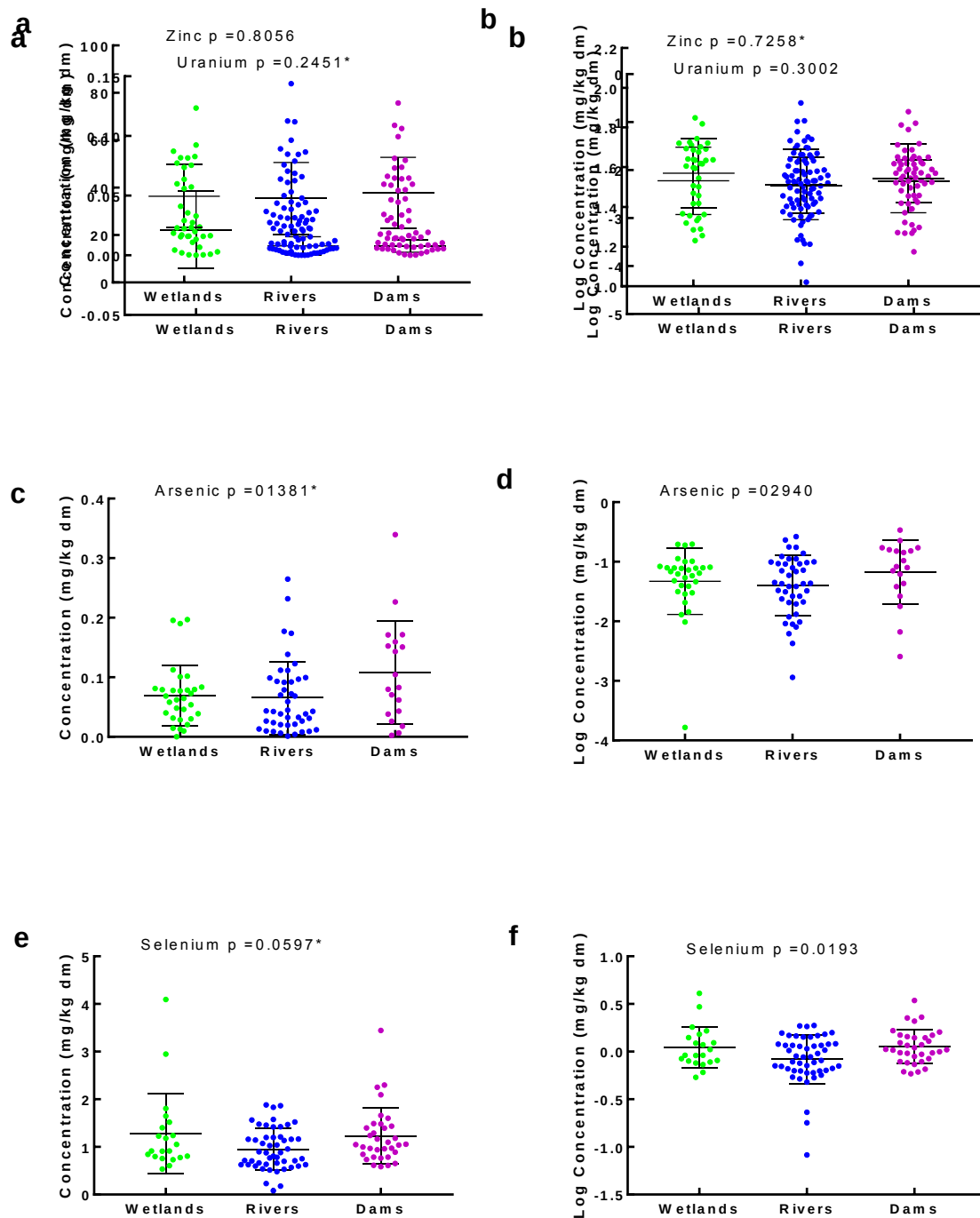


Figure 3.77 Scatterplots of uranium, arsenic, and selenium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.77a Figure a. Scatterplot of untransformed uranium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands and rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in uranium concentrations in the sampled Odonata.

Figure 3.77b Scatterplot of log-transformed uranium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.77c Scatterplot of untransformed arsenic data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands and rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in arsenic concentrations in the sampled Odonata.

Figure 3.77d Scatterplot of log-transformed arsenic data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.77e Scatterplot of untransformed selenium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands and dams. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in selenium concentrations in the sampled Odonata.

Figure 3.77f Scatterplot of log-transformed selenium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

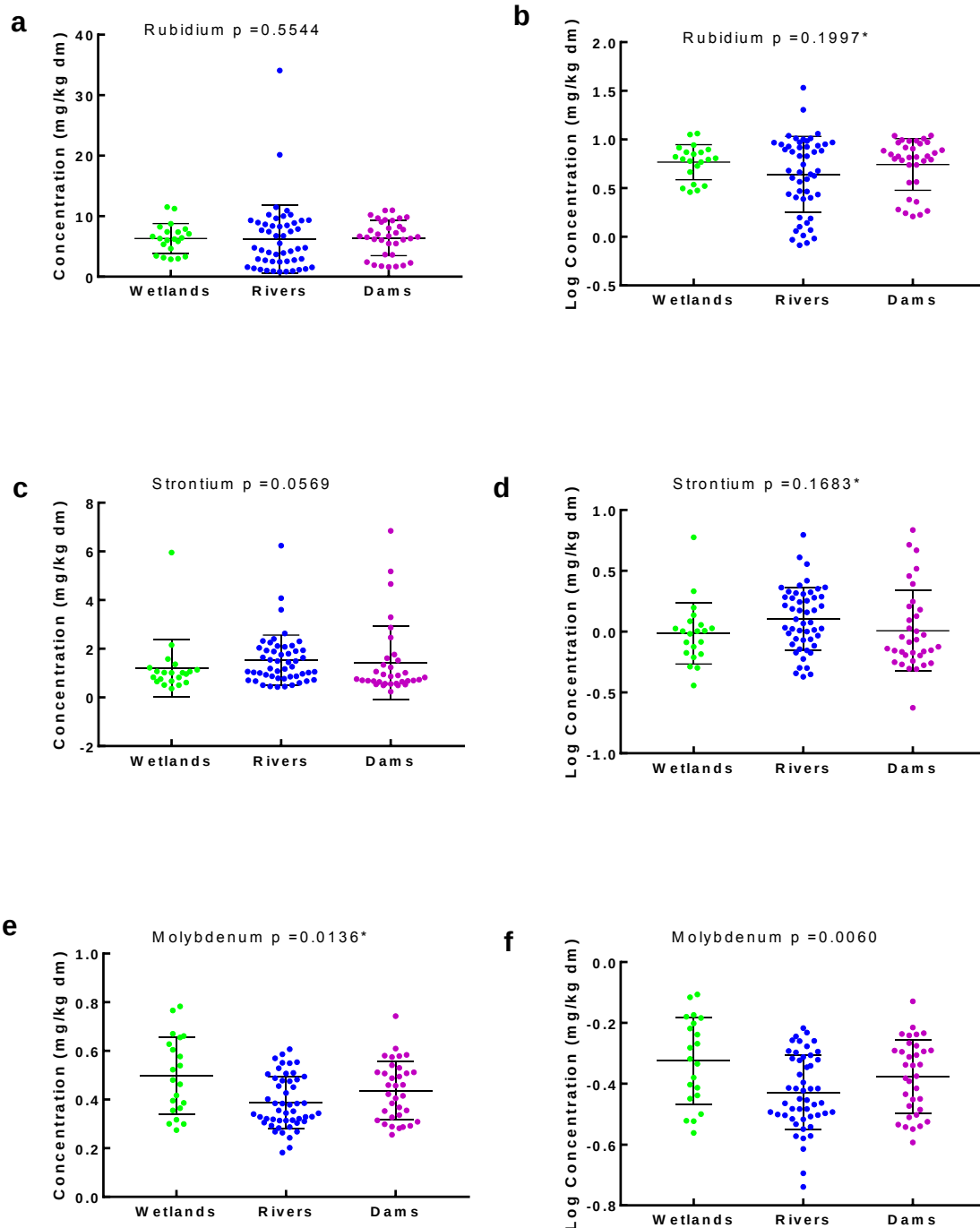


Figure 3.78 Scatterplots of rubidium, strontium, and molybdenum concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.8a Scatterplot of untransformed rubidium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.8b Scatterplot of log-transformed rubidium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in rubidium concentrations in the sampled Odonata.

Figure 3.8c Scatterplot of untransformed strontium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.8d Scatterplot of log-transformed strontium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in strontium concentrations in the sampled Odonata.

Figure 3.8e Scatterplot of untransformed molybdenum data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between Wetlands vs. Rivers. Therefore, habitat type did play a significant role in molybdenum concentrations in the sampled Odonata between Wetlands and Rivers.

Figure 3.8f Scatterplot of log-transformed molybdenum data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

