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The relationship between landscape functional patch type and soil ecotoxicity along a pollution gradient

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1612780

MSc Dissertation

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Abstract

Anthropogenic stressors such as mining and smelting disrupt soil processes and degrade landscape functionality, creating long-term barriers to ecological rehabilitation. The Landscape Function Analysis (LFA) model is widely used to monitor landscape recovery. It works by delineating a fixed portion of the landscape into regions of resource accumulation (patches) and areas of resource loss (interpatches). Each region is then visually and physically assessed for its soil-surface attributes, which relate to indices of landscape function (stability, infiltration, and nutrient cycling). However, the LFA model is field-based, so it cannot directly capture landscape function in terms of chemical and ecotoxicological degradation, and these are integral aspects of ecosystem function. This study aimed to determine whether complementing soil physicochemical and ecotoxicological indicators with LFA metrics could improve the assessment of ecosystem function in contaminated environments.

This research was conducted along a heavy metal pollution gradient at an area next to a smelter complex, using the Trigger–Transfer–Reserve–Pulse (TTRP) model as a conceptual foundation. Three complementary approaches were applied: (1) measuring soil properties and deriving LFA indices in patches and interpatches; (2) assessing the toxicity of soil leachates to the euryhaline cladoceran *Daphnia magna* (*D. magna*) and the marine bacterium *Aliivibrio fischeri* (*A. fischeri*); and (3) evaluating soil and leachate phytotoxicity using vascular plant bioassays.

The results indicated significant variation in soil chemistry along the gradient, with decreasing organic matter ($p = 0.003$) under higher contamination. The correlations between the LFA indices and soil parameters were weak, indicating that surface indicators were insufficient to reflect subsurface chemical impairment. For toxicity to *D. magna* and *A. fischeri*, the results revealed significant toxicity in leachates from highly contaminated sites, with increased immobilisation of *D. magna* ($p = 0.021$) and inhibition of *A. fisc*heri luminescence ($p < 0.001$) corresponding to higher concentrations of Cu, Zn, and Pb. Some leachates from areas with moderate LFA scores still exhibited strong toxicity, indicating that the visual surface condition may underestimate chemical stressors. The vascular plant bioassays revealed strong inhibition of germination ($p < 0.05$) and root growth ($p < 0.05$) in soils and leachates from highly contaminated areas, with root elongation being the most sensitive endpoint. These vascular plant patterns were consistent with aquatic bioassay results and correlated with elevated metal

concentrations and electrical conductivity (r = 0.67 and r = 0.92, respectively). Differences between patch and interpatch soils diminished with increasing contamination.

Collectively, these findings showed that heavy metal contamination and salinity decouple surface structure from subsurface processes, disrupting the TTRP feedback that maintains landscape resilience. This study proposes an expanded LFA framework for smelter-contaminated sites, incorporating chemical and ecotoxicological diagnostics alongside traditional surface metrics. The integrated approach captures both visible and latent dimensions of ecosystem function, offering a more mechanistic and sensitive tool for monitoring rehabilitation and ecological recovery in degraded areas.

Keywords: Smelter-related contamination; Landscape Function Analysis; spatial heterogeneity; ecotoxicology; patch types; soil contamination.

Declaration

I, Celine Bisnath, declare that this Dissertation is my own, unaided work except where acknowledged to be the work of others. It is being submitted for the Master of Science Degree at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



28th day of October 2025

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Chapter one – Introduction and literature review

1.1 Introduction

The landscape is a complex system that operates at various organisational scales (Turner, 1989). Each scale can function as a singular system, ranging in size from a few micrometres to kilometres of cover. However, the connecting link is that each scale is fundamentally linked to the other through resource flow and interactions between multiple organisms (Metzger *et al*., 2021). In a system without stressors, each scale operates to produce a functional ecological system; however, this is often not the case, and various stressors can "tip the ecological scale" (Brovkin *et al*., 2021). Anthropogenically induced stressors, such as industrial and agricultural activities, often result in landscape degradation. Upon realising this, the topic of rehabilitation and restoration of the environment has increased almost tenfold in the last century (Arneth *et al*., 2012). Therefore, monitoring procedures are needed to document the effectiveness and trajectory of restoring and rehabilitating a degraded landscape.

Landscape degradation is defined as a decline in the condition of a landscape, which results in the loss of ecosystem services and the overall functioning of the landscape (FAO and UNEP, 2021). With a focus on landscape degradation due to mining and smelting activities, mining companies are now legally bound to rehabilitate the affected landscape so that it is no longer a source of pollution (Festin *et al*., 2019). Some degree of restoration of ecosystem function can be achieved through rehabilitative measures, which include re-creating a landscape with a similar function to deliver ecosystem services (Bradshaw, 1984). Land rehabilitation requires monitoring of the degraded landscape long before mine closure. This is to determine the trajectory of rehabilitative measures and whether objectives are met (Tongway, 2008). The Landscape Function Analysis (LFA) protocol meets this requirement by serving as an effective monitoring tool.

The LFA protocol was developed for use in semi-arid environments. It is used as an indicator-based method to monitor the state of a degraded landscape in comparison to a more functional landscape that has desirable attributes for the end goal of ecological restoration. This is done by quantifying the ability of a landscape to conserve vital resources and recycle them into the landscape system, thereby providing a self-serving feedback system (Tongway, 1995). The ecological theory behind the LFA method considers spatial heterogeneity along

the broad landscape scale and finer scales by delineating the landscape into areas of resource accumulation (patches) and resource loss (interpatches) (Tongway and Hindley, 2004). In effect, it also encapsulates ecohydrological processes by considering the movement of water and vital resources between these patches and interpatches.

While the LFA method serves as a useful measure of the extent to which a site needs to improve, in comparison to a suitable reference site, the results of this assessment are based on subjective observations of interconnected processes along the landscape. A landscape exposed to stressors, such as a smelter-contaminated site, has a varied extent of resilience that may be specific to the biotic and abiotic characteristics of that site (Zamulina *et al*., 2022). The LFA protocol does not directly consider the physicochemical properties and biological diversity of the landscape in question and instead infers insight into these processes through visual and physical soil indicators. It has been well researched that the presence of chemical contaminants can reduce the normal processes within the soil (Ettler *et al*., 2016). Therefore, the visual and physical soil indicators assessed by the LFA model may not be reliable indicators of landscape function in contaminated conditions since microbial and plant activity may be suppressed even when the surface structure appears to function normally (Erskine *et al*., 2013). A single template monitoring approach cannot be used to efficiently address concerns related to contamination and rehabilitative methods. Therefore, effective monitoring procedures need a holistic approach to determine the extent of dysfunction (Noss, 1990).

This project takes a top-down approach to simplify the complexity of a heavy metal-contaminated landscape by examining spatial heterogeneity from the broad scale down to the patches and interpatches identified along the fine scale. Each patch and interpatch will then be subject to different methods of characterising ecotoxicological hazards, and an assessment of soil physicochemical properties. As more information about a system is available, more realistic models can be developed to facilitate the rehabilitation and restoration of the landscape in question (Gomez *et al*., 2014).

1.2 Literature review

1.2.1. Soil quality and ecosystem services

According to Doran *et al*. (1994), soil quality is defined as the “capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health”. This implies that biological

activity is dependent on soil quality. Heavy metal contaminants are not biodegradable (Cai *et al*., 2019) and can persist in the soil for many years after mine closure. Excessive levels of heavy metals can impede the physical, chemical, and biological processes occurring in the soil, limiting the structure of its ecosystem and therefore, its ability to provide ecosystem services. These contaminants can accumulate in the soil via dust deposition or in stream sediments via runoff from metallurgical effluents. Apart from reducing soil quality, metals can also enter the food chain and endanger animal life and welfare (FAO and UNEP, 2021).

Soil ecosystem services are the benefits that humans get from key soil functions (FAO and UNEP, 2021). These include, but are not limited to, decomposition of plant residues, nutrient cycling, carbon sequestration, agricultural activities, flood control, and climate regulation (Morgado *et al*., 2018). In the event of soil pollution due to the increased concentrations of heavy metal contaminants, soil acidification and salinisation, these ecosystem services may start to break down. This can result in nutrient imbalances, loss of biodiversity, and leaching of contamination to the groundwater (Bünemann *et al*., 2018).

Soil functioning is mediated by the interactions between abiotic and biotic components within the landscape (Morgado *et al*., 2018). For example, microbial communities at the belowground level can promote plant growth by the production of plant hormones (Koide, 1991), increase the bioavailability of nutrients to plants (Yang *et al*., 2018), and promote resistance and protection against diseases and other stressors (Lucas *et al*., 2014).

1.2.2. The impact of mining and metallurgical activities on soil health

Mining and smelting activities are a source of land (soil), water, air, and noise pollution. In particular, the effects on soils and sediment are most deleterious and long-lasting since they receive the bulk of contamination (FAO and UNEP, 2021). Soil contamination occurs through the release of organic and inorganic pollutants. Inorganic pollutants can be released into the environment during the mining and smelting process in the form of metal-rich dust and sulphur dioxide emissions. These metallic pollutants are known as potentially toxic elements (PTEs) (Pourret *et al*., 2021). In terms of their chemical properties and subsequent biological effects, PTEs are defined as metals or semi-metals that have the potential for toxicity due to their bioavailability in the soil (Duffus, 2002). Potentially toxic metals can be released into the environment during the smelting process via dust deposition, and runoff from rainfall and metallurgical effluents (Jeong *et al*., 2021). These elements include arsenic (As), bromine (Br), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead

(Pb), vanadium (V), and zinc (Zn) (Siegel, 2002). To maintain consistency with previous literature, these elements will collectively be referred to as “heavy metals (contaminants)” and “pollutants” for the remainder of this dissertation.

Copper smelters worldwide can emit an average of 200 kg of dust for every tonne of Cu produced (Goonan, 2004). Emissions contain small particles of dust, consisting of a range of metal/loids depending upon the ore source, such as Cu, As, Pb, antimony (Sb), and bismuth (Bi) (Davenport *et al*., 2002), as well as the release of sulphur dioxide. These particles may be windblown or precipitate into the soil from the atmosphere, fog, or rain (Ettler *et al*., 2016). The mixing of sulphur dioxide with water can also result in acid rain events. The uppermost layer of the soil is a sink for the accumulation of these toxic particles. It should be noted that soils naturally contain concentrations of heavy metals from the weathering of the soil parent material; however, soils can become significantly enriched due to anthropogenic sources of contamination, which in this case are mining and smelting processes. This leads to an overall decrease in soil quality. Smelter deposition of metal-rich dust can also release Pb, Cd, and mercury (Hg), which are major threats within soils (Gautam *et al*., 2016). This can lead to an inhibition of biological processes such as decomposition of organic matter, nutrient cycling, soil respiration, and bioturbation (Moreno, 2009). Chemical processes such as cation exchange capacity, regulation of soil pH, and the adsorption and desorption of heavy metal ions may also be affected (Singh and Steinnes, 2020). Physical processes are also affected, such as reduced water infiltration, soil structure and the formation of aggregates, which may increase soil erosion (Sheoran *et al*., 2010).

1.2.3. Landscape Function Analysis (LFA)

Landscape Function Analysis is a monitoring technique that builds on previously conceptualised ecological frameworks that describe the landscape as a system. It incorporates tools to identify a landscape that has been degraded (Tongway and Hindley, 2004). The framework underpinning the LFA methodology is known as “Trigger-Transfer-Reserve-Pulse” (TTRP) and views a landscape as a system of connected processes, within a range of targeted observations (Ludwig and Tongway, 1997). In a functional landscape, a **trigger**, such as rainfall, will result in the **transfer** of vital resources across the landscape in an upslope-to-downslope direction, or downwind, as well as vertically into the soil via infiltration. A **reserve** functions like a bank to accumulate these vital resources in discrete areas called patches, subsequently resulting in a growth **pulse**. This pulse adds to the overall retention of

resources within the landscape and their slow release to the soil biota. The benefits of these resources are fed back into the system via biological and physical processes, resulting in a balanced self-sustaining landscape where resource retention is promoted, and loss is low and slow (Tongway and Hindley, 2004).

Associating the observable patterns from the landscape soil surface with its underlying ecohydrological process is the essence of the LFA methodology. The TTRP framework consists of a field methodology and an interpretational framework. It follows a hierarchical structure by first considering the broad scale and then the fine scale of the landscape. This structure allows for a holistic view of the landscape, making it desirable for use on mine sites. The field methodology consists of the landscape organisation (LO) step at the broad scale and a soil surface assessment (SSA) at the fine scale. Each method is assessed along a gradsect (a transect laid along the gradient of the landscape). The SSA is made up of eleven indicators of visually assessed soil surface attributes (elaborated in the next sub-section). These are assessed along the landscape and input into the LFA model, with the outputs being three indices of landscape soil surface function: stability, infiltration, and nutrient cycling.

For the interpretational framework, each index does not hold any significance in isolation and needs to be compared to repeated measures of the same gradsect over time and compared to indices from a reference site that serves as the desired endpoint for rehabilitation. When compared, each index can help to determine the current stage and trajectory of rehabilitation or restoration procedures. This enables questions of resilience and proximity to a critical threshold (Tongway and Hinley, 2004). The LFA method serves as an objective monitoring tool for use by farmers and scientists alike, provided that the observer is consistent in following the guidelines for use. Any accelerated loss of water and resources will unbalance a system, resulting in a dysfunctional landscape (Tongway and Hindley, 2004): soil may be less stable, infiltration lowered, and nutrient cycling reduced. This uneven spatial distribution of nutrients is expressed in the heterogeneity of the landscape, which is key to the interpretational framework (Tongway and Ludwig 1990).

In the event of dysfunction due to mining activities, cross-landscape transfer processes may promote pollution redistribution after initial deposition. Some heavy metal contaminants are readily washed or blown out of the local landscape, whilst others may be concentrated in patches of organic matter or adsorbed onto clays (Elliot *et al*., 1986). Soil excavation, continuous erosion, or the slow release of pollutants into the soil may also impact the cycling

of nutrients in the landscape (Durães, 2018). The TTRP framework does not include the relationship between contaminant dynamics and the transfer processes along the landscape but has the potential to be modified to serve that purpose (Tongway, 2021: personal email communication).

An example of a successful rehabilitation exercise on a mine site is a bauxite mine in Australia, at Gove in the Northern Territory (Tongway, 2010). This study used LFA indices, which were derived at various intervals up to its 26-year mark of closure, to assess the effectiveness of rehabilitation along the mined landscape on which the soil layers had been carefully replaced and planted. However, this site was not impacted by contamination, and it also had intensive rehabilitation exercises, which are rarely affordable in southern Africa.

A copper mine in Zimbabwe (Dowo *et al*., 2013) used LFA indices to assess the level of land rehabilitation up to its 10-year mark of closure. After rehabilitation, indigenous woody species were found to increase along the previously mined site. This increased the ability of the landscape to be more self-sustaining, suggesting that rehabilitation practices were effective.

In South Africa, Van de Walt *et al*. (2012) used LFA to determine the functionality of plant communities along natural and rehabilitated areas (up to 10 years old at the time) on a platinum mine. It was found that the rehabilitated areas had greater indices of nutrient cycling and infiltration compared to natural landscapes along the mine site. The higher indices were due to restorative measures such as the use of fertiliser and irrigation. This positively impacted landscape functionality. However, more patches of vegetation occurred along the natural site as compared to the rehabilitated site.

Daniell *et al*. (2019) used LFA to monitor the progress of rehabilitation of a gold tailings storage facility (TSF) at its four-year mark. The LFA indices did not indicate much improvement since the earlier results. Haagner *et al*. (2008) found similar results when using LFA indices to extract information about the trajectory of rehabilitation on a gold TSF over a short monitoring period. They found it worthwhile to combine the LFA method with the point-centred quadrat method (which monitors the layer of herbaceous cover along the landscape). Limitations when using the LFA methodology come about when trying to extract monitoring information from artificial and polluted landscapes as opposed to natural substrata, and/or over a short monitoring period. An example of this is the accumulation of litter due to the presence of heavy metals inhibiting nutrient cycling (Martin, 2012). In this

case, the LFA protocol would capture the presence of litter as a soil protective measure, thereby promoting landscape functionality; however, a direct assessment of the soil would reveal a reduction in nutrient cycling. Therefore, this limitation may be improved when including other monitoring methods that directly assess the soil processes.

The praise for LFA is its cost-effectiveness and ease of use *in situ* (Tongway, 2004), instead of time-intensive analysis of field samples *ex-situ* that may incur significant costs. Single-variable assessments of landscape quality, such as vegetation cover, soil tests, or biodiversity measures, cannot fully encapsulate landscape complexity (Tongway, 1995). This emphasises the need for the LFA model, which considers the entire landscape as a system of interconnected processes. However, some studies have disputed the reliability of the LFA method based on the lack of any correlation with traditional measures of soil function on semi-arid (Australian) coal mine sites. (Erskine *et al*., 2013).

1.2.4. Soil surface assessment indicators and soil surface indices (SSIs)

The soil surface assessment consists of assessing eleven soil surface assessment indicators according to a specific class value, revealing the quality of each patch or inter-patch (Tongway and Hindley, 2004) (a description of each indicator and associated class values is found in **Appendix A**, **Table A3**). **Soil cover** considers the impact of rain-splash erosion to determine the extent of vegetation impeding raindrops. **Perennial vegetation cover** looks at grasses, shrubs, and trees as surrogates for the below-ground biological activity. **Litter cover** considers the activity of decomposition and bioturbation processes at the soil surface, which indicates nutrient cycling. **Cryptogram cover** assesses the presence of biological soil crusts on the soil, which indicates erosion control. **Crust brokenness** assesses the extent of potential and current erosive features. **Soil erosion** investigates the severity of erosion. **Deposition of materials** indicates the movement and retention of sediment and litter through the landscape, from the upslope to the downslope direction. **Surface roughness** determines the ability of the soil to capture and retain vital nutrients at a fine scale. **Surface resistance to disturbance** assesses how easily the soil can be disturbed, to permit or resist erosion. The **slake** and **soil texture tests** are field tests which evaluate the soil stability for water infiltration capacity. The indicators are then related to their specific soil surface index (SSI) to communicate the overall soil quality along the landscape. The soil surface indices include measures of stability, nutrient cycling, and soil infiltration.

The eleven soil surface assessment indicators do not directly measure physicochemical and biological processes that maintain soil condition and instead infer information on these processes through proxies of soil function (Tongway and Hindley, 2004). This is informative in a physically degraded landscape, where landscape heterogeneity is a mirror of ecological function (Tongway and Ludwig 1990); however, a similar approach should not be extrapolated to chemically contaminated environments. The relationship between the soil surface indicators and actual soil function gets warped in the presence of chemical contaminants, since these pollutants can cease nutrient cycling and inhibit microbial activity (Erskine *et al*., 2013), even when the soil surface features may imply a well-functioning system.

1.2.5. Factors that could affect the LFA indices and soil properties

As discussed in section **1.2.2**, the presence of heavy metal contaminants within the soil can have deleterious effects on soil quality. These effects can also be seen in the distorted outcome of the LFA indices and skewed soil properties. For instance, monitoring studies conducted along a study site at the Palabora Copper Mine in Limpopo, South Africa (Neveling, 2011), have indicated that the smelter stack emits wind-blown particles dominated by S, Cu, Ni, and Co. The site also received metal and nutrient-rich effluents from the metallurgical plant and sewage plant, respectively. This has resulted in highly polluted soils and vegetation patches closer to the smelter, and less polluted soils and vegetation further away.

Vegetation structures, such as trees and their associated woody litter, also trap dust particles (Dimitrijević *et al*., 2016). Concentrations of heavy metals in leaf litter samples may affect nutrient cycling within vegetation patches when compared to bare patches, and this may differ according to the level of pollution in that patch and chemical interactions with decomposing macro-organic matter. Focusing on the Palabora Copper Mine again, Kendall (2012) found that leaf litter sampled from areas in the direction of smelter deposition contained elevated concentrations of heavy metals and did not show any evidence of decomposition.

Plant growth, litter decomposition and nutrient cycling may all be affected by the presence of metal contaminants in the soil, as well as altered pH and electrical conductivity (EC) or salinity at differing soil depths. A study of soils affected by copper smelter dust in the sub-tropical environment of Chile indicated the presence of highly leached soils due to acidic

contamination (Jarošíková *et al*., 2018). Another study on the copper smelters along the Zambian Copperbelt found high concentrations of heavy metals in all the soils sampled, which also resulted in greater weathering of the soils (Ettler *et al*., 2016).

Oxidation Reduction Potential (ORP) at differing soil depths may change in response to the presence of moisture and metal contaminants in the soils. Metal solubility in soils is strongly influenced by the relationship between pH and ORP (Tack *et al*., 1996), and this may influence the availability of soluble metals (Cu, Ni, Co, Pb, and Br) to plants and thus, the quality (metal content and decomposability) of litter patches.

The surface layer of the soil profile contains the highest concentration of heavy metals (Ettler, 2016); however, some heavy metals can penetrate deeper into the soil profile. This vertical movement of contaminants occurs through leaching, or even bioturbation by the soil biota, and will depend on the mobility of the specific heavy metal. Ginocchio *et al*. (2004) investigated heavy metal pollution along the soils near a copper smelter in Chile and found that the concentration of heavy metals, with an emphasis on Cu, was greater under shrubby plant cover, but the bioavailability of these metals was lower relative to their abundance. This was due to the ability of soil organic matter to bind to heavy metals, thereby reducing their mobility in the soil.

The release of sulphur dioxide during the smelting process can also increase the bioavailability of heavy metals via direct accumulation along the landscape, or in the form of acid rain. Ettler *et al.* (2011) found that the binding of sulphates to lead, which has a lower rate of mobility compared to other heavy metals, drastically increased the mobility of lead within the soil.

1.2.6. Soil ecotoxicology

Ecotoxicology is a field that examines the deleterious effects of substances on various living organisms (Van Gestel, 2012). A single species can be used as a test organism to determine safe or lethal concentrations of a toxicant. Each test species forms part of a battery of tests which must be practical and cost-effective, easily standardised, reproducible, and ecologically meaningful (Van Gestel *et al*., 2016). The OECD (Organisation for Economic Cooperation and Development) and ISO (International Standardisation Organisation) are foundational guidelines that set the standard for these laboratory ecotoxicological bioassays. The validity of each test is based on whether an authorised test organism is used, if the test follows a set duration, and if there is a defined endpoint to the test (Alves and Cardoso, 2016). This review

will focus on three lines of toxicity testing: aquatic microbial tests using *Aliivibrio fischeri*, aquatic invertebrate tests using *Daphnia magna*, and terrestrial tests using seed germination and the inhibition of root elongation of vascular plants.

1.2.7. Microbial tests using *Aliivibrio fischeri* (Vibrionaceae)

Aliivibrio fischeri (*A. fischeri*) is a marine, bioluminescent gram-negative bacterium species. It is used for toxicity testing in saline water, soil leachates and sediments due to its bioluminescent properties and response to pollutants (Abbas *et al*., 2018). *Aliivibrio fischeri* naturally emits luminescence due to the enzyme luciferase, which is linked to the cellular metabolism of the bacterium species. When exposed to toxic compounds, cellular metabolism is affected, resulting in an inhibition of bioluminescence. The extent of inhibition of bioluminescence is directly related to the toxicity of the sample (Parvez *et al*., 2006).

Various factors of a toxic sample can result in the inhibition of *A. fischeri* bioluminescence. For instance, the concentration of toxic heavy metals in solution, such as Cu, Pb, Ni, and Cd, can lead to the production of reactive oxygen species (ROS). This is linked to a reduction in cellular metabolism and can lead to reduced bioluminescence through oxidative stress and cell damage (Bergua, 2021).

A highly acidic solution can result in the denaturation of luciferase and directly inhibit bioluminescence (Silva *et al*., 2021). A decrease in pH can also increase the solubility of heavy metal contaminants, thereby producing ROS and reducing cellular metabolism (An *et al., 2012)*. Additionally, increased salinity can cause osmotic stress and reduce cellular metabolism. Focusing on effluents from mining and smelting activities, sulphide compounds can readily reduce to sulphates, which affects the electron transport chain of *A. fischeri* and inhibits luciferase function (Planer-Friedrich, 2008).

Limitations of using *A. fischeri* include a limited sensitivity to heavy metal contaminants such as Mn, Cd and Zn, as found by Teodorovic *et al*. (2009) - this study investigated industrial water quality using *A. fischeri* and *Daphnia magna* (*D. magna* is explained in the next sub-section) and concluded that *A. fischeri* is not a reliable indicator. Additionally, Restrepo *et al.* (2017) investigated the correlation between landfill leachate solutions and toxicity to *A. fischeri* and found that *A. fischeri* did react after exposure, but it was the least sensitive amongst all the species assessed. Loureiro *et al*. (2005) investigated the toxic effect of soil leachates derived from a mine in Portugal and found that *A. fischeri* was sensitive to low

doses of heavy metals, while having no toxic effect at high doses. Therefore, the use of *A. fischeri* may result in a generalisation of toxicity (Parvez *et al*., 2006).

1.2.8. Aquatic testing with *Daphnia magna* (Daphniidae)

Daphnia magna is a freshwater cladoceran and widely used for ecotoxicity testing due to its salinity tolerance, sensitivity to a wide range of pollutants, ease of handling, and ecological relevance (Persoone *et al*., 2009). This review focuses on the acute immobilisation tests of *D. magna,* which assess the percentage of *D. magna* immobilised after exposure to a pollutant for set periods. Immobilisation of *D. magna* is defined as the complete cessation of swimming after a gentle agitation (Blewett *et al*., 2018).

Factors of a test solution that promote the immobilisation of *D. magna* include the concentration of bioavailable heavy metal contaminants, with these leading to inhibition of respiration, neurotoxicity, decreased enzyme activity, and lowered ion regulation, leading to immobilisation (Knops *et al*., 2001). The bioavailability of these contaminants is dependent on the pH, oxygen reduction potential, and soil organic matter of the solution. An acidic and aerobic solution enhances the solubility of most heavy metals, thereby increasing their toxicity to *D. magna*. Additionally, a high salt content can cause osmotic stress and dehydration of *D. magna* (Gonçalves *et al*., 2007).

Providing a food source for *D. magna* during the acute assessment of immobilisation has been scrutinised in the literature. Some studies have recommended that the presence of food during tests would increase the real-life applicability of the test (Barata *et al*., 2002). Others refute the presence of food since *D. magna* are filter feeders, and the presence of food within a contaminated substance could reduce or increase the uptake of bioavailable pollutants due to adsorption, thereby increasing the rate of immobilisation, which would mask the actual toxicity of the media (Weltens *et al.,* 2000).