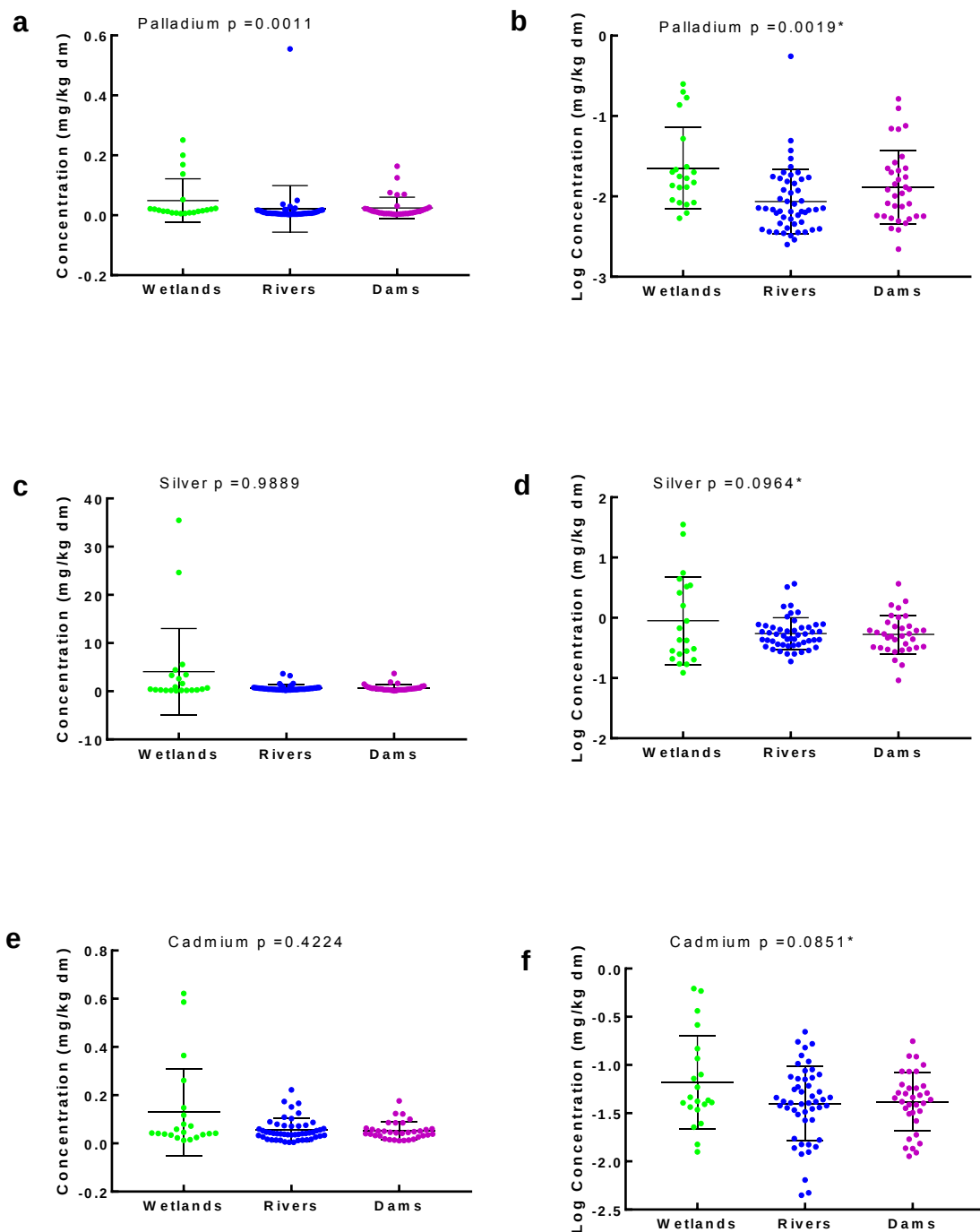


Figure 3.79 Scatterplots of palladium, silver, and cadmium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.79a Scatterplot of untransformed palladium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.79b Scatterplot of log-transformed palladium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except rivers. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers. Therefore, habitat type did play a significant role in palladium concentrations in the sampled Odonata between Wetlands and Rivers.

Figure 3.79c Scatterplot of untransformed silver data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.79d Scatterplot of log-transformed silver data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in silver concentrations in the sampled Odonata.

Figure 3.80e Scatterplot of untransformed cadmium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.80f Scatterplot of log-transformed cadmium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$). Therefore, habitat type did not play a significant role in cadmium concentrations in the sampled Odonata.

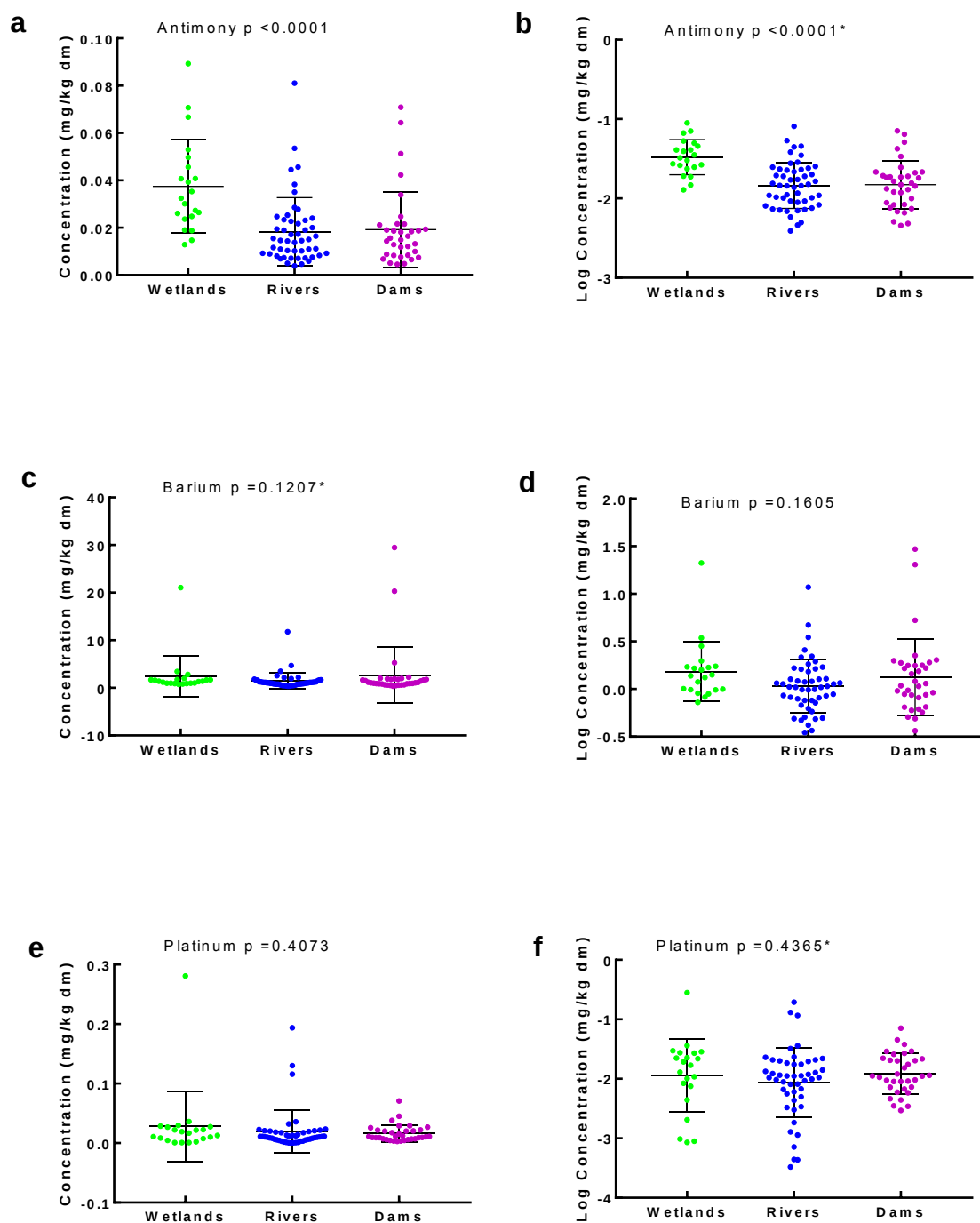


Figure 3.81 Scatterplots of antimony, barium, and platinum concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.81a Scatterplot of untransformed antimony data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significantly differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams.

Figure 3.81b Scatterplot of log-transformed antimony data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.81c Scatterplot of untransformed barium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were not normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significantly differences ($P > 0.05$).

Figure 3.81d Scatterplot of log-transformed barium data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.81e Scatterplot of untransformed platinum data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.81f Scatterplot of log-transformed platinum data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significantly differences ($P > 0.05$).

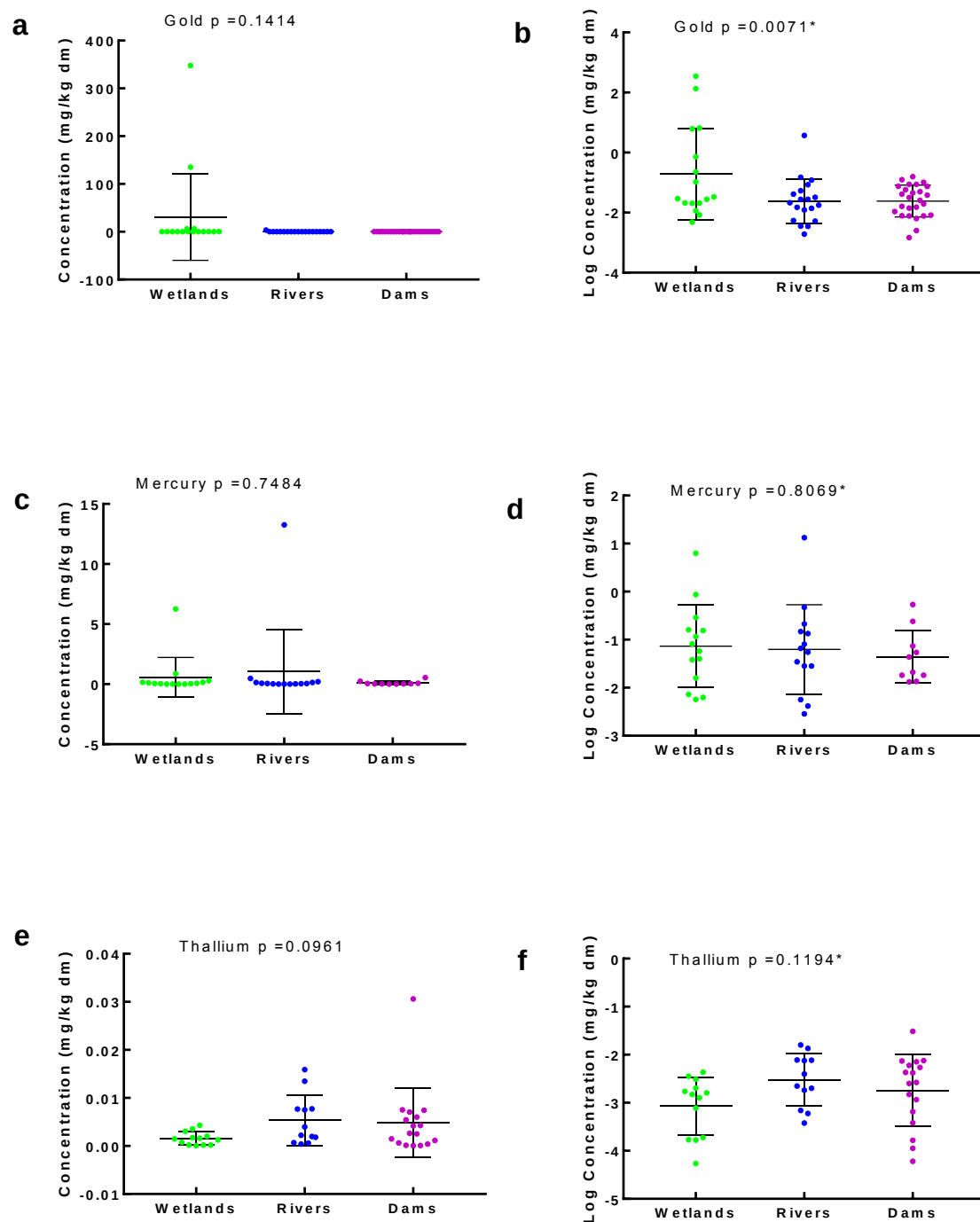


Figure 3.82 Scatterplots of gold, mercury, and thallium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.82a Scatterplot of untransformed gold data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.82b Scatterplot of log-transformed gold data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except rivers. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Wetlands vs. Dams.

Figure 3.82c Scatterplot of untransformed mercury data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.82d Scatterplot of log-transformed mercury data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$).

Figure 3.82e Scatterplot of untransformed thallium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.82f Scatterplot of log-transformed thallium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$).

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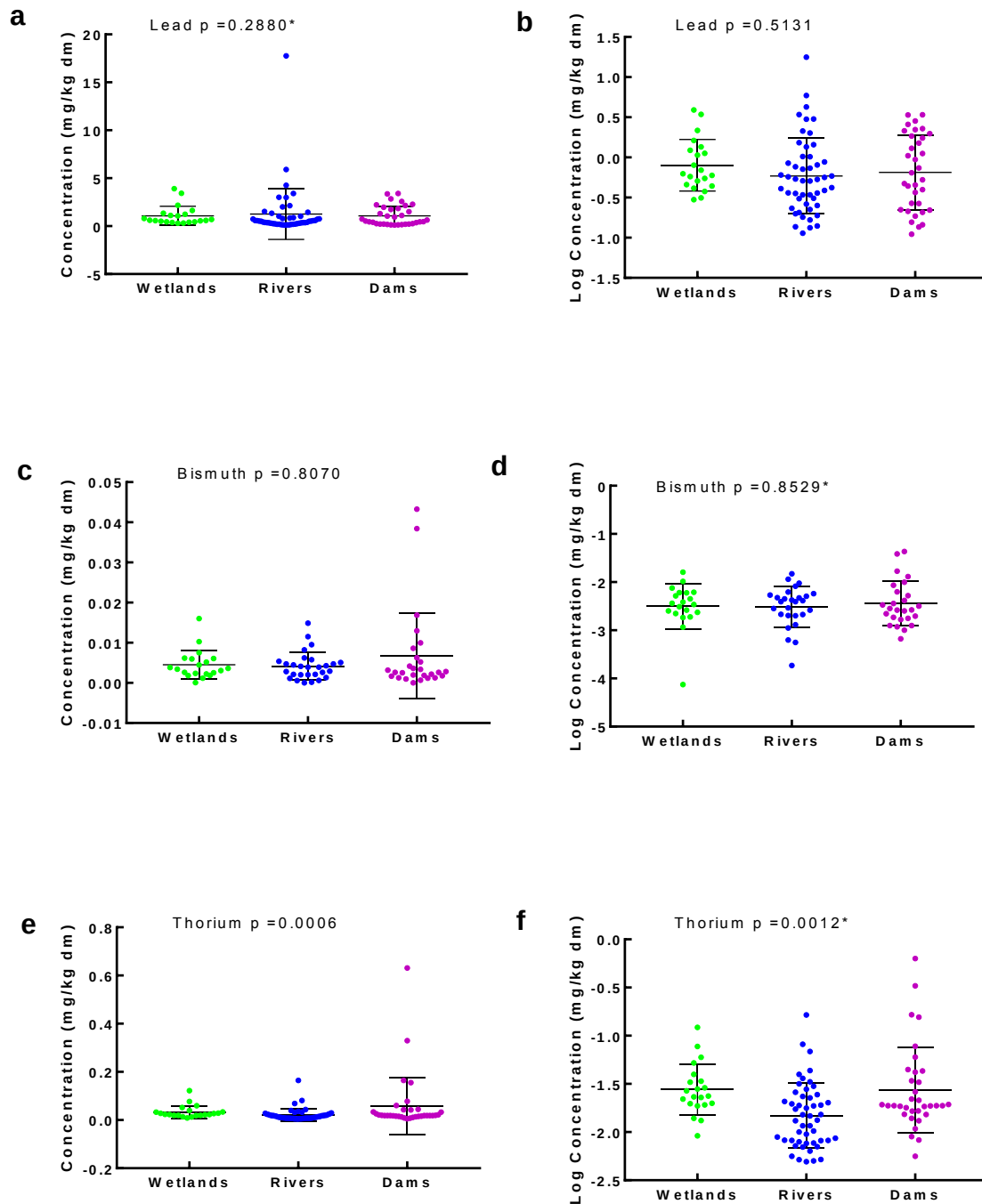


Figure 3.83 Scatterplots of lead, bismuth, and thorium concentrations (means and standard deviations indicated) according to habitat type (a, c and e untransformed data; b, d and f log-transformed data); One-way ANOVA p-values indicated). Valid test results are indicated by *

Figure 3.83a Scatterplot of untransformed lead data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands and rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$).

Figure 3.83b Scatterplot of log-transformed lead data according to habitat type. The mean and standard deviation are indicated.

The log-transformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.83c Scatterplot of untransformed bismuth data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.83d Scatterplot of log-transformed bismuth data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed except wetlands and rivers. Unpaired, one-way ANOVA showed no significant differences between habitat types ($p > 0.05$). Tukey's multiple comparisons showed no significant differences ($P > 0.05$).

Figure 3.83e Scatterplot of untransformed thorium data according to habitat type. The mean and standard deviation are indicated.

The untransformed concentrations were not normally distributed, this graph will not be discussed further.

Figure 3.83f Scatterplot of log-transformed thorium data according to habitat type. The mean and standard deviation are indicated.

All concentrations were normally distributed. Unpaired, one-way ANOVA showed significant differences between habitat types ($p < 0.05$). Tukey's multiple comparisons showed significant differences ($P < 0.05$) between: Wetlands vs. Rivers and Rivers vs. Dams.

3.7 Nonmetric multidimensional scaling habitat results

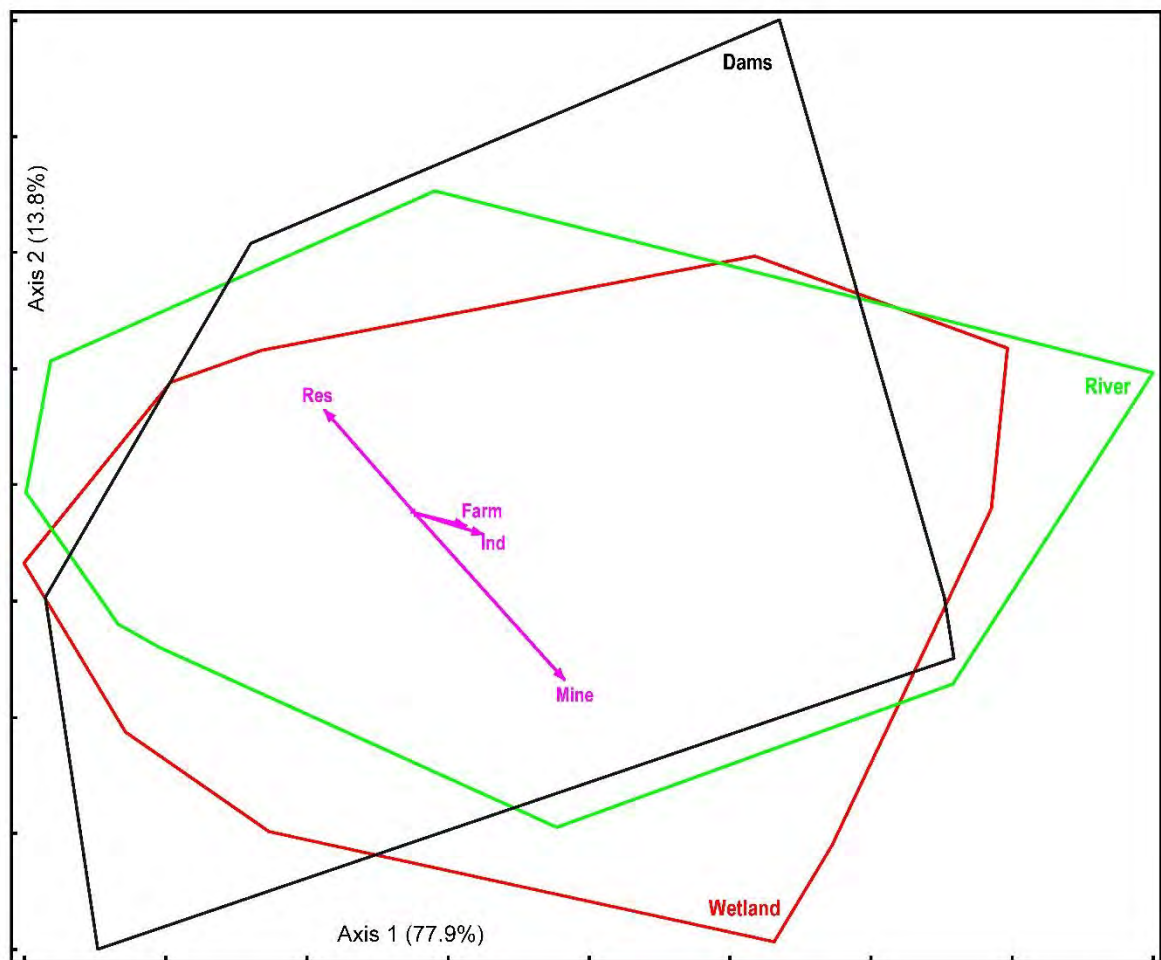


Figure 3.84 Nonmetric multidimensional scaling (NMS) of relativized (by element) elemental concentration data. Gower (ignore 0) was used as distance measure. Six axes were allowed, with a maximum of 500 iterations. Starting conditions were random. There were 250 runs with real data, followed by 250 runs with randomised data to determine the p-value for the Monte Carlo test. Only two dimensions were needed for this ordination. Final stress was 13.6, and the final instability was 0.0000. Axis 1 explains 77.9% of the variation, and axis 2 explains 13.8%.

Convex hulls for the 3 habitat types are shown (Figure 3.84). The environmental factors are shown as purple vectors. The convex hulls all overlapped.

Chapter 4: Discussion

“Drink in the beauty and wonder at the meaning of what you see.”

– Rachel Carson

4.1 Introduction

As discussed in Chapter 1, metallic elements in trace amounts are not harmful to organisms, but in excess can cause health problems, decrease survival and fitness, and increase mortality. The current study was the first done on the concentrations and patterns of metallic elements in adult dragonflies, anywhere in the world. Research done on the presence and concentrations of perfluorinated organic compounds in adult dragonflies (Lesch et al., 2017) has led to this study. For this study, I caught and analysed 105 dragonflies belonging to 15 species (Table 3.1) from 21 sites (Table 3.2) across South Africa, and analysed the samples for 33 elements (Table 1.2). All samples were successfully analysed. In Chapter 3, the results were analysed, and plotted according to four variables; site, species, body size, and habitat type. Scatterplots were used to illustrate and compare the concentrations. In addition, multivariate analyses were done to discern patterns of relative elemental compositions, and relate the patterns to variables already mentioned. Environmental variables included were; distance from industries, residential areas, mines, and farms. In this chapter, the results will be discussed and compared with other studies.

4.2 Elements

The means of selected metallic element concentrations for each variable can be seen in Tables 3.3, 3.4, 3.5, and 3.6. The elements listed in these tables are discussed in detail in Chapter 1, Section 1.3. All metals were briefly discussed in Tables 1.2 and 1.3.