1.2.9. Terrestrial tests using seed germination and inhibition of root elongation

Seed assays are used to assess the impact of contaminated soils or leachates on plant development due to their cost-effectiveness and serve as an early warning system for terrestrial distress (Bareke, 2018). Two test endpoints discussed in this review are peak germination and the inhibition of root elongation. Peak germination endpoints imply the percentage of seeds that can germinate after exposure to a contaminated substance, and the

inhibition of root elongation endpoint is the length of primary roots after exposure to contaminants compared to root length after exposure to a control substance (Wang, 1991). Germinating seeds are sensitive to environmental changes since cell division, metabolism, and nutrient transport begin to take place during this time (Bareke, 2018). Root emergence and development are also sensitive during germination due to cell division and differentiation in the root apical meristem and zone of maturation (Bengough, 2006).

According to standard protocols, seed assays are considered valid when using at least one monocot and one dicot species, due to the differing germination types and root development patterns, thereby increasing the ecological relevance of each test (OECD, 2006; Test number 208). Additionally, monocot seeds are considered more resilient to toxicants due to antioxidant responses driven by the fibrous root system (Al Hassan *et al*., 2017). Whereas dicot seeds are considered more sensitive due to the single root system (Pemberton *et al*., 2001). Decreased pH and increased concentration of heavy metals and salts are factors that can affect seed germination and inhibit root elongation by disrupting cell division and metabolism (Bareke, 2018).

1.3 Rationale, problem statement and research aim

The landscape function analysis (LFA) model is currently used on mine sites to assess landscape function through physical and visible indicators from patches and interpatches along the landscape. However, its effective use in heavy metal contaminated landscapes is still uncertain. Assessing the physicochemical properties of the soil can identify the presence of contaminants, but this does not expose their ecological consequences. Ecotoxicological bioassays assess the response of aquatic and terrestrial organisms to polluted soils and leachates, and this provides a direct quantification of biological impairment. This study seeks to address the limitations of using the LFA model in heavy metal-contaminated landscapes.

Following this brief rationale and problem statement, the aim of this dissertation is to investigate whether integrating soil physicochemical and ecotoxicological assessments from patches and interpatches along a pollution gradient can complement the ability of the LFA model to evaluate landscape function.

1.4 Research question and objectives

The overarching research question posed in this dissertation is: To what extent can the assessment of soil physicochemical and ecotoxicological properties, along a gradient of pollution, complement the LFA protocol?

This question will be answered by the following objectives and tasks, which are presented in three data chapters of this dissertation:

- Chapter 2 forms the foundation of this dissertation since the LFA results and soil properties will be used in the subsequent chapters. This data was collected along gradsects within three zones of pollution, and each gradsect was organised into patch and interpatch types. The research question posed in this chapter is: "What is the relationship between LFA indices (stability, infiltration, and nutrient cycling) and soil properties of patches and interpatches across a gradient of soil pollution?"
- Chapter 3 evaluates the acute ecotoxicity of soil leachate solutions from patches and interpatches, of polluted and unpolluted gradsects, to the immobilisation of *Daphnia magna* Straus, 1820 and inhibition of bioluminescence of *Aliivibrio fischeri* Urbanczyk *et al*., 2007. The key research question posed in this chapter is: "How does the ecotoxicity of soil leachates to *D. magna* and *A. fischeri* change between patches and interpatches across a gradient of soil pollution, and how is this related to the properties of each solution?"
- Chapter 4 assesses the phytotoxicity of soils and leachate solutions to the germination and root elongation of six species of vascular plants. Soils and leachates are derived from patches and interpatches from gradsects on three pollution zones. Species used are: *Amaranthus hybridus* L., *Dactyloctenium australe* Steud., *Lepidum sativum* L., *Medicago sativa* L., *Brassica nigra* L., and *Sorghum bicolor* (L) Moench. The key research question posed in this chapter is: "How does patch/interpatch type influence the phytotoxicity of soils and leachate solutions to plants, and how is this related to the properties of each substrate?

Chapter two – Assessing landscape function and soil properties of patch types from a gradient of pollution in a semi-arid lowveld savanna

2.1 Introduction

Landscape degradation due to anthropogenic activity impacts soil health and landscape functionality and may affect entire ecosystem function and resilience (Bradshaw, 1984). Ecosystem function is defined as the ability of the abiotic and biotic features of an ecosystem to interact with each other in a way that promotes the exchange of energy and nutrients within the food web (Tilman *et al*., 2014). The physicochemical and biological properties of an ecosystem are closely related to drive its function.

Heavy metal pollutants can alter the functionality of a landscape system, and this could be exacerbated by spatial heterogeneity, which unequally distributes pollutants through the landscape. Understanding the relationship between spatial heterogeneity and the change in soil properties of polluted systems is integral to developing an effective landscape monitoring protocol (Lovett *et al*., 2005). The Landscape Function Analysis (LFA) procedure serves as a systematic monitoring procedure by organising a landscape into patch and interpatch units and assessing their soil surface condition (Tongway and Hindley, 2004).

The LFA procedure captures the movement of vital resources, such as water, soil, and nutrients, through the landscape; these resources flow through interpatch areas and are captured within patch areas. In a polluted landscape, heavy metal contaminants can indirectly disrupt the normal flow of water and vital resources. This occurs through an increase in salinity, thereby affecting water availability and uptake (Singh and Steinnes, 2020), altering pH and soil aggregate formation, leading to soil acidification and a reduction in the porosity and infiltrative capacity of the soil (Sheoran *et al*., 2010), and through reducing biological activity and root growth, leading to hard and compacted soils (Moreno, 2009). Therefore, if the LFA protocol is going to be used in heavy metal-contaminated landscapes, it needs to be validated in the presence of heavy metals.

This chapter focuses on identifying patches and interpatches, their associated LFA indices, and fundamental soil properties from a range of soil contamination. The research question I

will address is: What is the relationship between LFA indices (stability, infiltration, and nutrient cycling) and soil properties of patches and interpatches across a gradient of soil pollution?

To answer this research question, the objective of this chapter is:

1. To evaluate how the LFA indices relate to key soil properties assessed within each patch and interpatch across the pollution gradient.

And to do this, I will:

   a. To arrange each gradsect across the gradient of soil pollution into its respective patch and interpatch type using the landscape organisation step of the LFA protocol.
   b. To assess the soil surface condition with LFA indices of stability, infiltration, and nutrient cycling for each patch and interpatch identified across the pollution gradient.
   c. To analyse the soil properties (in terms of pH, electrical conductivity, soil organic matter, and soluble concentrations of Cu, Pb, Br, Ni, and SO_4) of each patch and interpatch identified along the pollution gradient at depths of 0-2cm and 2-5cm.

2.2 Methods

2.2.1. Study site

Field analysis and data collection took place on three sites in Phalaborwa Municipality, Limpopo, South Africa (**Figure 2.1**). Consideration was given to finding sites with a similar slope, aspect, geology, soil catenas and vegetation type. Site 1 (hereafter referred to as the high pollution zone) (23°58'40.31"S and 31° 9'7.48" E) and site 2 (hereafter referred to as the medium pollution zone) (23°58'49.22"S and 31° 8'59.18" E) were found in the game reserve of the Palabora Copper Mining Company (PMC), down-gradient of the smelter complex located to the South of Phalaborwa. A field reference site (hereafter referred to as the unpolluted zone) (23°55'29.30"S and 31° 7'24.33" E) was found in a rangeland to the Northwest of the town of Phalaborwa.

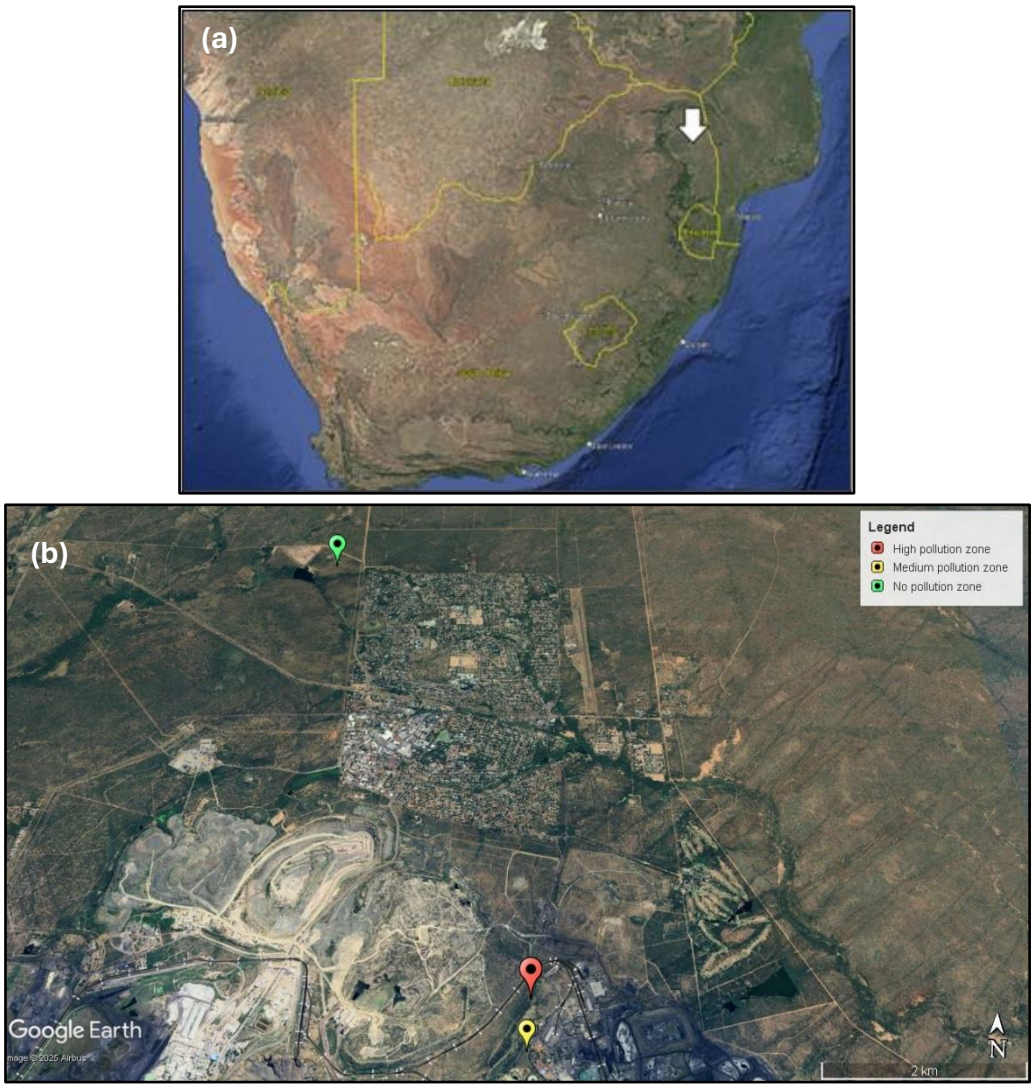


Figure 2.1: (**a**) Location of the study sites in the Limpopo province, South Africa (white arrow). (**b**) The red and orange pins indicate the high and medium pollution zones within the Phalaborwa Copper Mine, respectively. The green pin indicates an unpolluted zone found 6 km from PMC. [Google Earth image: September 2024]

Palabora Copper Mining Company was an open pit mine and smelter from 1964 to 2002, after which an underground mine was developed, adding another 20 years to its lifespan. Found next to the Kruger National Park, the mine also functions as a private game reserve, so large herbivores such as elephant, buffalo, and impala were found on site. The high and medium pollution zones were found on a previously studied site referred to as Loole (after the Loole Spruit that runs through the site). The smelter complex emits particles of metal-rich dust into the surrounding area, which the Loole Spruit receives, along with runoff of polluted water from the metallurgical complex. The soil types and extent of spillage were previously surveyed (Red Earth cc., 2015). The altitude of Phalaborwa municipality ranges from 383 masl to 398 masl. Average temperatures range from 20° to 30° C in summer (wet season) and 16° to 23° C in winter (dry season) (**Figure 2.2**). The wet season typically occurs between

September to April, with an average rainfall of 480 mm per annum (January being the rainiest). In contrast, the dry season starts in May and ends in August. Average humidity ranges from 52% to 87% (**Figure 2.2**). There is a dominant north-westerly direction of wind (Matimolane *et al*., 2024).

The prevailing vegetation type of Phalaborwa is a semi-arid Mopaneveld woodland (Siebert *et al*., 2021). Much of the natural vegetation along the high and medium pollution zones was lost because of smelter-related disturbance and emissions, and large herbivores. Soils along each zone range from terrestrial to riparian due to the presence of streams that traverse each area. Common tree species on the study sites include *Philenoptera violacea*, *Dichrostachys cinerea*, *Combretum apiculatum*, *Colophospermum mopane*, and *Terminalia sericea*. Common grass species making up most of the ground cover include *Aristida argenta, Digitaria eriantha*, *Cenchrus clandestinus*, and *Urochloa mossambicensis*.

Topography on the study site ranges from a gentle 2 – 5 degrees to a moderately steep 8 – 12 degrees. The parent material of the soils along the Loole study site includes dolerite and carbonate, granite, sandstone, alluvium and colluvium deposits and anthropogenic deposits (Red Earth cc., 2015).

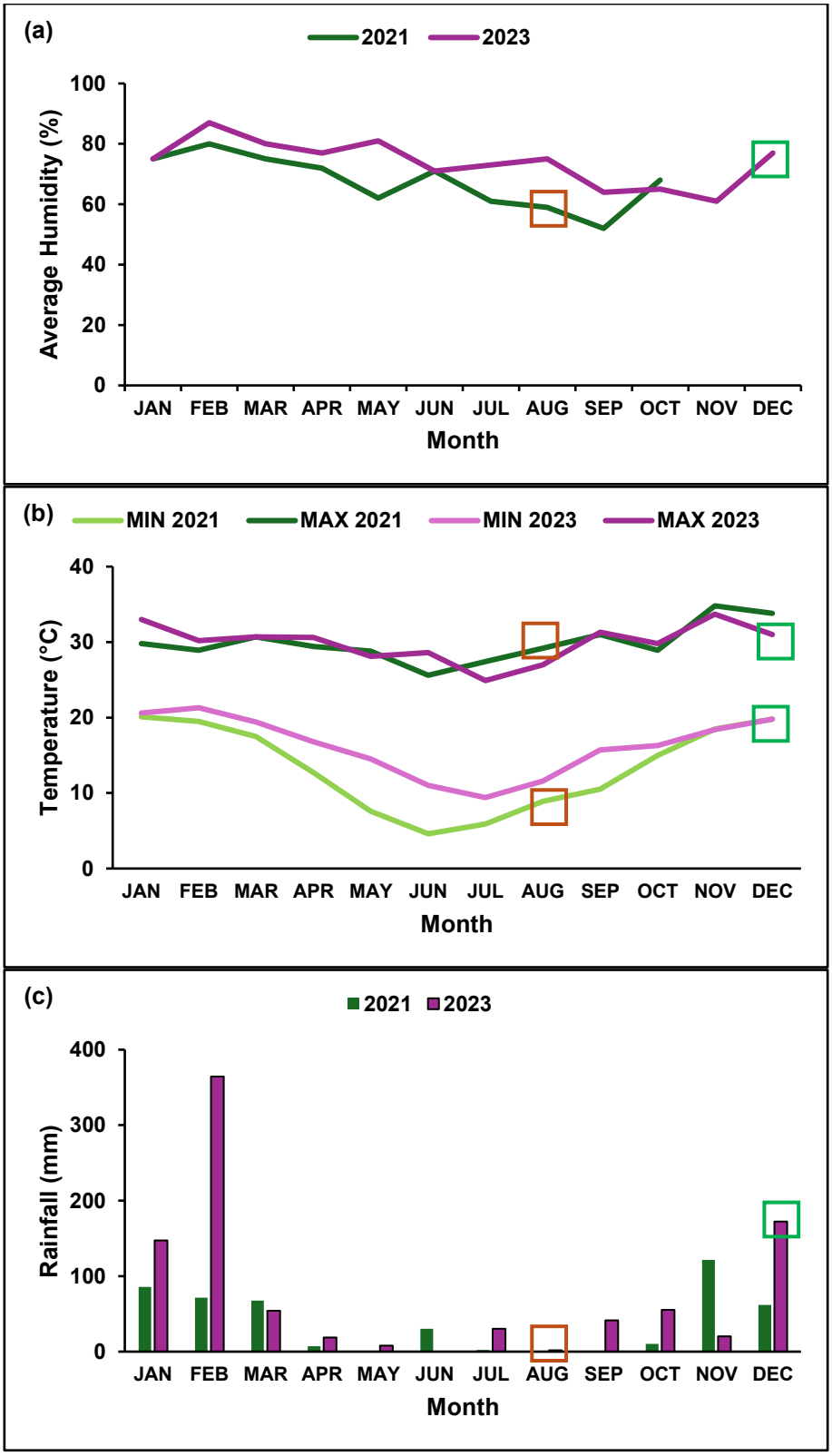


Figure 2.2: (**a**) Monthly average humidity (%) for 2021 and 2023. No data was available for November and December 2021. (**b**) Monthly maximum and minimum temperatures for 2021 and 2023. (**c**) Monthly rainfall for 2021 and 2023. All readings were recorded at 08:00. Data obtained from South African Weather Services (SAWS), Phalaborwa Gate weather station [0681266E6].

2.2.2. Study period

This project combines field data and soil samples collected in 2021 and 2023. The high and medium pollution zones were assessed during the dry season of 20-23 August 2021, while the unpolluted zone was assessed during the early wet season just before the main rains on 20-22 December 2023 (**Figure 2.2c**).

The results of the LFA protocol are a product of biotic and abiotic features of the landscape. Since these features change with the time of year, the LFA results are not directly comparable to the high and medium pollution zones. Therefore, this project does not compare landscape function between pollution zones, but uses the stored soils collected from the patches and interpatches, identified within respective zones, to assess ecotoxicity and soil properties under laboratory conditions.

2.2.3. Fieldwork

2.2.3.1 Layout of gradsects

The term "gradsect" is used in place of "transect" as per Gillison and Brewer (1985), since the environmental data is collected along a gradient through the landscape. Each gradsect was initially proposed to cover the same length along each zone. However, time constraints and the presence of elephants and buffalo along the site impeded this design for the gradsect on the medium pollution zone. The gradsect along the high pollution zone was 160 m to encompass the distance from the top of the catena (10 m in from the road) to the stream at the bottom. The gradsect along the medium pollution zone was split into three segments of 61 m, 60 m, and 40 m, to avoid buffalo on site. The gradsect along the unpolluted zone was 160 m long (4 m in from the road at the top of the catena, to the stream at the bottom).

The selection of the high and medium pollution zones was informed by previous mappings of soil contamination along the site (Weiersbye & Davies, 2017; unpublished maps for PMC) (**Appendix A, Figure A1**). The high and medium pollution zones are directly downwind and downgradient of the smelter complex (**Figure 2.3a**). It has been established from studies on copper smelters in similar environments in sub-Saharan Africa (Ettler *et al*., 2011; Kříbek *et al*., 2018: Namibia and Zambia) that smelter pollution is widespread, and that "non-polluted" sites are not feasible. Therefore, the unpolluted zone was found to the far northwest of the PMC property to ensure a negligible level of contamination due to smelter deposition (**Figure 2.3b**).

Zones of pollution were the Contaminated Land guidelines of the National Environmental Waste Act of South Africa (NEMWA), which establishes the national trigger (screening) values for investigation in South Africa. Soil Screening Values (SSV) for SO_4^{2+}, Ni, Co, and Cu were used to delineate the high and medium pollution zones within the PMC property (**Table 2.1**).



Figure 2.2: Layout of gradsects during fieldwork: (**a**) the high pollution zone (red – 160 m long) and the medium pollution zone[a] (orange – 61 m, 60 m, and 40 m long) (**b**) the unpolluted zone (green – 160 m long). Each gradsect was laid at right angles to the slope contours. Blue lines on each picture show the canalled streams. [Google Earth image: October 2024]

Table 2.1: Definitions of "**low**," "**medium**", and "**high**" sulphate and metal contamination along the Loole site and the unpolluted site. Levels of contamination are in mg/kg or ppm on a soil dry mass basis for the surface 0 – 5 cm depth.

	Low contamination	**Medium contamination**	**High contamination**
SO_4^{2}	<400	>4000<6000	>6000<9000
Ni	<91	>91<200	>200<500
Co	<300	>300<500	>500<3000
Cu	<100	>100<5000	>5000<20000

Before visiting each site, the length and position of each gradsect were planned on Google Earth. The objective was to align the transect at right angles to the slope to survey the flow of water and nutrients downslope (Tongway and Hindley, 2004). Field work at each site started at approximately 08h00 and ended at 17h00. Each site needed two days to assess for the

[a] The gradsect along the medium pollution zone was broken into three segments due to time constraints and the presence of elephants and buffalo on the gradsect line during fieldwork.

Landscape organisation and the Soil Surface Assessment. An exception was for the medium pollution zone since animals and time constraints led to the segmentation of this gradsect.

2.2.3.2 Landscape Organisation

The Landscape Organisation (LO) step of the LFA is done at a broad scale and on the slope of the landscape (Tongway and Hindley, 2004). Patches and interpatches along the gradsect are identified and then marked by placing flags at their starting and end points (**Figure 2.4a**). In this way, the distance of each patch/inter-patch along the gradsect and its length and width were easily observable. Surface obstructions greater than 2 cm were characterised as a patch (**Figure 2.4b**). Patches are areas that collect resources, while interpatches represent areas of resource loss (**Figure 2.4c**). The LO step results in a tabulated form of the extent of resource loss and accumulation within the landscape. The number of patches, patch and interpatch type, length and width and the distance between each patch were recorded.

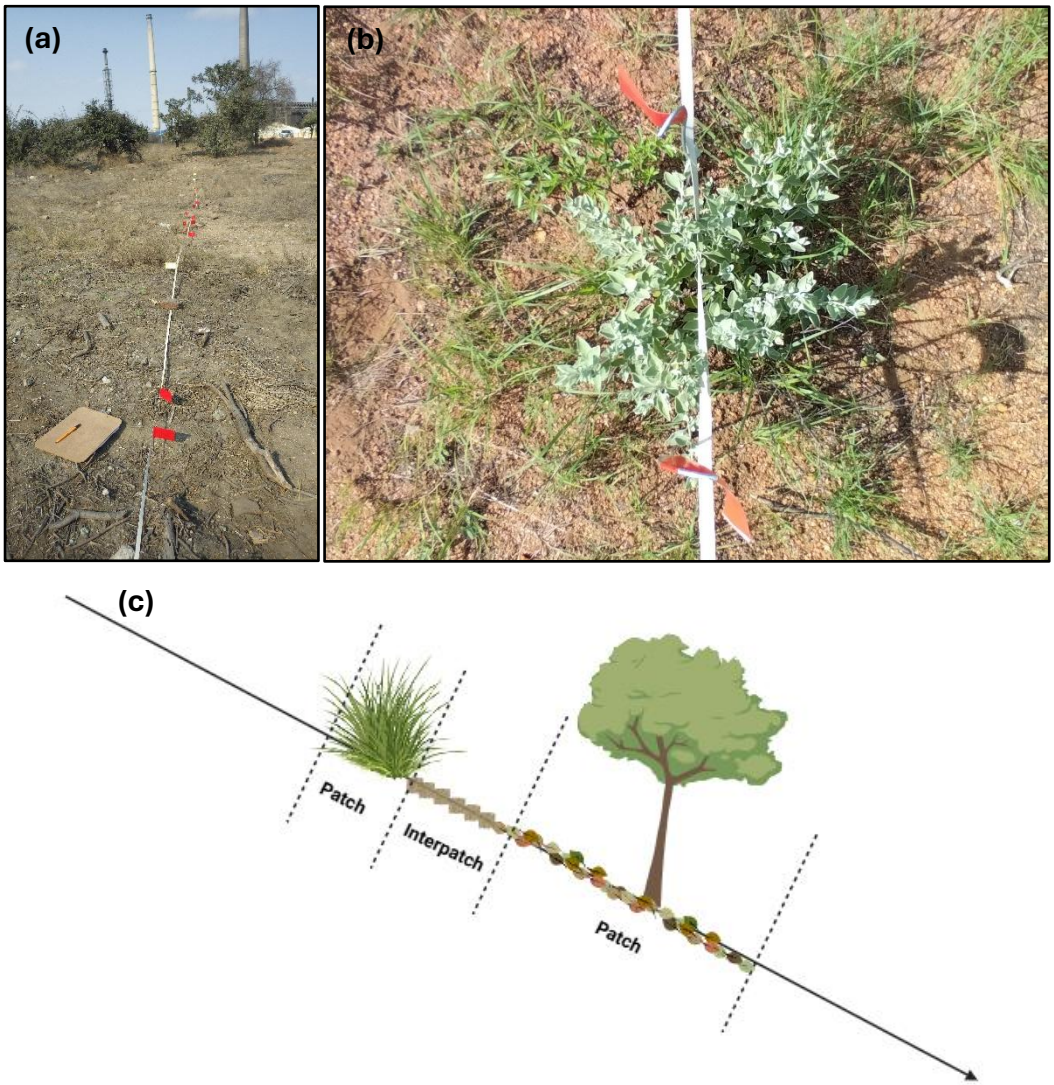


Figure 2.3: (a) Landscape organisation along the high pollution zone. **(b)** Example of a patch identified along the unpolluted zone **(c)** Schematic diagram for the definition of a patch and interpatch (diagram not drawn to scale).

2.2.3.3 Soil Surface Assessment

After the landscape organisation was concluded along the entire length of each gradsect, a Soil Surface Assessment (SSA) was then conducted at each patch and inter-patch identified. A one-metre mini-transect was placed at 90° to the gradsect, and 11 indicators of soil quality were used to score each patch and inter-patch (**Table 2.2**). Scores for each indicator are available in **Appendix A, Table A1.** These indicators were then categorized into three indices of soil quality: stability, water infiltration, and nutrient cycling. A maximum of five and a minimum of three replicates were collected for each patch/inter-patch. Only patches and interpatches that contained at least three replicates were included in this chapter.

Table 2.2: The eleven soil surface condition indicators that were visually assessed on each patch and interpatch. Each indicator corresponds to a Soil Surface Index (SSI) in terms of soil Stability, Infiltration, and nutrient cycling.