4.2.1 Arsenic

As discussed in Chapter 1 Section 1.3.4, arsenic is extremely toxic. Its elevated presence in the environment is likely caused by mining activities, excessive ground water irrigation, silviculture, and agricultural activities that make use of fungicides, herbicides, and insecticides. Arsenic concentrations did not vary by habitat type (Figure 3.77), species, or size (Figure 3.52). The concentrations of arsenic at sites 1 (Nottingham) and 2 (Richards Bay) were noticeably lower compared with the other sites (Figure 3.9). Site 1 is located in the silvicultural areas of KwaZulu-Natal. The low concentrations here are unexpected since arsenic is used in wood preservatives and insecticides. In contrast, Site 16 (Knysna) is also located near silviculture activities, but did have high arsenic concentrations. Other sites that showed high arsenic concentrations included Site 5 (Durban), 7 (Wilderness), 11 (Montague), and 15 (New Castle). The relatively elevated concentrations at these sites were unexpected since no clear mining or industrial activities were present except, perhaps, for Site 5. Site 5 is located in the middle of Durban city. It is about 5 km from Durban harbour. The city has many industrial activities, which could explain the high arsenic concentrations. However, for other elements, Site 5 concentrations were low.

There are therefore indications that dragonflies would be good indicators of environmental arsenic.

4.2.2 Mercury

Mercury is one of the most toxic metallic elements, and is a recognised environmental pollutant. It is naturally released into the environment through earthquakes, erosion, and leaks in the earth's crust. However elevated mercury concentrations likely originated from coal and gas power stations, gold mining, iron-and steel production industries as well as waste disposal and smelters. Other sources and symptoms are discussed in Chapter 1, Section 1.3.1. There were no clear differences between the mean concentrations of mercury for the different habitat types, nor for the size categories. However, Species 8 (*Trithemis furva*) had higher concentrations of mercury compared with any other species (Figure 3.64.c). This species was sampled at more than one site and thus its higher mean concentration of mercury should be noted.

Noticeable high mean concentrations of mercury were found at Site 20 (Visarend Vreugde) and 21 (Prozesky) (Figure 3.22). Both the sites were located near Potchefstroom. Site 21 is a wetland close to a wastewater treatment plant. The high concentrations could thus be attributed to polluted wastewater containing mercury. Site 20 is next to the Vaal River that is heavily polluted from mining and industrial activities (McCarthy, 2011). In addition, illegal gold mining and historic gold mines have contributed to the elevated concentrations of mercury in this area. De Klerk et al. (2013) assessed mercury exposure in seasonal wetlands and found elevated concentrations during low flow seasons. Furthermore, two studies found elevated concentrations of mercury in water, soil, and sediments from various locations in the North-West Province (Lusilao-Makiese et al., 2013, 2014). Oosthuizen and Somerset (2010) found elevated concentrations of mercury in individuals from low-income households. One possibility might have been that the individuals were occupationally exposed. This indicated that not only is the environment affected by excess mercury, but people and animals as well. This could possibly explain why the mean concentrations I found in the dragonflies were higher here than elsewhere.

Adults dragonflies may therefore be a good indicator of environmental mercury since the relatively higher concentrations in the North-West Province concurred with the findings of others. Whether *Trithemis furva* has a tendency to accumulate higher concentrations should be further explored, given that it is a widely distributed species (Table 2.1).

4.2.3 Lead

Lead is a highly toxic element that tends to bio-accumulate in biological tissues. A major source of lead pollution is from mining activities (Reglero et al., 2009). Old water pipes made from lead can contaminate water flowing through it. In 2014, in a town in the United States of America, as a cost-saving measure, water was pumped from the Flint River rather than Lake Huron, without taking the corrosion-control treatments of old pipes required into consideration (Bellinger, 2016). Since then, a number of studies indicated elevated lead blood levels in humans, with severe consequences (Hanna-Attisha et al., 2016; Kennedy, 2016; Laidlaw et al., 2016). Leaded gasoline is also a source of air pollution. Other possible sources and symptoms of chronic and acute lead poisoning are discussed in Section 1.3.2.

Results indicate that lead concentrations did not differ for species, habitat type, or size. Notably higher mean lead concentrations were found at Site 16 (Knysna) (Figure 3.24). This was unexpected since Knysna is not a known mining area. All other sites where low in

comparison. Site 17 (Ventersdorp) and Site 15 (New Castle) had slightly higher concentrations than the remaining sites. This could be because of mining activities in the areas.

Dragonflies might therefore be a good indicator of elevated environmental lead.

4.2.4 Cadmium

As discussed in Section 1.3.3, cadmium is an accumulative poison and is a by-product of zinc and lead mining. It is also present in fertilizers. Results indicate that cadmium concentrations did not differ for species, habitat type, or size. The highest mean concentration of cadmium was found at Site 5 (Durban). This could possibly be because of the industrial activities within the city. Site 16 (Knysna) cadmium concentrations was half that of Durban.

There are therefore indications that dragonflies would be good indicators of environmental cadmium.

4.2.5 Chromium

As discussed in Section 1.3.5, chromium is an essential element but in excess can be extremely toxic. Elevated chromium is likely caused by mining activities, including iron mining in South Africa. In addition, chromium is used in a number of industries such as steel manufacturing. Results indicate that chromium concentrations is slightly higher in species 11 (*Trithemis kirbyi*) (Table 3.3), and in wetlands (Table 3.5). The size of the species had no effect. Site 1 (Nottingham) had notably lower mean concentrations than other sites, followed by sites 11 (Montague) and 3 (Riversdale) (Figure 3.30). This was expected because of the absence of any noticeable industries nearby. Site 10 (Rustenburg) had a higher mean concentration than the other sites. Rustenburg's extensive mining activities are not limited to platinum, but include chromite mining (Cawthorn et al., 2005). Chromite is a major source of the chromium.

There are therefore indications that dragonflies would be good indicators of environmental chromium.

4.2.6 Copper

As discussed in Section 1.3.6, copper is an essential element, but can cause serious health problems if concentrations within tissues exceed physiological regulation capacity. Elevated copper in the environment is likely caused by mining activities and a number of industries. Another source of copper pollution is from its use as a fungicide. Results indicate that copper concentrations did not differ for species, habitat type, or size. Site 16 (Knysna) had notably higher mean concentrations of copper compared with other sites (Figure 3.8). Chromated copper arsenate is used as a wood preservative in silviculture (Hingston et al., 2001) and might possibly be the reason for the elevated copper concentrations in Knysna. I did not sample near Phalaborwa where there is a large copper mine. Copper mines cause air pollution and airborne particles can concentrate in areas a distance from the copper mine or smelting unit. Investigations into cattle deaths in an area close to the copper mine in Phalaborwa found that cattle died because of chronic copper intoxication (Gummow et al., 1991). Furthermore, elevated copper concentrations have been found to cause sperm abnormalities in Impala from the Kruger National Park (Ackerman et al., 1999).

There are therefore indications that dragonflies would be good indicators of environmental copper.

4.2.7 Nickel

As discussed in Section 1.3.7, nickel is an essential element that can cause health problems at high concentrations. Elevated nickel concentrations are likely caused by mining activities, industrial activities, and the burning of fossil fuels. Results indicate that nickel concentrations did not differ for species, habitat type, or size. Site 10 (Rustenburg) had notably higher mean concentrations than other sites (Figure 3.7). This would be expected since Rustenburg is in the middle of a number of mines. Other sites had comparable mean concentrations except for site 11 (Montague) that had the lowest mean concentration.

There are therefore indications that dragonflies would be good indicators of environmental nickel.

4.2.8 Selenium

As discussed in Section 1.3.8, selenium is toxic in excess, but essential at lower concentrations, none the less. Elevated selenium concentrations are likely caused by fertilizers that have leached into rivers, and from mining activities. Results indicate that selenium concentrations did not differ for species, habitat type, or size. Site 2 (Richards Bay) notably had the lowest mean concentration of selenium (Figure 3.11). Site 17 (Venterdorp) had the highest concentration. This might be explained by mining activities in the North-West Province, geological background, as well as extensive agricultural practices. Mean concentrations at sites in the Eastern Cape and KwaZulu-Natal were much lower compared with the other sites.

There are therefore indications that dragonflies would be good indicators of environmental selenium.

4.3.9 Aluminium

As discussed in Section 1.3.9, aluminium can be toxic and is non-essential to life. Elevated aluminium concentrations are likely due to mining and industries. Results indicate that aluminium concentrations are slightly higher in species in the small category (Table 3.6) and in wetlands (Table 3.5). Species 1 (*Orthetrum cafferum*), 2 (*Nesiothemis farinosa*), 4 (*Orthetrum chrysostigma*) and 6 (*Crocothemis erythraea*) all had slightly lower mean concentrations (Table 3.3). Site 17 (Rustenburg) had the highest mean concentration which may partially be explained by mining activities (Figure 3.3). Site 2 (Richards Bay) had the lowest concentration of aluminium. This is surprising since one of the largest aluminium smelters in the world is located at Richards Bay. The collection site though, is located about 3 km upstream from the rail terminal, and 7 km from the nearest large industrial complex. This may indicate that dragonflies are small-scale, localised indicators for aluminium, and more detailed sampling would be required.

There are therefore indications that dragonflies would be good indicators of environmental aluminium.

4.2.10 Gold

As discussed in Section 1.3.10, gold is a non-essential element and even though it is not easily absorbed into biological tissues, it can cause a health hazard if taken up. Elevated gold concentrations can be caused by mining activities. Results indicate that gold concentrations were higher in Species 8 (*Trithemis furva*) (Table 3.3). In addition, this species was mostly

located in wetlands habitats (Table 3.5). Species 11 (*Trithemis kirbyi*) and 12 (*Trithemis arteriosa*) had no quantifiable gold. Site 21 (Prozesky), notably, had a very high mean concentration of gold (Figure 3.21). The maximum concentration was 83 mg/kg dm. This site is a wetland close to a wastewater treatment plant and mining activities. At sites 6 (WFS 5), 7 (Wilderness), 8 (Mooi Brug), 10 (Rustenburg), 11 (Montague), 12 (East London), and 13 (Coffee Bay) the concentrations of gold was below detection level. Most unusual is the absence of gold at Site 10 that is a known mining area.

Although the distribution of gold could not be explained from current knowledge on gold pollution (of which there is very little published), there are indications that dragonflies would be good indicators of environmental gold.

4.2.11 Sodium

As discussed in Table 1.3, sodium is an essential element that can be toxic in excess. Elevated concentrations can be caused by mining activities and a number of industries. Results indicate that sodium concentrations were slightly higher in species 5 (*Sympetrum fonscolombii*) and 8 (*Trithemis furva*) (Table 3.3). Sites around Potchefstroom all had high mean concentrations of sodium (Figure 3.1). This was expected since this area is known for mining. Sites 15 (New Castle) and 16 (Knysna) had high concentrations of sodium. Sites 10 (Rustenburg) and 11 (Montague) had the lowest concentration of sodium. It was expected of a site such as Montague, however it was expected that Rustenburg would have elevated concentrations of sodium because of the excessive mining activities in the area.

Since sodium is a physiologically regulated element, dragonflies might not be a good indicator based on the results presented here.

4.2.12 Phosphorus

As discussed in Table 1.3 elevated concentrations of this element is caused by mining, a number of industries and fertilizers. Results indicate that phosphorus concentrations did not differ for species, habitat type, or size. Sites around Potchefstroom (14, 17, 20 and 21) had relatively higher mean concentrations of phosphorus (Figure 3.4). This can, as mentioned, be because of the mining activities in the area. As noted elsewhere, sites 15 (New Castle) and 16 (Knysna) had higher concentrations of metallic elements such in the case of phosphorus. Site 2 (Richards Bay) and Site 10 (Rustenburg) had the lowest phosphorus concentration. However, it is surprising to find phosphorus is such low concentrations at a site such as Rustenburg that is surrounded by mining activities.

Since phosphorous is a physiologically regulated element, dragonflies might not be a good indicator based on the results presented here.

4.2.13 Magnesium

As discussed in Table 1.3, magnesium is essential to life, however in too high or too low concentrations it can cause health problems. Elevated concentrations of magnesium can be caused by fertilizers that end up in rivers as agricultural runoff water, and a number of industries. Results indicate that magnesium concentrations did not differ for species, habitat type, or size. All sites were relatively high in magnesium concentration, except the sites in the Eastern Cape, sites 2 (Richards Bay) and 5 (Durban) in KwaZulu-Natal, and Site 10 (Rustenburg) in the North-West Province (Figure 3.2). The low concentrations in the Eastern Cape were expected since the sites are not close to extensive agricultural practices and

industries. However, sites 5 and 10 were expected to have had higher concentrations of magnesium because of the number of industrial activities at Site 5 and the agricultural activities at Site 10. That said, Site 10 is an abandoned manmade cement dam. It was isolated and no rivers were in a radius of 10 km, but could have contained polluted ground water.

Since magnesium is a physiologically regulated element, dragonflies might not be a good indicator based on the results presented here.

4.2.14 Calcium

As discussed in Table 1.3, calcium is an essential element. However, in the oxalate form it can be toxic. Elevated concentrations of calcium can be caused by industries that manufacture cement, and smelters. Results indicate that calcium concentrations were not dependent on habitat types, but slightly higher concentrations were found in smaller individuals (Figure 3.42.a). Species 8 (*Trithemis furva*) indicated slightly higher mean concentrations (Figure 3.42.c). Sites around Potchefstroom (14, 17, 20, and 21) had relatively higher mean concentrations of calcium (Figure 3.6). The origin of the concentrations is unknown but some industrial activities are nearby. A large gypsum dump is located upstream from Site 21. As noted before, sites 15 (New Castle) and 16 (Knysna) had higher concentrations of metallic elements such as in the case of phosphorus, and now calcium as well. Site 1 (Nottingham) had the lowest mean concentration of calcium. Low concentrations of calcium can be expected from this site since the area is mostly forestry.

However, since calcium is a physiologically regulated element, dragonflies might not be a good indicator based on the results presented here.

4.2.15 Potassium

As discussed in Table 1.3, potassium is an essential element. However, in excess it can cause extreme illness. Elevated concentrations can be caused by mining (especially gold mining), industries including pharmaceuticals, and fertilizers that end up in rivers as agricultural runoff. Most soil types contain potassium that is also an essential component of fertilizers. Results indicate that potassium concentrations did not differ for species, habitat type, or size. Sites such as 3 (Riversdale) and 13 (Coffee Bay) that would be expected to have less pollution, however, had elevated concentrations of potassium (Figure 3.5). Sites 1 (Nottingham), 2 (Richards Bay) and 4 (Graaff Reinet) had the lowest mean concentrations compared with other sites. The lower mean concentrations at the sites can possibly be a result of negligible mining and agricultural activities. Site 16 (Knysna) stood out again as the site with the highest elemental concentration, indicating potassium pollution.

Since potassium is a physiologically regulated element, dragonflies might not be a good indicator based on the results presented here.

4.2.16 Zinc

As discussed in Table 1.3, zinc in trace amounts is essential to life. However, in higher concentrations it can be toxic and interfere with the binding of other essential elements leading to health effects. Elevated concentrations of this essential element can be caused by mining and industries such as battery manufacturing and alloy production. Results indicate that zinc concentrations were not dependent on species, habitat type, nor for size. Notably high mean concentrations of zinc were found in sites around Potchefstroom (14, 17, 20, and 21) (Figure 3.10). The evaluated concentrations can possibly be because of nearby mining. All sites in

KwaZulu-Natal, Site 10 (Rustenburg) and Site 4 (Graaff Reinet) had lower concentrations of Zinc. It is clear that Site 10, even though it is located near mining activities, had low mean concentrations of elements. Sites 16 (Knysna) and 15 (New Castle) stayed true to their high concentrations of other metallic elements.

Despite zinc being a physiologically regulated element, dragonflies might be a good indicator based on the results presented here.

4.2.17 Rubidium

As discussed in Table 1.3, rubidium has no biological role, and causes health effects at high concentrations. Results indicate that rubidium concentrations were not affected by species, habitat type, or size. All sites in the Eastern Cape and Site 3 (Riversdale) in the Western Cape had relatively lower mean concentrations of rubidium (Figure 3.12). However, since no major use for rubidium is known, the concentrations found at all sites were surprising.

There are therefore indications that dragonflies would be good indicators of environmental rubidium.

4.2.18 Strontium

As discussed in Table 1.3, strontium is not known to be a toxic element nor is it essential to life. Elevated concentrations of this element can be caused by mining activities and industries that produce fireworks and warning flares. Results indicate that strontium concentrations did not differ for habitat type or size. However, slightly higher concentrations of strontium were found in Species 2 (*Nesciothemis farinose*) (Figure 3.55.d). Site 7 (Wilderness) had the highest mean concentration of strontium (Figure 3.13). This could not be explained since Wilderness is not a known industrial area. Sites around Potchefstroom (14, 17, and 21) and Site 3 had notably lower concentrations of strontium.

There are therefore indications that dragonflies would be good indicators of environmental strontium.

4.2.19 Molybdenum

As discussed in Table 1.3, molybdenum is an essential element in low concentrations but can be toxic in higher doses and cause severe health and birth related effects such as foetal deformities. Elevated concentrations of this element can be caused by mining and alloy production. In addition, it is used in fossil fuel industries that contribute to air pollution. Results indicate that molybdenum concentrations did not differ for size variations. However, Species 5 (*Sympetrum fonscolombii*) had relatively higher concentrations of molybdenum (Figure 3.56.c). Site 17 (Ventersdorp) had the highest mean concentration of this element (Figure 3.14). As mentioned before this site is located near mining activities. Other sites around this area, except for Site 10 (Rustenburg), had elevated concentrations of molybdenum as well, indicating a possible common source. The lowest mean concentration of this element was at Site 11 (Montague) in the Western Cape. There is no mining activity nearby, possibly explaining the low molybdenum concentrations. However, two other sites in the Western Cape, sites 7 (Wilderness) and 16 (Knysna), did show elevated mean molybdenum concentrations. These sites have unusually high mean concentrations of other metallic elements as well, indicating an unknown and possibly common pollution source.

There are therefore indications that dragonflies would be good indicators of environmental molybdenum.

4.2.20 Palladium

As discussed in Table 1.3, palladium has a low toxicity but can be poisonous at high doses, and may be carcinogenic. Elevated concentrations of this element can be caused by the production of alloys, production, and combustion of fuel, and in various chemical industries. Results indicate that palladium concentrations did not differ for species, habitat type, or size. Sites 20 (Visarend Vreugde) and 21 (Prozesky) stood out from the rest (Figure 3.15). The elevated concentration of Site 21, as mentioned before, can be a result of the wastewater treatment plant next to the site. Site 20 concentrations, however, can partly be due to the mining and industrial activities upstream from this site. Some sites such as Site 12 (East London) had concentrations below detection limits.

There are therefore indications that dragonflies would be good indicators of environmental palladium.

4.2.21 Silver

As discussed in Table 1.3, silver is non-essential to life and toxic at high concentrations. Elevated concentrations of this element can be caused by mining practices (especially lead, zinc, copper and gold mining), alloy production, ENMs, and in electronics. Results indicate that silver concentrations did not differ for species or size. However, slightly higher concentrations were found in samples from wetlands (Figure 3.79.d). One site stood out from all the rest. Site 21 (Prozesky) had notably higher mean concentrations of silver than all other sites (Figure 3.16). This elevated concentration can be because of the wastewater treatment plant that is not far from this site.

There are therefore indications that dragonflies would be good indicators of environmental silver.

4.2.22 Antimony

As discussed in Table 1.3, antimony is an element toxic to life. Elevated environmental concentrations can be caused by the production of alloys and lead, and when used as an active ingredient in flame-retardants. Results indicate that cadmium concentrations did not differ for species or size. However, slightly higher concentrations of antimony occurred in wetlands (Figure 3.81). Sites around Potchefstroom (14, 17, 20, and 21) had high concentrations of antimony (Figure 3.18). Site 16 (Knysna) again showed elevated mean concentrations of the element. Site 5 (Durban) indicated high concentrations of antimony. As mentioned before, this site is located in the middle of a city that is surrounded by industries and it has a harbour nearby that may make use of flame resistant products.

There are therefore indications that dragonflies would be good indicators of environmental antimony.

4.2.23 Barium

As discussed in Table 1.3, barium is believed to be non-toxic in low concentrations. Elevated concentrations can be caused by mining activities, alloy production, and the use of fireworks. Results indicate that barium concentrations did not differ for species, habitat type, or size. Three sites stood out. Sites 10 (Rustenburg), 7 (Wilderness), and 1 (Nottingham) had the

highest mean concentration of barium (Figure 3.19). Wilderness was a most interesting site. Elevated concentrations of barium and strontium were found here. Both elements are used in the manufacturing of fireworks and signal flares, but this is unlikely to be the common source. It was no surprise that Site 10 (Rustenburg) had relatively elevated concentrations of barium, since this area is known for its extensive mining. However, the high mean concentration of barium at Site 1 (Nottingham) that is mostly known for its forestry was surprising.

There are therefore indications that dragonflies would be good indicators of environmental barium.

4.2.24 Platinum

As discussed in Table 1.3, platinum is non-toxic in its metallic form. However, some of its associated compounds are poisonous. Elevated concentrations are caused by mining, alloy production, and in the industrial production of chemicals, electronics, glass, and aircraft. Results indicate that platinum concentrations did not differ for species, habitat type, or size. Site 21 (Prozesky) had the highest mean concentration of platinum (Figure 3.20). As mentioned before, Prozesky is a wetland that is located next to a wastewater treatment plant. Mining are prominent in the area. About 15 km away is Site 20 (Visarend Vreugde) that also had elevated mean concentrations, indicating a probable mutual pollution source. In addition, Visarend Vreugde is located next to the Vaal River downstream of a number of industries. The lowest concentration was found at Site 13 (Coffee Bay). This comes as no surprise since this area is considered “Wild Coast” and has little industrial or mining activities. The area is more rural and informal.