Soil surface condition indicators:	Soil Surface Index (SSI):
1. **Rain-splash protection**	STABILITY
2. **Perennial vegetation cover**	INFILTRATION, NUTRIENT CYCLING
3. **Litter cover**	STABILITY, INFILTRATION, NUTRIENT CYCLING
4. **Biological soil crust cover**	NUTRIENT CYCLING, STABILITY
5. **Physical crust brokenness**	STABILITY
6. **Erosion type and severity**	STABILITY
7. **Deposited materials**	STABILITY
8. **Soil surface roughness**	INFILTRATION, NUTRIENT CYCLING
9. **Surface resistance to disturbance**	STABILITY
10. **Slake test**	INFILTRATION, STABILITY
11. **Soil texture**	INFILTRATION

2.2.3.4 Soil sample collection

After conducting the SSA, soil samples were collected at depths of 0 – 2 cm and 2 – 5 cm. Soils were collected at this shallow soil surface depth since the intention was to assess the soil surface in relation to the corresponding soil surface index (SSI) measured at the respective query zone. For statistical purposes, the goal was to acquire at least three soil replicates for each patch and interpatch category identified along each zone. Soil samples were collected by first clearing off any surface litter/stones/vegetation (without damaging any live plants) and then excavating a 10 x 10 cm square area (**Figure 2.5a**). Each sample was collected with a hard plastic shovel to avoid contamination by metals in the tools. Soils were then placed in a double plastic Ziplock bag and stored in a cooler box while on site. The

Ziplock bags were each labelled with a paper label on the outer bag to prevent any contamination by metals diffusing from the ink into the soil (**Figure 2.5b**). The soil samples were then transported to the Ecological Engineering and Phytoremediation (EEP) laboratory, School of Animal, Plant, and Environmental Sciences at the University of the Witwatersrand within a few days for soil preparation and analysis.



Figure 2.4: (a) Example of a 10 x 10 cm area excavated on each patch and interpatch to collect a soil sample**. (b)** Soil samples were collected in plastic Ziploc bags to prevent cross-contamination.

2.2.4. Laboratory analysis

Visible roots were first removed from each soil sample. For analysis of soil properties, each sample was checked for initial weight before airdrying and reweighing the soil sample. The dried soil was then sieved to the 2 mm fraction with a wire mesh sieve (**Figure 2.6a and b**). The resulting gravel (> 2 mm) and soil (< 2 mm) were weighed. Subsequent soil preparation was done according to the procedures in the following text.

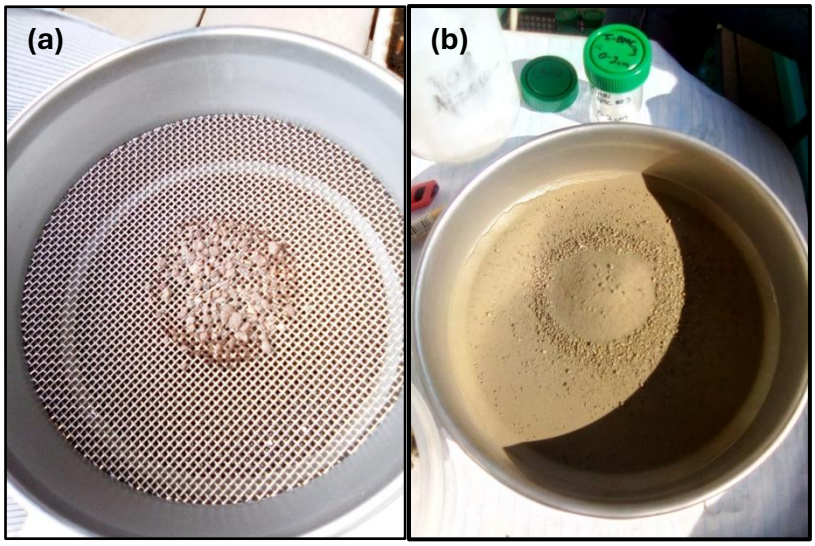


Figure 2.5: Sieving of a soil sample with a 2 mm wire mesh sieve into (**a**) gravel fraction and (**b**) < 2 mm fraction.

2.2.4.1 Leachate analysis for pH, electrical conductivity, and soluble contaminant concentrations

Leachate analysis was assessed to simulate rainwater runoff and ponding on-site. Therefore, two leachate extraction methods were used: the Field Leach Procedure (FLP) and the Synthetic Precipitation Leaching Procedure (SPLP). These are commonly used in mine waste and contaminated land screening protocols for assessing the potential risk of contaminants leaching into stream water or after heavy rainfall conditions (Hageman, 2004). Each leachate was diluted to a ratio of 1:20, as recommended by the US EPA (US Environmental Protection Agency, 2002) when dealing with soil and not mine waste.

For the FLP extractions, standard methods were used, according to the USGS (US Geological Survey) FLP (Hageman, 2005), which accounts for soluble contaminants under unpolluted rainwater and stream water conditions. This extraction method required soils to be sieved to the 2 mm fraction. Each soil sample was then weighed to 10.0 g and placed into polyethene leaching bottles that had been prepared with an acid wash (0.1 M nitric acid) and then rinsed with deionised water. Thereafter, 200.0 mℓ of deionised water at room temperature (25° C) was added to each bottle. This mixture was then stirred on a rotating shaker for 10 minutes at 150 rpm and allowed to settle for another 10 minutes. Once settled, the leachate was filtered through ash-free non-adsorbent Millipore water filter papers (pore size: 0.45 µm) and a plastic funnel (**Figure 2.7**).

The SPLP (US EPA, 1994) assesses the bioavailability of contaminants under conditions that simulate industrial acid rainfall conditions. These conditions are known to occur at the PMC

smelter (Kendell, 2012). A modified SPLP (which does not contain sulphuric or nitric acid) was performed as a sequential leach test and used soil extracts from the FLP. For this test, 100.0 ml of deionised water was adjusted with 0.1 M of hydrochloric acid (HCl) to a pH of 4 and added to the leached soil extracts from the FLP step. This mixture was first shaken by hand for 30 seconds, then stirred on a rotating shaker for 18 hours at 30 rpm and allowed to rest for an hour. Once settled, the leachate was filtered through ash-free non-adsorbent Millipore water filter papers (pore size: 0.45 µm) and a plastic funnel (**Figure 2.7**).

The extracted leachates were then stored in a cold room at 5° C. The extracts were brought to room temperature when testing for pH and electrical conductivity (and its derivatives in terms of total dissolved solids and salinity) using an Orion Star A329 meter with pH and conductivity electrodes. Soluble metal and sulphate concentrations from each extract (at room temperature) were semi-quantitatively determined with colorimetric test strips (Merck Supelco and Macherey-Nagal test strips. Solutions tested for sulphate, copper, lead, nickel, cobalt, and bromine).



Figure 2.6: Leachate extraction method using ash-free non-adsorbent Millipore water filter paper and a plastic funnel.

2.2.4.2 Soil organic matter

Soil organic matter (SOM) was measured with the mass-loss method according to Robinson (1922), as described in Leelamanie *et al*. (2015). This involved 100.0 mℓ of freshly made-up 10 vol (3%) hydrogen peroxide (H_2O_2) to 20.00 g of the dry 2 mm size fraction of the sieved soil, simultaneously stirring with a glass rod. The assay was conducted in plastic beakers in a fume cabinet and allowed to rest for 18 hours. After oxidation of the organic matter, each soil

sample was photographed to show the presence of efflorescence minerals. The hydrogen peroxide decomposed to water and was evaporated in a fan oven at ~ 50 °C, with the plastic beakers sealed with a paper towel over the top (anchored with a rubber band) to prevent loss or gain of soil particles. The samples were dried until there was no further loss in mass (4 days). The difference in weight was the fraction of SOM within each sample.

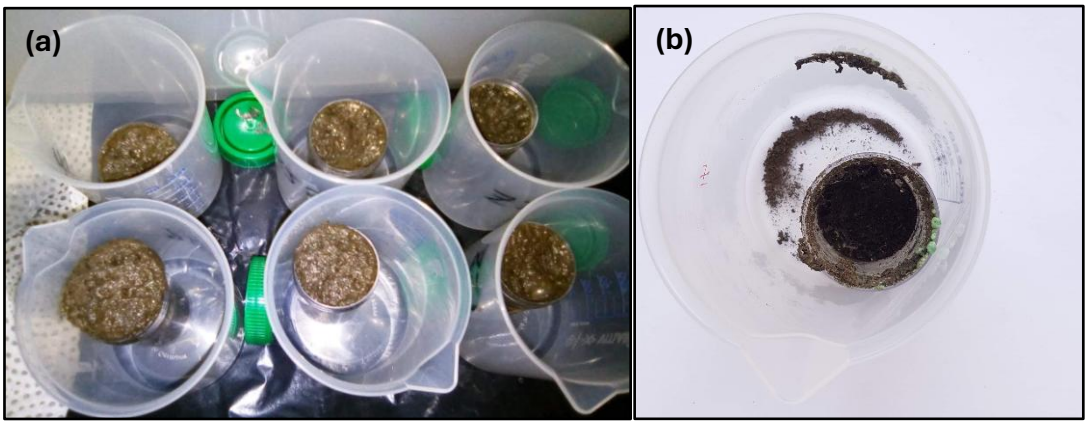


Figure 2.7: (**a**) Measurement of soil organic matter by the mass loss method. (**b**) An example of green and white efflorescence minerals in a soil sample from the high pollution zone.

2.2.5. Data and statistical analysis

Landscape organisation (LO) and soil surface assessment (SSA) data from the field were input into the LFA Microsoft Excel spreadsheet embedded with formulae to calculate the LO and SSA indices (Tongway and Hindley, 2004). A Shapiro-Wilk test was used to test the normality of the data. Non-parametric statistical tests were used since the data was not normally distributed, and these tests were also preferred due to their sensitivity to the effect of outliers, as was the case for this dataset. Since the LFA protocol analyses field data at two levels (broad site level using the LO and the finer soil surface level using the SSA), analysis of the soil properties was done similarly: first, broad differences between pollution zones were tested with a Kruskal Walis test, followed by a non-parametric *post hoc* Dunns test. Thereafter, differences amongst the patches and interpatches within each pollution zone were tested with a Mann-Whitney U test. A further level of analysis shows the results of individual patch and interpatch categories (with each category consisting of three replicated field samples). A multiple regression analysis was conducted to understand the relationship between the soil surface indices (in terms of stability, infiltration, and nutrient cycling) and the measured soil properties (in terms of pH, electrical conductivity, soil organic matter, and

soluble concentrations of Cu, Pb, Br, Ni, and SO4). All results were considered significant at $p < 0.05$. Data was input and analysed on Microsoft Excel (Microsoft Corporation, 2023), and RStudio was used specifically for statistical analysis (R Core Team, 2022).

2.3 Results

2.3.1. Landscape organisation

Patches/interpatches and their distribution along each pollution zone were identified with the landscape organisation step (LO) of the Landscape Function Analysis (LFA) protocol (**Table 2.3**). Four patch types (areas of resource accumulation) and four interpatch types (areas of resource loss) were found along the high pollution zone. Only two patch types and a single interpatch type were found along the medium pollution zone – it should be noted that the 160 m gradsect along the medium pollution zone had to be split into three offset segments due to the presence of elephants and buffalo during fieldwork.

Along the unpolluted zone, seven patch types and two interpatch types were identified. The unpolluted zone was found to have a diverse range of patches and interpatches, as seen in similar descriptions for the broad-leaf patch (BLP), fine-leaf patch (FLP), the black cryptogram patch (BCP) and the mossy cryptogram patch (MCP). Similar patches and interpatches along the unpolluted zone were included in the analysis to indicate the diversity along the area. Overall, patch types were of a greater proportion compared to interpatch types along each pollution zone.

Table 2.3: Patch and interpatch categories identified along each pollution zone. The average length and percentage cover of each patch and interpatch are also given.

		Identity (Code)	Mean length (m)	Percentage cover along gradsect	Description
High pollution zone	Patch types	Grassy litter patch (GLP)	1.87	21.1	Grass-dominant litter that has been transported between grass patches.
		Tree litter patch (TLP)	2.86	11.5	Dominant tree leaf litter that was found under sub-canopy tree patches or was transported.
		Grass patch (GP)	1.37	18.9	Collection of grass tufts.
		Woody litter patch (WLP)	0.64	3.6	Cleared *Dichrostachys cinerea* bushes, which had slight decomposition and transported branches.
	Interpatch types	Thick bare crust interpatch (BMC [T])	1.34	4.3	Thick bare mineral crust with an indurated layer of copper along the surface.
		Stone interpatch (SP)	0.67	1.6	Scattered rocks and stones.
		Thin bare crust interpatch (BMC [t])	1.14	10.1	Thin bare mineral crust (BMC) with loose sand along the surface.
		Bare sand interpatch (BP)	1.88	28.9	Bare sand patch.
Medium pollution zone	Patch types	Subcanopy litter and grass patch (PVC)	10.23	43.9	Collective tree and grass species.
		Grassy sward patch (GS)	20.00	28.6	Dense sward of tall grass.

Table 2.3 continued to the next page.

	Interpatch types	Sandy interpatch (SaP)	6.43	27.6	Loose sand patch.
Unpolluted zone	Patch types	Grass patch (GP)	0.97	17.8	Collection of tussock grasses.
		Broadleaf patch (BLP)	2.47	21.2	The area under broad-leaf tree sub-canopies.
		Fine leaf patch (FLP)	1.50	14.7	The area under fine-leaf tree sub-canopies.
		Forb patch (FP)	1.26	9.3	Perennial forb patches.
		Woody shrub patch (WSP)	0.57	2.1	Woody sub-shrub patch.
		Riparian weed patch (RWP)	6.50	8.0	A continuous band of herbaceous weeds found near a stream of water.
		Grassy sward patch (GS)	4.50	5.5	A continuous band of grass.
	Interpatch types	Black cryptogram interpatch (BCP)	1.08	17.2	Black cryptogram layer present on a gravel wash surface.
		Mossy cryptogram interpatch (MCP)	0.43	4.3	Mossy cryptogram layer over a gravel wash surface.