There are therefore indications that dragonflies would be good indicators of environmental platinum.

4.2.25 Thallium

As discussed in Table 1.3, thallium is a toxic element that can be absorbed through the skin. Elevated concentrations of this element may be caused by mining and coal-fired power stations. Results indicate that thallium concentrations did not differ for species, habitat type, or size. Site 15 (New Castle) had the highest mean concentration of thallium (Figure 3.23). New Castle has had elevated concentrations of most metals due to the iron and steel smelting plant located here. Site 16 (Knysna) had high concentrations of thallium as well. Some sites, including sites 2 (Richards Bay), 3 (Riversdale), 4 (Graaff Reinet), 7 (Wilderness), 10 (Rustenburg), 11 (Montague), 12 (East London) and 13 (Coffee Bay) had concentrations below detection limit.

There are therefore indications that dragonflies would be good indicators of environmental thallium.

4.2.26 Bismuth

As discussed in Table 1.3, bismuth has no biological role and presents no real threat to the environment. Elevated concentrations can be caused by mining activities and industries that manufacture cosmetics and medicine. Results indicate that bismuth concentrations did not differ for species, habitat type, or size. Elevated concentrations were found at sites 15 (New Castle) that had the highest mean bismuth concentration of all sites (Figure 3.25). This indicated that the coal mines and Arcelor Mittal smelters in New Castle (Thornhill and Richardson, 2014) might be a possible source. Low concentrations of this element were found

at sites 2 (Richards Bay) and 4 (Graaff Reinet). These sites have low concentrations of many elements. Richards Bay site is near a protected area.

There are therefore indications that dragonflies would be good indicators of environmental bismuth.

4.2.27 Thorium

As discussed in Table 1.3, thorium is a non-essential element and considered radioactive and toxic. Elevated concentrations of this element can be caused by nuclear power stations. Results indicate that thorium concentrations did not differ for species, habitat type, or size. The highest concentration of thorium was found at Site 15 (New Castle) (Figure 3.26). As mentioned before, the coalmines and Arcelor Mittal smelters in New Castle (Thornhill and Richardson, 2014) might be a possible source for the elevated thorium concentrations. However, more research in this area is needed. Sites in the Western Cape indicated lower concentrations.

There are therefore indications that dragonflies would be good indicators of environmental thorium.

4.2.28 Uranium

As discussed in Table 1.3, uranium is a non-essential element that is considered radioactive and poisonous. Elevated concentrations can be caused by mining and nuclear power stations. Results indicate that uranium concentrations did not differ for species, habitat type, or size. Sites 17 (Ventersdorp) and 21 (Prozesky) indicated the highest mean concentrations of uranium (Figure 3.27). This can partly be caused by the mining activities in the area and the wastewater treatment plant next to Prozesky. However, it was a good sign to find concentrations of uranium at many sites were extremely low, even below detection limit.

There are therefore indications that dragonflies would be good indicators of environmental uranium.

4.2.29 Titanium

As discussed in Table 1.3, titanium is a non-essential element. This metal, although non-essential, is normally not toxic or poisonous. Elevated concentrations of this metal can be caused by power generating stations and a number of industries. Results indicate that titanium concentrations did not differ for species, habitat type, or size.

It is not clear if dragonflies would be good indicators of environmental titanium.

4.2.30 Vanadium

As discussed in Table 1.3, vanadium is an essential element. However, like many other essential elements, either too little or too much may cause health problems. Elevated concentrations of this element can come from mining and the production of steel where it is alloyed. Results indicate that vanadium concentrations did not differ for size variations. However, slightly higher concentrations of vanadium concentrations were found in Species 5 (*Sympetrum fonscolombii*) (Figure 3.44.d) and in wetland habitats (Figure 3.74.d). Sites around Potchefstroom (14, 17, 20 and 21) had higher mean concentrations of vanadium (Figure 3.29). The origin of the concentrations is unknown but some industrial activities are found around Potchefstroom that may be the source. As seen for other elements, sites 15

(New Castle) and 16 (Knysna) had higher concentrations. Site 10 (Rustenburg) also had relatively high concentrations of vanadium, possibly associated with mining.

There are therefore indications that dragonflies would be good indicators of environmental vanadium.

4.2.31 Manganese

As discussed in Table 1.3, manganese is an essential element; however, it can cause health problems in certain forms and at high concentrations. Elevated concentrations of this element may be caused by mining activities and fertilizers that contain this element that end up in rivers as agricultural runoff. Results indicate that manganese concentrations did not differ for dragonfly size. However, slightly higher concentrations of manganese were found in species 5 (*Sympetrum fonscolombii*) and 8 (*Trithemis furva*) (Figure 3.46.d). The highest concentration of manganese was found at Site 17 (Ventersdorp) (Figure 3.31). This site is near both mining activities and agricultural activities that may use fertilizer that contain manganese. Sites in KwaZulu-Natal all had low mean concentrations.

Although manganese is an essential and physiologically regulated element, there are indications that dragonflies would be good indicators of environmental manganese.

4.2.32 Iron

As discussed in Table 1.3, iron is an essential element to life. However, in excess it can be extremely toxic. Elevated concentrations of this element may be caused by mining, smelting, and a number of industries, including pharmaceutical production. Results indicate that iron concentrations did not differ for species or size. However, slightly higher iron concentrations were associated with wetland habitats (Figure 3.75.c). The mean iron concentration was high at almost all sites, except at Site 2 (Richards Bay) and Site 4 (Graaff Reinet) (Figure 3.32).

Although iron is an essential and physiologically regulated element, there are indications that dragonflies might be good indicators of environmental iron.

4.2.33 Cobalt

As discussed in Table 1.3, cobalt is an essential element and is suspected to cause health problems in high amounts. Elevated concentrations of this element may be caused by mining and related activities. Results indicate that cobalt concentrations did not differ for species, habitat type, or size. The highest mean concentration of cobalt was found at Site 20 (Visarend Vreugde) (Figure 3.33). This site is next to the Vaal River that is known to be polluted (Grafford and Avenant-Oldewage, 2010). The lowest concentration was found at Site 4 (Graaff Reinet) that so far has been the least polluted site.

There are therefore indications that dragonflies would be good indicators of environmental platinum.

4.2.34 Summary

The results indicated that dragonflies might be suitable indicators for some elements but not others, although more research is needed for confirmation. This is summarised in Table 4.1.

Table 4.1 Summary of the recommendations regarding the suitability of dragonflies as indicators of environmental levels of selected elements.

Recommended		Not recommended	Unsure
Palladium	Selenium	Sodium	Titanium
Copper	Platinum	Phosphorous	
Manganese	Thallium	Magnesium	
Cadmium	Iron	Calcium	
Molybdenum	Aluminium	Potassium	
Cobalt	Rubidium		
Thorium	Gold		
Silver	Strontium		
Nickel	Vanadium		
Zinc	Antimony		
Barium	Beryllium		
Uranium	Mercury		
Lead			

4.3 Sites

The data indicated some interesting results, in particular the repeated patterns of high and low concentrations of elements at certain sites. Some of the more interesting sites will be discussed.

4.3.1 Prozesky

Prozesky (Site 21) indicated elevated mean concentrations of a number of heavy metals such as antimony, silver, palladium, cobalt, mercury, gold, uranium, and calcium. The collection site is located close to a wastewater treatment plant. This may be the main source of the elevated metallic elements concentrations. In addition, mining can contribute to the pollution in this area. Prozesky is a wetland that has a number of bird species feeding and nesting within it, this makes it a popular bird watchers location. The elevated concentration of the metallic elements at this site is a cause of concern for the already threatened bird populations. Van der Schyff et al. (2016) conducted a study to determine the concentration of metallic elements in bird eggs from sites located around the Vaal River catchment. Some of the sites are close to Potchefstroom where Prozesky is located. They found elevated concentrations of gold and uranium in the bird eggs. The mean concentration of gold in dragonflies from Prozesky was 83 mg/kg dm, the same order of magnitude as gold in bird eggs from the same region (van der Schyff et al., 2016).

Figure 3.35, also ordinated Site 21 away from most of the others, associated with relatively higher gold and mercury.

4.3.2 Knysna

Knysna (Site 16) was one of the sites that had elevated mean concentrations of most of the metals. Some of the metallic elements included phosphorus, potassium, copper, zinc, lead, iron, and arsenic. Arsenic is used in wood preservatives (Table 1.3 and Section 1.3.4) and it was no surprise to find elevated concentrations as Knysna is known for its silviculture (Russell, 1996). In contrast, Watling and Watling (1976) analysed trace metallic elements in three oyster species and found trace metal concentrations to be below detection limit. However, the study done by Watling and Watling was almost 40 years ago and since then pollution could have increased in the area, or there were analytical improvements. There has not been much research on metallic element pollution in this area. However, possible sources of metallic

elements include silviculture activities, the wastewater treatment plant located 400 m away from the sampling site, and an informal settlement located upstream.

Figure 3.35 ordinated Site 16 away from most other sites towards the right, which may now be considered as the 'dirty' side. The convex hulls indicated a strong association at Site 16 for relatively higher arsenic and lead contributions. However, other metallic elements such as potassium, copper, and zinc were also associated, but less so when compared with other sites.

A clear source of the elevated metallic elements remains unclear and there is probably more than one. More research in this area is needed.

4.3.3 Rustenburg

Rustenburg (Site 10) is located in the centre of a town that is surrounded by extensive mining. Barium, chromium, and nickel had elevated mean concentrations compared with other sites. The cause of the three metallic elements contamination is likely the mining activities in and around Rustenburg. It was however, surprising to find the mean concentrations of cadmium, copper, lead, zinc, and most especially platinum to be lower compared with other sites. South Africa is the largest producer of platinum on earth and Rustenburg is a town surrounded by platinum mines (Emsley, 2003). The biggest platinum mine on earth is located about 3 km from the town centre. It was expected that the mean platinum and other concentrations at especially this site would be higher. The sampling site was independent from rivers or streams, indicating that dragonflies are indicative of the conditions of their immediate but not their larger environments. Site 10 ordinated to the left of Figure 3.35, associated with the 'cleaner sites', supporting the deduction that dragonflies are site-specific indicators.

More research is needed on this aspect, and in this intensively mined area.

4.3.4 Durban

Durban (Site 5) is located close to the harbour and surrounded by industrial activities. This site had surprisingly low concentrations of all elements used in industries, except for arsenic and cadmium (Table 3.4). The mean concentrations of most element were lower than expected. One possible explanation is that the water body from where the dragonflies likely emerged as adults was not connected to any polluted river or stream. Water-source wise, it may be completely isolated (as was Site 10). The only other route of exposure to pollution may have been from airborne pollution. Olaniran et al. (2014) analysed the Umgeni and Umdloti Rivers in Durban for lead, mercury, cadmium, aluminium, and copper and found concentrations exceeding the South African Department of Water Affairs and Forestry (1995) and World Health Organization (2004) tolerance limits for freshwater quality. The rivers are about 5 km away from the sampling site. This supports the notion that dragonflies are indicators of their immediate environment and not the entire surrounding area.

Site 5, like Site 10, both sites located inside cities that have many potential pollution sources, both ordinated towards the 'cleaner' side of Figure 3.35, mutually supporting the deduction that dragonflies are indicators of their immediate environments.

4.3.5 New Castle

Newcastle (Site 15) is a town in KwaZulu-Natal. The site was on a function venue. Elevated concentrations of thallium, thorium, zinc, arsenic, phosphorus, sodium, magnesium, potassium, rubidium, and lead stood out from many of the other sites. No research has been published in the scientific literature on metallic elements concentrations from this town. The site is located close to agricultural activities that could explain the elevated selenium, zinc, potassium, and magnesium concentrations. The elements are all used as additives in fertilizers (Emsley, 2003). However, the most likely source is the mining and industrial activities in the area. Newcastle is surrounded by coal mining such as Uitkomst coalmine and Arcelor Mittal smelters that could contribute heavily to pollution in this area (Thornhill and Richardson, 2014).

What was interesting is that this site, just like Site 5 (Durban) and Site 10 (Rustenburg) had an isolated water source free from inflowing water from nearby streams and rivers. Both Durban (Site 5) and Rustenburg (Site 10) are known for mining and or industrial activities but showed lower concentrations of metallic elements. However, the Newcastle site is also isolated but had elevated concentrations. Furthermore, the site was on the opposite side of town, located away from the wastewater treatment plant, mines and Arcelor Mittal. It is suspected that the pollution from decades of mining and industry may have polluted the entire area, resulting in the pollutant patterns and compositions measured here. Site 15 was located towards the right and 'dirty' side of Figure 3.35. More research is therefore needed in this area.

4.3.6 Nottingham

Nottingham (Site 1) is located near the Drakensberg and is popular for silviculture, cattle farming, and trout fishing. Samples from this site indicated elevated concentrations of magnesium and barium. The most unexpected result was the relatively low mean concentration of arsenic. Arsenic is used in wood preservation and it was expected that the mean concentrations at this site would have exceeded concentrations found at some other sites. However, the mean arsenic concentration at this site was the second lowest indicating that arsenic is perhaps not used intensively in silviculture here. On the other hand, the elevated magnesium concentrations could have come from farming via fertilizers. Site 1 ordinated towards the 'clean' side of Figure 3.35. More research would be required as to sources involved.

4.4 Species

Metallic element concentrations in 15 dragonfly species were successfully analysed. However, three species did not have enough individuals to be included in the ANOVA analyses. Thus, only 12 species were used to determine the concentration differences and relative contribution patterns between species. For more detailed species-specific differentiation, more individuals of different species from the same sites should be analysed concurrently.

All species had different combinations of habitat preferences, behaviour, and sizes. The species composition may differ depending on the location in South Africa (Tarboton and Tarboton, 2015). Results indicated that species 5 (*Sympetrum fonscolombii*) and 8 (*Trithemis furva*) tended to bio-accumulate metallic elements more effectively. However, the differences between species were not significant. The results indicated that the species of dragonflies were irrelevant when analysing for metallic elements at the national scale of this study. To some extent, the results contradict the findings of (Burghelae et al., 2011). They found that

each species of beetle they used to analyse for metallic elements between two sites accumulated different metallic elements, differently. Tables 4.2 (rows 9 and 10) provide details of two studies done on the metallic element bioaccumulation efficiency in different arthropods (to be discussed later).

The largely negligible differences between sites with respect to species, size, or habitat type (Figures 3.70, 3.71, 3.84) suggests that dragonflies effectively reflect ambient metallic elements, equivalent to birds, earthworms or fish (Newman, 2015). Based on this study, I suggest that other arthropod taxa may also be considered in future studies, and they are summarised in Table 4.3.

Table 4.2 Studies on factors that may affect the concentrations of metallic elements within invertebrates.

	Factor	Article title	Precis	Reference
1	Diet influence	Use of macro and trace elements as biological markers in the lesser grain borer, <i>Rhyzopertha dominica</i> (Coleoptera: Bostrichidae).	Mahroof and Phillips (2012) conducted a study where they compared 10 trace element concentrations between insects kept under laboratory conditions that had a selective diet, and adult beetles collected from the periphery of granaries or inside forested woodlands. They found that the amount of zinc quantified in lab-reared beetles was significantly lower than insects collected outside. This study focused on the concentration of metallic elements within the food they consumed.	Mahroof and Phillips, 2012.
2	Habitat type	Relationships among potassium, calcium and trace elements in tree leaves and associated canopy arthropods	The concentrations of phosphorous, calcium, manganese, iron, copper, zinc, rubidium, and strontium were analysed in three tree foliages and associated arthropod assemblages. The concentrations of metals associated with the foliage indicated patterns. However, the concentrations within arthropods associated with the assemblages showed no clear pattern. Greater concentrations of manganese and strontium were found within foliage litter in the watershed, which had no disturbances. Hickory tree foliage contained higher metal (zinc, copper, iron, manganese) concentrations in comparison with other species of trees. Among the different tree species, the foliage from Dogwoods had higher calcium concentrations, while lower manganese concentrations were noted.	Risley, 1990.
3	Battle between sexes and seasons	The use of chemical composition as a population marker in insects: a study of the Brimstone butterfly	Dempster et al. (1986) analysed Brimstone butterflies (<i>Gonepteryx rhamni</i> L.) for elements phosphorus, calcium, iron, copper, zinc, titanium, phosphor, chlorine, and sulphur. Elements such as phosphorus, calcium, sulphur, zinc, chlorine, and potassium were well suited to discriminate variables for this butterfly. They found differences in chemical composition between males and females, and individuals sampled from different sites. The year that the samples were caught as well as the seasons indicated	Dempster et al., 1986.

			significant differences. However, the variations became less distinctive over time as adults age and feed.	
4	Habitat type	Habitat type-based bio-accumulation and risk assessment of metal and As contamination in earthworms, beetles and woodlice	The effect that different environmental factors (including different habitats) had on concentrations of arsenic, cadmium, copper, lead, and zinc in earthworms, beetles, woodlice, and soil. Vermeulen et al. (2009) concluded that the bio-accumulated concentrations in the organisms do not necessarily agree with the soil characteristics. Thus, is not efficient to differentiate habitats on the soil characteristics alone.	Vermeulen et al., 2009.
5	Transfer of metals to offspring	Elemental marking of arthropod pests in agricultural systems: single and multigenerational marking	The transfer of specified elements from adult to reproductive tissues such as eggs can be used as an elemental marker. Trace elements such as rubidium and caesium proved especially effective. Elements that the host plants (field crops) have been treated with can help serve as a marker in arthropod pests and their offspring movements.	Hayes, 1991.
6	Organ-specific concentration patterns	Ants as bio-accumulators of metals from soils: Body content and tissue-specific distribution of metals in the ant <i>Crematogaster scutellaris</i>	Ants (<i>Crematogaster scutellaris</i>) were used to determine which organ was the most effective in bio-accumulating metals and if the concentrations matched that found in the soil associated with the ant's location. Known polluted and non-polluted sites were used. Gramigni et al. (2013) concluded that of the six metals analysed for, five proved effective in the use of whole body and tissue specific analyses. The concentrations correlated with the soil concentrations. Furthermore, metals concentrated in the midgut, the Malpighian tubules and fat body proved to be effective in bioaccumulation metals and can thus be used to analyse.	Gramigni et al., 2013.
7	Food web and difference in species	Heavy metals in terrestrial macro invertebrates: Species differences within and between trophic levels	Concentrations of lead, zinc, and cadmium were analysed in herbivorous, herbivorous + detritivorous, detritivorous, and carnivorous terrestrial macro invertebrates collected at a disused lead and zinc mine site. Morgan et al. (1986) found no evidence of increased metal concentrations during transference from herbivores to carnivores. In addition, the highest metal concentrations were found in detritivores and not carnivorous as would be	Morgan et al., 1986.

			expected. It is possible that this is because of the removal or loss of metals (lead and zinc) within certain target organs and cells. Furthermore extreme differences in metal concentrations were found between closely related species, more so than to species from different tropic levels.	
8	Food web	Cadmium dynamics in terrestrial food webs of a coal ash basin	Skinner et al. (1978) found an increase of cadmium with higher trophic levels. However, detritivorous had the highest cadmium concentrations. In food webs, detritivores commonly play the role of decomposers, amplifying the importance of leave litter in the bioaccumulation of metals in terrestrial arthropods.	Skinner et al., 1978.
9	Differences in species and body size	Abiotic and biotic factors influencing in situ trace metal levels in macro-invertebrates in freshwater ecosystems.	Regarding a linear negative log relationship, certain trace metals (lead more so than cadmium and copper) indicated a direct relation towards species and respective body weight.	van Hattum et al., 1991.
10	Difference in species	Predatory aquatic beetles, suitable trace elements bio-indicators	Three species of predatory aquatic beetles <i>Laccophilus minutus</i> , <i>Hydroglyphus pusillus</i> , and <i>Rhantus suturalis</i> , were used to analyse aluminium, copper, arsenic, cadmium, cobalt, iron, manganese, molybdenum, lead, nickel, selenium and zinc in rice paddies and control sites (reservoirs). Burghelae et al. (2011) found that the concentration of heavy metals, with the exception of selenium and arsenic, was higher at the rice paddies than the reservoirs. Furthermore, each species of beetle accumulated different metals more effectively. <i>H. pusillus</i> accumulated iron, nickel and manganese, more effectively even at the control sites, and <i>R. suturalis</i> accumulated aluminium, molybdenum and lead more effectively especially in rice fields.	Burghelae et al., 2011.
11	Food web	Trophic transfer of metals along freshwater food webs: evidence of cadmium bio-magnification in nature	Croteau et al. (2005) studied copper and cadmium to identify relative trophic positions and food web structures. They found 15 times higher cadmium concentrations within the scope of two higher trophic positions. In addition, the cadmium concentrations in invertebrates were twice that in fish. Copper on the other hand had no such results.	Croteau et al., 2005.