Patchiness and connectivity along the gradsects of each pollution zone are described in **Table 2.4**. According to the Landscape Organisation index, 55% of the high pollution zone is covered by patches. The medium pollution zone has a 72% patch cover, while the unpolluted zone has a 79% patch cover. A greater number of patches per 10 m were found along the unpolluted zone compared to the high and medium polluted zones. On average, interpatch length was greater along the gradsects of the high and medium polluted zones.

Table 2.4: Landscape organisation results from gradsects in the three pollution zones: high, medium, and unpolluted (and the month when each zone was assessed), from a copper smelter.

	High pollution zone (Aug 2021)	**Medium pollution zone (Aug 2021)**	**Unpolluted zone (Dec 2023)**
Length of gradsect	160 m	160 m[b]	160 m
Number of patches/10 m	3.1	0.6	5.0
Total patch area	661.5 m^2	638.3 m^2	560.5 m^2
Landscape organisation index	0.55	0.72	0.79
Average interpatch length	1.50 m	6.43 m	0.83 m

2.3.2. Soil surface assessment

The soil surface assessment (SSA) was conducted on each patch and interpatch by visually assessing 11 indicators of soil surface condition. Scores for each indicator were manually entered into the custom LFA spreadsheet designed for the SSA, and three indices of soil surface function were derived: stability, water infiltration, and nutrient cycling. These indices do not hold any significance alone but need to be used in a time series comparison and compared against a reference site which has the desired endpoint for rehabilitation (Tongway and Hindley, 2004). However, this chapter aimed to simply determine the relationship between the soil surface indices and soil properties from patches and interpatches along each pollution zone. Additionally, the unpolluted zone (which served as a field reference site) was sampled in a different year from the polluted site, so the SSA along the polluted zones could not be compared to the unpolluted zone. The indicator scores used to derive each soil surface index (SSI) are available in **Appendix A**, **Table A1**, along with pictures of each patch and interpatch that was assessed.

[b] The length of the 160 m gradsect along the medium pollution zone was broken into three segments of 60 m, 60 m, and 40 m.

The resulting indices of stability, water infiltration, and nutrient cycling are summarised in **Figure 2.9**. Differences between the polluted zones and the unpolluted zone could not be assessed since the SSA was conducted in 2021 and 2023, respectively. Differences between patches and interpatches could be compared within each pollution zone. For the high pollution zone, all index values for the patches were significantly higher than the interpatches (stability: $p = 0.005$; infiltration: $p < 0.0001$; and nutrient cycling: $p < 0.001$), and similarly for the medium polluted zone (stability: $p = 0.026$; infiltration: $p = 0.026$; and nutrient cycling: $p = 0.025$); see **Appendix A, Table A6** for full results of Mann-Whitney U tests). Statistical tests were done tentatively for the interpatch types along the medium pollution zone since these contained a sample size of three. For the unpolluted zone, water infiltration and nutrient cycling for patches were significantly higher than the interpatches ($p = 0.002$ and $p = 0.02$, respectively). However, there was no difference in the stability index between the patches and interpatches.

Figure 2.9 is found on the next page.

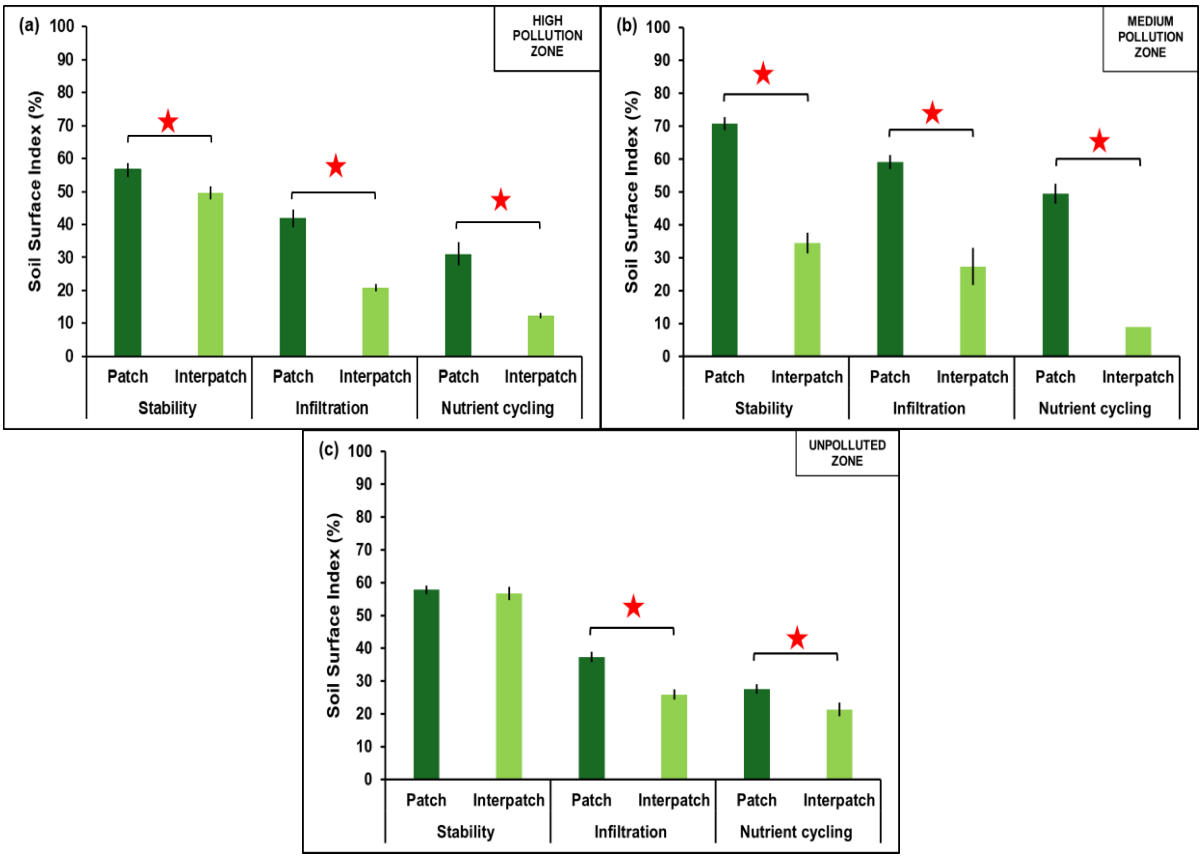


Figure 2.8: Distribution of Soil Surface Indices (SSIs) in terms of stability, water infiltration, and nutrient cycling for **(a)** the high pollution zone[c] (assessed in 2021), **(b)** the medium pollution zone[d] (assessed in 2021), and **(c)** the unpolluted zone[e] (assessed in 2023). Statistical tests were only valid between patches and interpatches within each site since the polluted zones were evaluated at a different time than the unpolluted zone. A red star indicates a significant difference between the patch and interpatch SSI response (Mann-Whitney U test: $p<0.05$).

The broad combinations of patches and interpatches (**Figure 2.9**) were then split into respective patch/interpatch categories. The results in **Figures 2.10**, **2.11**, and **2.12** show the mean (± standard error) of a triplicate response of the SSIs for each patch/interpatch category. For the high pollution zone, the statistical tests in **Figure 2.9** already revealed that there is a significant difference between patches and interpatches. Within the patches, the woody litter patch (WLP) scored the highest results for each SSI (stability = 62%, infiltration = 55%, and nutrient cycling = 49%), whereas the grassy litter patch (GLP) generally scored the lowest (stability = 52%, infiltration = 32%, and nutrient cycling = 21%) (**Figure 2.10a, b, and c**).

[c] High pollution zone: $N_{patches} = 12$; $N_{interpatches} = 12$
[d] Medium pollution zone: $N_{patches} = 6$; $N_{interpatches} = 3$
[e] Unpolluted zone: $N_{patches} = 21$; $N_{interpatches} = 6$

For the interpatches, the thin bare mineral crust interpatch (BMC[t]) scored the lowest stability and nutrient cycling index (39% and 10%, respectively). The stony interpatch (SP) scored the lowest infiltration index (17%) while also scoring the highest nutrient cycling index (15%). The thick bare mineral crust interpatch (BMC[T]) scored the highest stability and infiltration index (54% and 23%, respectively).

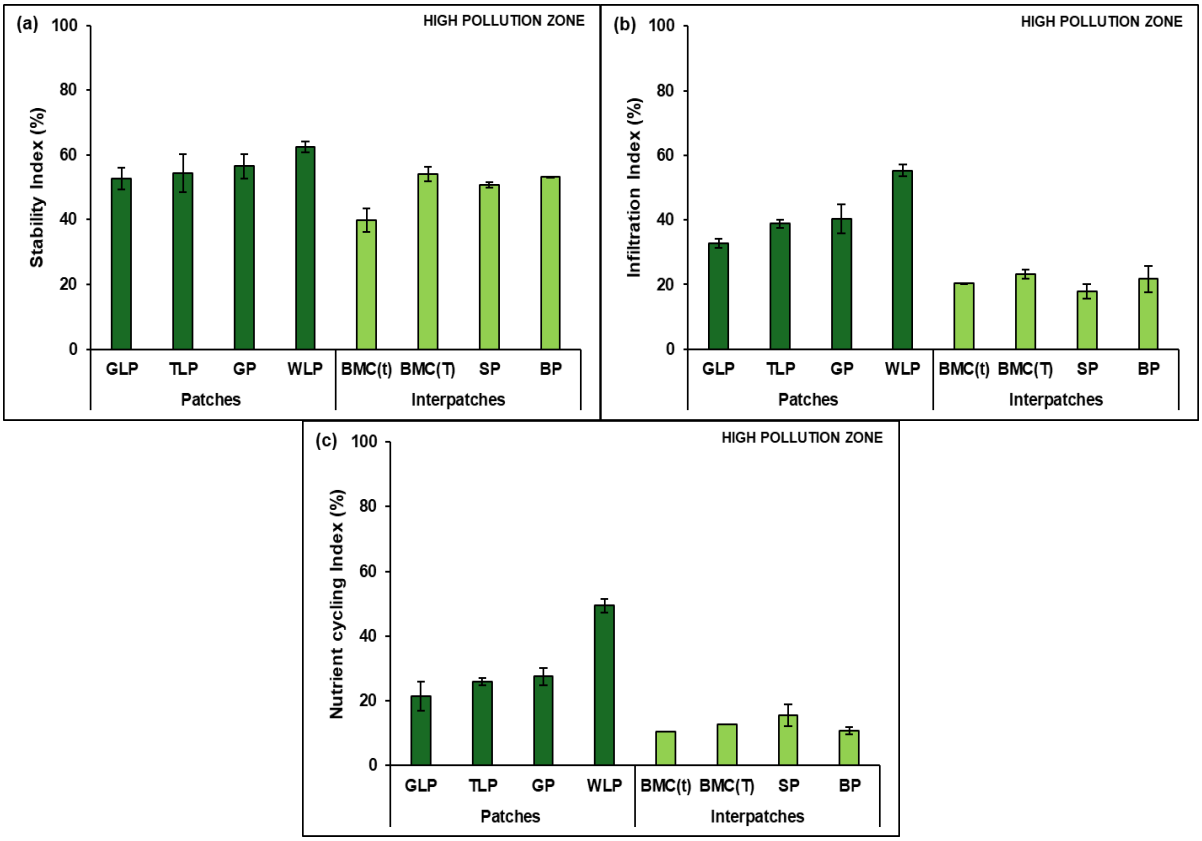


Figure 2.9: Mean (± standard error) of soil surface indices in terms of **(a)** stability, **(b)** infiltration, and **(c)** nutrient cycling in each patch and interpatch category [f] found along the high pollution zone (assessed in 2021). Each patch/interpatch category is the result of field triplicate samples.

Only three patch types were assessed in total for the medium pollution zone, which reduced the robustness of the results derived from the zone. However, these results were included to facilitate preliminary results that may encourage future analysis. The sub-canopy grass and litter patch and the grassy sward patch performed similarly for each SSI measured, whereas the sandy interpatch scored the lowest index values overall (**Figure 2.11**).

[f] GLP – grassy litter patch; TLP – tree litter patch; GP – grass patch; WLP – woody litter patch; BMC(T) – thick bare mineral crust interpatch; BMC(t) – thin bare mineral crust interpatch; SP – stone interpatch; BP – bare sandy interpatch.