12	Food web	Taking the trophic bypass: aquatic-terrestrial linkage reduces methyl-mercury in a terrestrial food web	Bartrons et al. (2015) analysed terrestrial arthropods for methylmercury. They found that terrestrial arthropods that consume aquatic prey had lower methylmercury concentrations than arthropods that ate mostly terrestrial prey. They stated that this could be because the lower trophic position of arthropods that directly feed on midges relative to those that fed mostly on terrestrial prey and that had, on average, higher trophic positions. This creates a shorter food web allowing less bioaccumulation to take place.	Bartrons et al., 2015.
13	Body size	Heavy metal content of forest litter arthropods as related to body-size and trophic level	Thirteen forest-dwelling arthropod species were analysed for metallic elements near a zinc factory in the Netherlands. The species were grouped into three body-size categories. Analysis of covariance was used to correct for weight effects on body burden. The results indicate that body-size influence the bioaccumulation of certain metals such as lead, but not others such as zinc and cadmium.	van Straalen and van Wensem, 1986.
14	Species	First report of perfluoroalkyl substances in South African Odonata	Dragonflies were sampled at six sites in Limpopo and North-West Provinces, South Africa. They indicated measurable concentrations of PFAs, in particular perfluorooctanesulfonic acid. All species used indicated bio-accumulated concentrations. This was the first such study ever published on persistent organic pollutants in adult dragonflies.	Lesch et al., 2017.

Research discussed in Table 4.2 all had different outcomes regarding relative trophic position, body size, habitat associations, and transfer of metallic elements between generations of arthropods. However, no adult dragonfly studies, except Lesch et al. (2017) could be found. The current study is the first to investigate metallic element concentrations in adult dragonflies, while also incorporating multiple variables such as body-size, species, habitat type, and relative distances from possible pollution sources. It is unclear exactly how many arthropod species there are, indicating an endless list of the possible arthropods that can be used to determine the metallic element concentrations in the environment. None the less, Table 4.3 discusses the advantages and disadvantages of some possible arthropod taxa that may be considered in future studies.

Table 4.3 Advantages and disadvantages of selected arthropod taxa that may be considered in future studies.

Scientific name (order)	Common name	Advantages	Disadvantages
Scorpiones	Scorpions	• Easily identifiable • Easy to find • Widespread and abundant in arid habitats • Predatory species	• Mostly active at night • Venom can be dangerous to humans
Acari (seven different orders)	Ticks and mites	• Widespread • Parasitic or free-living scavenging carnivores	• Diverse group • Difficult to identify
Amblypygi	Whipscorpions	• Close association with soil • Predatory • Found in subtropical and arid zones	• Not easy to find • Difficult to identify
Myriapoda	Centipedes and Millipedes	• Centipedes are predators • Widespread	• Millipedes feed on plant material • Difficult to identify
Opiliones	Harvestmen	• Close association with soil • Mostly predatory species • Possibility of super predator	• Some species feed on dead plant material • Difficult to identify
Solpugida	Sunspiders	• Easily recognizable • Diurnal predators	• They are not abundant
Hymenoptera	Wasps	• Predatory species that hunt for prey to feed their offspring • Sometimes pollinators	• Adult solitary wasps mainly feed on nectar

(Holm, 2017; Picker et al., 2004)

4.5 Size categories

Dragonflies were categorized into four body sizes; very small, small, medium, and large. The very small and large body size categories had few individuals and were therefore excluded for most comparative assessments. Most of the elements tended to have higher bio-accumulated mean concentrations in the small body size category. Only for selenium and arsenic were the concentrations higher in the medium category. This relates with the results found by van Straalen et al. (1986) who found that smaller sized individuals bio-accumulated some

elements such as lead better than larger sized organisms (Table 4.2 row 13). However, body size does not necessary influence the bioaccumulation of elements such as zinc and cadmium. In this study, the difference between the small sized individuals and the medium sized individuals showed minimal differences. When pooled, the differences were not statistically significant, indicating that the medium and small body-sized individuals bio-accumulated metallic elements equally well (Figure 3.70). This result adds to the value that adult dragonflies have (all of whom are aerial predators) as bio-indicators of site-specific environmental pollution, since the differences were notable between sites, but not between size categories. From a body size point of view, dragonflies seem well suited as indicators for different sites.

4.6 Habitat types

The sites were divided into three general habitat types; wetlands, rivers, and dams. Samples from wetlands indicated bioaccumulation of silver, cadmium, antimony, iron, gold, and aluminium slightly more effectively than the other habitat types. Thallium was the only element to bio-accumulate in wetland samples the least. The ANOVA showed no significant differences between concentrations from wetlands, dams, and rivers ($p > 0.05$). However, the Tuckey test did show a difference for aluminium, chromium, iron, cobalt, nickel, molybdenum, lead, antimony, gold, thallium concentrations in samples collected from wetlands compared with samples collected from dams and rivers, respectively. The results indicated that the habitat type from where the samples originated had little effect on metallic element concentrations on a larger scale, but may affect concentrations at smaller scales. This agrees somewhat with the results of Risley (1990) and Vermeulen et al. (2009) who found that metallic element concentrations correlated with environmental factors and arthropod species (Table 4.2, row numbers 2 and 4).

4.7 Advantages and disadvantages of using dragonflies as indicators

As discussed in Chapter 1 Section 1.4, many arthropod can be used as indicators of environmental pollution. The reasons behind the use of each species are discussed in Section 1.4.2 and Tables 1.4, 1.5, and 1.6. The reasons behind the use of dragonflies will be discussed in Table 4.4.

Table 4.4 Advantages and disadvantages of using adult dragonflies as metallic element indicators.

Advantage	Discussion
Easy to collect with entomological nets	• Adult dragonflies are relatively easy to catch and preserve. • They can easily be captured using an entomological net. • They are also relatively easy to locate, with adult dragonflies flocking near fresh water. • Field guides on adult dragonflies help locate and identify dragonflies in a specific area. • In most cases, the diversity of species will be more than two species, each, normally, with multiple individuals. • The vegetation surrounding the water body plays a key role, in not only the species diversity but in the sampling process. • Dragonflies perch on grass or branches overhanging the water that is close to the edge, presenting predictable opportunities for capture.

	• Dragonflies are easy to handle after capturing and can simply be placed in a container with alcohol for preservation, or frozen.
Easy to identify	• Taxonomically, adult dragonflies are well documented, and many countries have reliable and user-friendly field guides. • Dragonflies are some of the larger insect orders and have flamboyant colours. • The sexes of most species can be distinguished in the field making it easier to sample selectively adult males.
Seasonality	• Adult dragonflies emerge from their moults during summer or late spring and are diurnal rather than nocturnal. • Dragonflies are active during the day, hunting, mating, and laying their eggs around fresh water systems. • This creates favourable opportunities for sampling dragonflies during the day. • During the day, dragonflies are easier to identify. • In addition, there is no need for the use of specialized capturing equipment such as night-lights. • They have a relatively short life span as adults (1-9 months) this favours the use of the invertebrates to indicate the effects pollution has on them, their offspring, and the environment.
Ethics	• In many countries, ethical implications for collecting, transporting and storage of lower invertebrates are less restrictive than for mammals, fish, and birds.
Occurrence	• Odonata can be located on all continents and most islands, with the exception of Antarctica. • There are no records of species from this region and if by chance a dragonfly might have wondered off course, finding itself in this polar region, it is unlikely that it will settle and reproduce due to the low temperatures and poor vegetation (according to Kalkman et al. (2008)). • A high diversity of dragonflies is found in the tropics (Kalkman et al., 2008). • Furthermore, assemblages of dragonflies are large enough for sampling without causing a dramatic population decline. • The assemblages are often made up of habitat generalist as well as specialist species. • This makes dragonflies good indicators of change (Simaika and Samways, 2009a).
Exposure	• Dragonflies inhabit both terrestrial and aquatic environments. According to Johnston (1995), organisms that inhabit both terrestrial and aquatic environments are exposed to multiple pollutants.
Diet and trophic level	• Dragonflies are ruthless hunters in both the larvae and adult live stages. • They feed predominantly on other invertebrates. • In addition, some larvae feed on small fish and tadpoles. • Dragonflies inhabit a high trophic status. • This favours the bioaccumulation of toxic chemicals higher up the food chain. • They are themselves prey for other predators and play an important role in the ecosystem.

Disadvantages	Discussion
General	• Very little is known about the effects of toxic substances on Odonata. • Adults can only be sampled during warmer months. • The prey of adult dragonflies will be myriad, opportunistic, and difficult to sample and analyse, complicating trophic transfer assessments. However, it is most likely that prey will be of local origins. • Some dragonflies relocate or are migratory, complicating site-specific assessments • However, the same constraints are also pertinent to other classes of indicators, such as birds, fish, and mammals.

(Corbet et al., 1999)

Chapter 5: Conclusions and recommendation

“In nature nothing exists alone.”

– *Rachel Carson*

5.1 Conclusions

A number of other organisms have been used as bio-indicators of environmental pollution. However, this is first study done on the concentration of metallic elements that bio-accumulate in adult dragonflies. The only other study that have used adult dragonflies as bio-indicators was by Lesch et al. (2017) analysing for PFAs. That study led to this study. The variables between samples indicated the following results:

- All species of dragonflies despite all having differences in behaviour, indicated suitability as indicators. However, *Sympetrum fonscolombii* and *Trithemis furva* bio-accumulated slightly higher concentrations of elements such as sodium and manganese.
- The body size difference between species did not affect the concentration of metallic elements that bio-accumulate within adult dragonflies.
- Dragonflies from different habitat types showed that sample origin had little effect on metallic element concentrations on a larger scale, but may affect concentrations at smaller scales.

Different dragonfly species with different body sizes originating from different habitat types are indicative that all dragonflies may be used to as indicators of metallic elements in their surroundings.

There were 21 sampling sites across South Africa. Depending on putative pollution sources for each sampling site, concentrations between sites showed differences.

- Sites close to wastewater treatment plants such as Prozesky and Knysna showed elevated concentrations metallic elements. However, the elements were not the same.
- Mining activities such as at Newcastle had exceptional high mean concentrations of some metallic elements, most likely associated with mining. The metallic elements included thallium, thorium, zinc, arsenic, phosphorus, sodium, magnesium, potassium, rubidium, and lead.
- Sites such as Durban and Rustenburg that was expected to indicate high metallic element concentrations because of surrounding industries and mining had unexpectedly lower metallic element concentrations compared with other sites. However, this may be because the water at these sampling sites was from isolated 'clean' sources.

To the best of my ability, I could not find any literature on the effects that elevated metallic elements have on adult dragonflies.

The aim of the study was to evaluate adult dragonflies as potential indicators of metallic elements in the environment. Based on my results, and pending further supporting studies, I conclude that dragonflies would be excellent indicators of metallic elements.

5.2 Recommendations

- A minimum of five samples from each site should be collected and analysed.
- Sites nearer to possible pollution sources such as mines and wastewater treatment plants should be analysed.
- Sampling at sites that are not isolated from incoming water sources such as streams, is preferable to get a more accurate indication of the concentration of metallic elements in a larger area.
- The difference in bioaccumulation potential should be establish between sexes.
- Research on the effects that elevated metallic elements have on adult dragonflies should be conducted to infer risk, and inform conservation.

“Knowing what I do, there would be no future peace for me if I kept silent.”

– Rachel Carson

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