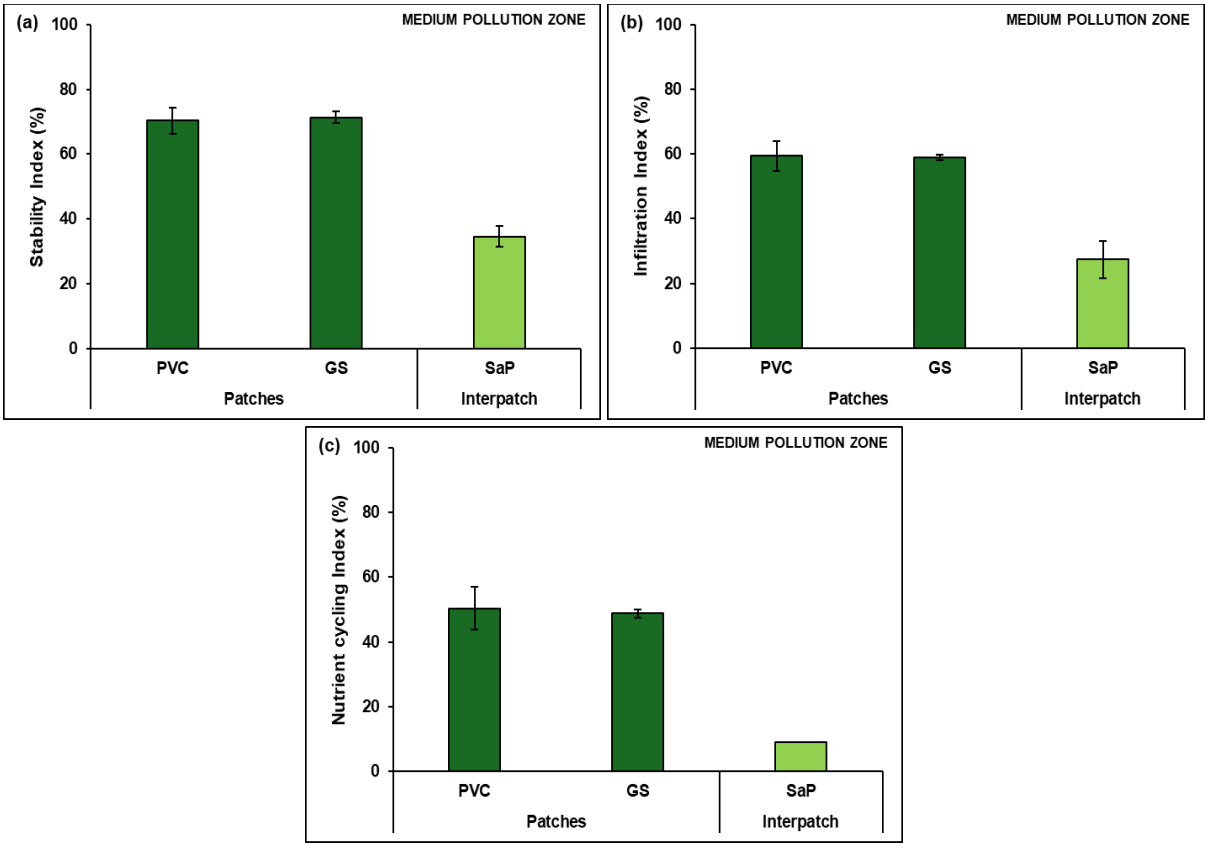


Figure 2.10: Mean (± standard error) of soil surface indices in terms of (**a**) stability, (**b**) infiltration, and (**c**) nutrient cycling in each patch and interpatch category [g] found along the medium pollution zone (assessed in 2021). Each patch/interpatch category is the result of field triplicate samples.

For the unpolluted zone, the stability index values did not differ significantly between patches and interpatches, so amongst each patch/interpatch category, the riparian weed patch (RWP) scored the highest stability index of 68%, with the grassy sward (GS) closely followed by scoring 62% (**Figure 2.12**). The fine leaf patch (FLP) scored the lowest stability index of 52%. The RWP weed patch scored the highest infiltration and nutrient cycling index of 48% and 37%, respectively, closely followed by GS with 46% and 34%. Among the interpatches, the black cryptogram interpatch scored the lowest infiltration and nutrient cycling index (24% and 19%, respectively). Overall, interpatch types scored lower stability, infiltration, and nutrient cycling values compared to the patch types.

Figure 2.12 is found on the next page.

[g] PVC – subcanopy litter and grass patch; GS – grassy sward; and SaP – sandy interpatch

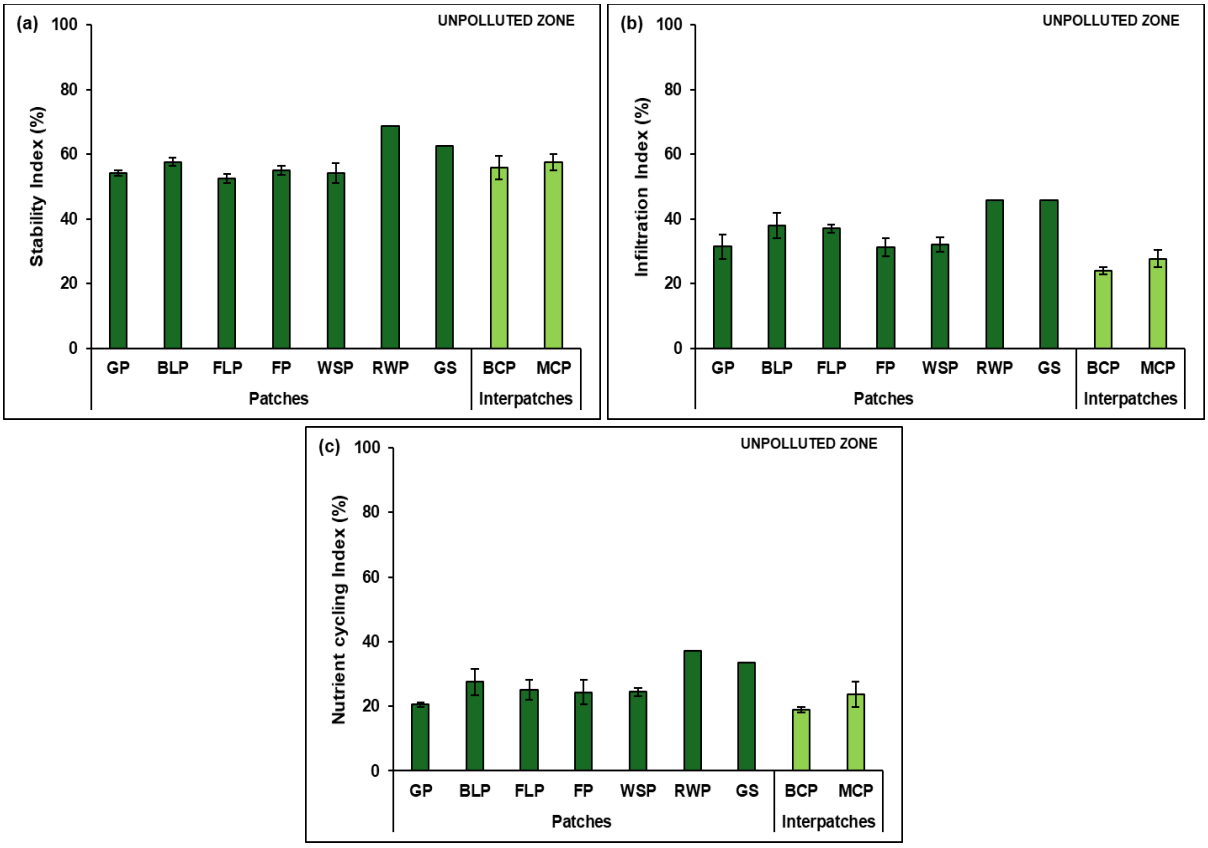


Figure 2.11: Mean (± standard error) of soil surface indices in terms of (**a**) stability, (**b**) infiltration, and (**c**) nutrient cycling in each patch and interpatch category [h] found along the unpolluted zone (assessed in 2023). Each patch/interpatch category is the result of field triplicate samples.

2.3.3. Soil properties

Each patch and interpatch soil sample underwent two sequential extractions: a Field Leach Procedure (FLP) – indicating the potential for leaching of contaminants after normal rainfall conditions or under clean stream water conditions, and a Synthetic Precipitation Leach Procedure (SPLP) – indicating the potential contaminants that may be released from the soil after industrial acid rainfall conditions. These extracts were tested for pH, electrical conductivity and soluble concentrations of sulphate, Cu, Ni, Co, Br, and Pb. Comparisons were made between pollution zones (since soil properties of the stored soil samples were compared from a pollution-level perspective and not an abiotic/biotic level perspective,

[h] GP – grass patch; BLP – broadleaf patch; FLP – fine leaf patch; WSP – woody shrub patch; RWP – riparian weed patch; GS – grassy sward; BCP – black cryptogram interpatch; and MCP – mossy cryptogram interpatch.

which was the case for the LFA results) and between patches and interpatches within each pollution zone.

For each soil property measured, data were analysed at two levels: first, the broad pollution zone level by comparing the three pollution zones and second, the patch type level by comparing patches and interpatches from each pollution level.

2.3.3.1 pH

Extracts of the FLP indicate the pH after clean steam water or rainfall conditions on-site. For the 0-2 cm depth (**Figure 2.13a**), pH values for patches along the high pollution zone were much lower when compared to the pH from the unpolluted zone ($p = 0.040$) (**Appendix A, Table A4** for Kruskal Wallis test results). For interpatches, the pH from the high pollution zone was significantly more acidic compared to the unpolluted zone ($p = 0.002$). For the unpolluted zone, the pH of the interpatches was lower compared to the pH of the patches ($p = 0.023$) (See **Appendix A, Table A3** for Mann-Whitney U tests)

For the 2-5 cm depth (**Figure 2.13b**), the pH of patches from the high pollution zone was significantly lower than the medium and unpolluted zone ($p = 0.009$ and $p < 0.001$; respectively). Interpatch FLP pH of the high pollution zone was significantly lower than the pH of the unpolluted zone ($p = 0.02$). See **Appendix A, Figure A5** for the FLP pH of each patch category making up the patches and interpatches from the high, medium, and unpolluted zones.

Figure 2.13 is found on the next page.

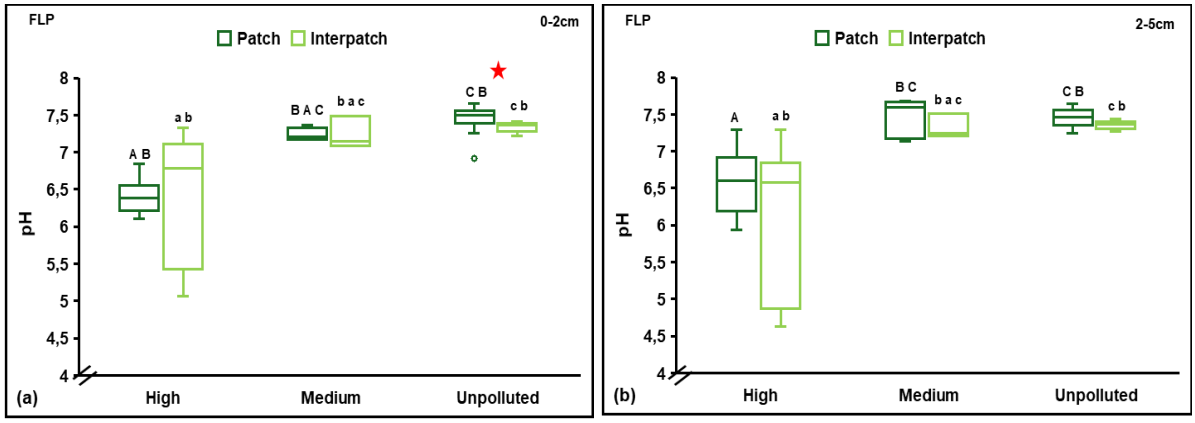


Figure 2.12: pH values of FLP leachate solutions for soil samples collected from patches and interpatches along the high[i] and medium[j] in 2021, and unpolluted[k] in 2023 at depths of (**a**) 0-2 cm and (**b**) 2-5 cm. Bars labelled with differing upper-case letters were found to be significantly different from each other, based on a post hoc Dunn's test ($p < 0.05$). A red star indicates a significant difference between patch and interpatch soils.

The pH from SPLP solutions implies the modified pH values (due to acid rain conditions) as they deviate from the actual pH given by the FLP solutions. For the 0-2 cm depth (**Figure 2.14a**), SPLP pH from patches of the high pollution zone was much lower compared to the medium and unpolluted zones ($p < 0.001$ and $p < 0.001$; respectively). For the interpatches, the SPLP pH from the high pollution zone was significantly lower than the SPLP pH from interpatches in the unpolluted zone ($p = 0.001$).

For the 2 – 5 cm depth (**Figure 2.14b**), SPLP pH from patches of the high pollution zone was lower than SPLP pH of patches of the medium and unpolluted zones ($p < 0.001$ and $p = 0.001$; respectively). For the interpatches, the SPLP pH from the high pollution zone was significantly lower than the SPLP pH in the unpolluted zone ($p = 0.003$). The SPLP pH of patches from the unpolluted zone was higher than the SPLP pH of the same zone ($p = 0.004$). See **Appendix A, Figure A6** for the SPLP pH of each patch category making up the patches and interpatches from the high, medium, and unpolluted zones.

[i] High pollution zone: $N_{patches} = 12$; $N_{interpatches} = 12$
[j] Medium pollution zone: $N_{patches} = 6$; $N_{interpatches} = 3$
[k] Unpolluted zone: $N_{patches} = 21$; $N_{interpatches} = 6$

Figure 2.14 is found on the next page.

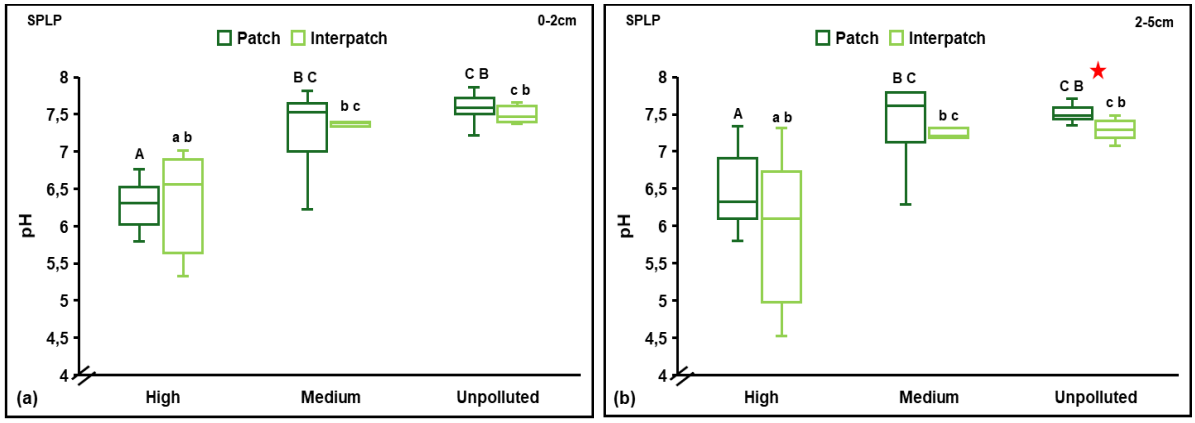


Figure 2.13: pH values of SPLP leachate solutions for soil samples collected from patches and interpatches along the high[l] and medium[m] in 2021, and unpolluted[n] in 2023 at depths of (**a**) 0-2 cm and (**b**) 2-5 cm. Bars labelled with differing upper-case letters were found to be significantly different from each other, based on a *post hoc* Dunn's test ($p < 0.05$). A red star indicates a significant difference between patch and interpatch soils.

2.3.3.2 Electrical conductivity

Electrical conductivity (EC) indicates the salinity or soluble salts available in the soil after normal rainfall conditions (FLP) and acid rainfall conditions (SPLP).

Along the 0-2 cm level (**Figure 2.15a**), the EC in FLP extracts measured along patches was significantly higher along the high pollution zone and medium pollution zone, with a range of about 200 µS/cm to 560 µS/cm, when compared to the unpolluted site, which ranged between 70 µS/cm to 200 µS/cm ($p < 0.001$). Only the interpatches along the high pollution zone with a range between 70 µS/cm to 3500 µS/cm were found to be significantly greater than the electrical conductivity values along the unpolluted zone with a range of around 60 µS/cm ($p = 0.01$). Along the 2-5 cm depth (**Figure 2.15b**), the EC readings along patches and interpatches along the high pollution zone were significantly greater than the patches ($p = 0.002$) and interpatches ($p = 0.03$) along the unpolluted zone. Results for each patch category making up the patches and interpatches can be found in **Appendix A, Figure A8.**

[l] High pollution zone: $N_{patches} = 12$; $N_{interpatches} = 12$
[m] Medium pollution zone: $N_{patches} = 6$; $N_{interpatches} = 3$
[n] Unpolluted zone: $N_{patches} = 21$; $N_{interpatches} = 6$

Figure 2.15 is found on the next page.

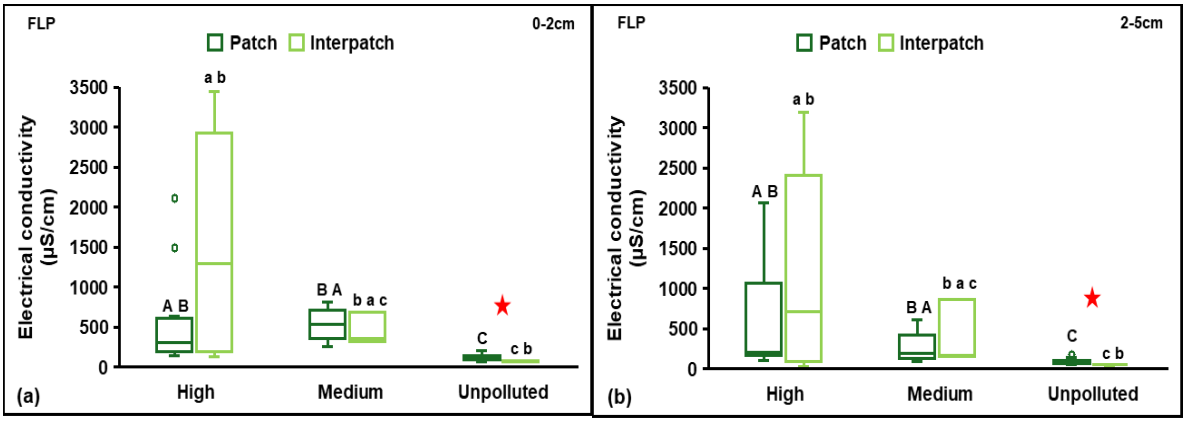


Figure 2.14: Electrical conductivity values of **FLP** solutions for soil samples from patches and interpatches along the high[o] and medium[p] in 2021, and unpolluted[q] zone in 2023 at depths of (**a**) 0-2 cm and (**b**) 2-5 cm. Patch soil samples labelled with differing upper-case letters were found to be significantly different along each site. Interpatch soil samples labelled with differing lower-case letters were significantly different along each site based on a post hoc Dunn's test ($p < 0.05$). A red star indicates a significant difference between the patches and interpatches.

The EC readings for SPLP extracts at 0-2 cm (**Figure 2.16a**) had soil samples from patches along the high and medium pollution zones that were significantly higher compared to the patches along the unpolluted zone ($p < 0.001$). The interpatches along the high pollution zone were also significantly higher than the unpolluted zone ($p > 0.001$). A significant difference was also found between patches and interpatch soil EC within the 0-2 cm depth, with interpatch soils having an exceptionally high mean EC of 3800 µS/cm +/- SE. Along the 2-5 cm depth (**Figure 2.16b**), the EC values for patch spikes ($p < 0.001$) and interpatch soils ($p < 0.05$) for the high and medium pollution zones were much higher than the unpolluted zone. Results for each patch category making up the patches and interpatches can be found in **Appendix A, Figure A8.**

Figure 2.16 is found on the next page.

[o] High pollution zone: $N_{patches} = 12$; $N_{interpatches} = 12$
[p] Medium pollution zone: $N_{patches} = 6$; $N_{interpatches} = 3$
[q] Unpolluted zone: $N_{patches} = 21$; $N_{interpatches} = 6$

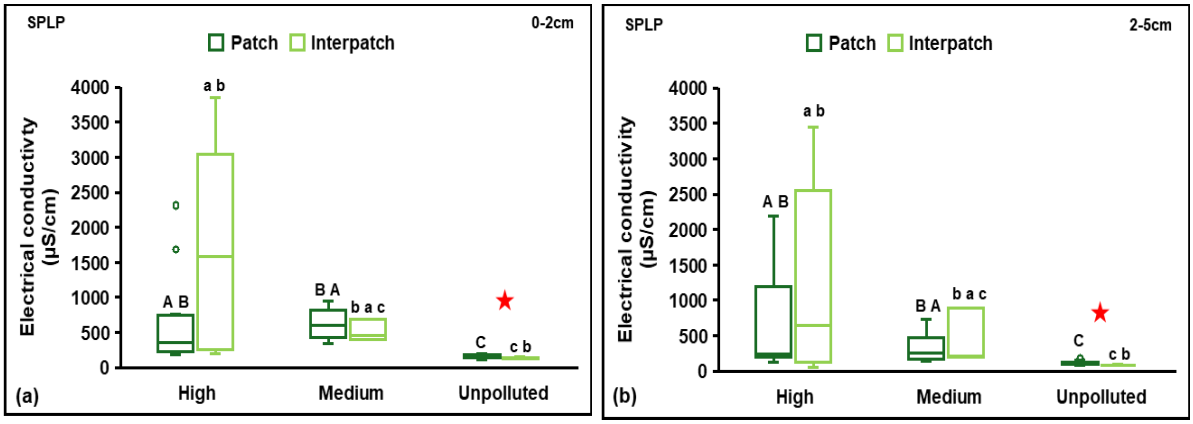


Figure 2.15: Electrical conductivity values of **SPLP** solutions for soil samples from patches and interpatches along the high[r] and medium[s] in 2021, and unpolluted[t] zone in 2023 at depths of (**a**) 0-2 cm and (**b**) 2-5 cm. Patch soil samples labelled with differing upper-case letters were found to be significantly different along each site. Interpatch soil samples labelled with differing lower-case letters were significantly different along each site based on a post hoc Dunn's test ($p < 0.05$). A red star indicates a significant difference between the patches and interpatches.

2.3.3.3 Soluble contaminant concentrations

The unpolluted zone FLP and SPLP solutions did not reveal any detectable levels of soluble contaminants in terms of Cu, Pb, Br, Ni, or SO_4. Therefore, site 3 could be confirmed as the unpolluted zone with respect to the contamination present at the PMC smelter.

When FLP solutions were tested for soluble contaminants (**Table 2.5**), only interpatch soils from the high pollution zone were found to contain any soluble metal concentrations after simulating normal stream or rainfall conditions. It is interesting to note that the deeper surface depths of 2-5 cm on the high pollution zone contained a higher number of soluble contaminants compared to the 0-2 cm depth. The medium pollution zone contained no detectable soluble contaminants.

Table 2.5 is found on the next page.

[r] High pollution zone: $N_{patches} = 12$; $N_{interpatches} = 12$
[s] Medium pollution zone: $N_{patches} = 6$; $N_{interpatches} = 3$
[t] Unpolluted zone: $N_{patches} = 21$; $N_{interpatches} = 6$

Table 2.5: Mean (± standard error) semi-quantitative concentrations of soluble Cu, Pb, Br, Ni, and SO_4 in FLP soil extractions from patches and interpatches at depths of 0-2 cm and 2-5 cm.

FLP	Depth	High pollution zone		Medium pollution zone	
		Patch (n = 12)	Interpatch (n = 12)	Patch (n = 6)	Interpatch (n = 3)
Cu (mg/ℓ)	0 – 2 cm	-	11.3 (4.36)	-	-
	2 – 5 cm	-	52.5 (25.8)	-	-
Pb (mg/ℓ)	0 – 2 cm	-	15.4 (6.67)	-	-
	2 – 5 cm	-	29.2 (13.0)	-	-
Br (mg/ℓ)	0 – 2 cm	-	2.08 (0.96)	-	-
	2 – 5 cm	-	4.38 (6.75)	-	-
Ni (mg/ℓ)	0 – 2 cm	-	2.50 (1.31)	-	-
	2 – 5 cm	-	5.00 (5.22)	-	-
SO_4 (mg/ℓ)	0 – 2 cm	200 (0)	250 (26.1)	-	-
	2 – 5 cm	200 (0)	283 (29.7)	200 (0)	200 (0)

The SPLP solutions revealed a greater number of soluble contaminants released after simulating an acid rainfall condition. Interpatch soil from the high pollution zone released a greater proportion of contaminants when compared to the patches of the high pollution zone. Depths of 0-2 cm contained a greater proportion of contaminants when compared to the 2-5 cm, except for the concentration of SO_4 in interpatch soils from the high pollution zone. The medium pollution zone revealed contaminants detected only in SPLP solutions. Results for each patch category making up the patches and interpatches can be found in **Appendix A.**

Table 2.6: Mean (± standard error) semi-quantitative concentrations of soluble Cu, Pb, Br, Ni, and SO_4 in SPLP soil extractions from patches and interpatches at depths of 0-2cm and 2-5cm.

SPLP	Depth	High pollution zone		Medium pollution zone	
		Patch (n = 12)	Interpatch (n = 12)	Patch (n = 6)	Interpatch (n = 3)
Cu (mg/ℓ)	0 – 2 cm	16.1 (7.98)	41.0 (12.9)	1.00 (0)	1.00 (0)
	2 – 5 cm	8.75 (3.12)	30.2 (12.4)	1.00 (0)	1.00 (0)
Pb (mg/ℓ)	0 – 2 cm	39.2 (7.01)	52.5 (11.0)	0.67 (0.21)	1.00 (0)
	2 – 5 cm	38.3 (8.06)	49.2 (12.1)	0.50 (0.22)	0.33 (0.33)
Br (mg/ℓ)	0 – 2 cm	10.3 (5.95)	6.08 (1.76)	-	-
	2 – 5 cm	4.08 (0.77)	5.25 (1.14)	-	0.67 (0.33)

Table 2.6 continued to the next page.

Ni (mg/ℓ)	0 – 2 cm	-	-	-	-
	2 – 5 cm	-	-	-	-
SO_4 (mg/ℓ)	0 – 2 cm	300 (67.4)	333 (66.7)	200 (0)	200 (0)
	2 – 5 cm	233 (22.5)	383 (75.7)	200 (0)	200 (0)

2.3.3.4 Soil organic matter

There was no significant difference in soil organic matter between the pollution zones (see **Appendix A, Table A3** for results of Kruskal-Wallis tests). For differences between patch types, patches in the high-pollution zone had significantly more soil organic matter than the interpatches at 0-2 cm ($p = 0.002$) (**Figure 2.17a**). For depths of 2-5 cm, patches from the high and medium pollution zones had significantly more soil organic matter than the interpatches ($p = 0.033$ and $p = 0.024$, respectively) (See **Appendix A, Table A4** for the results of Mann-Whitney U tests). Results for each patch category making up the patches and interpatches can be found in **Appendix A, Figure A9.**

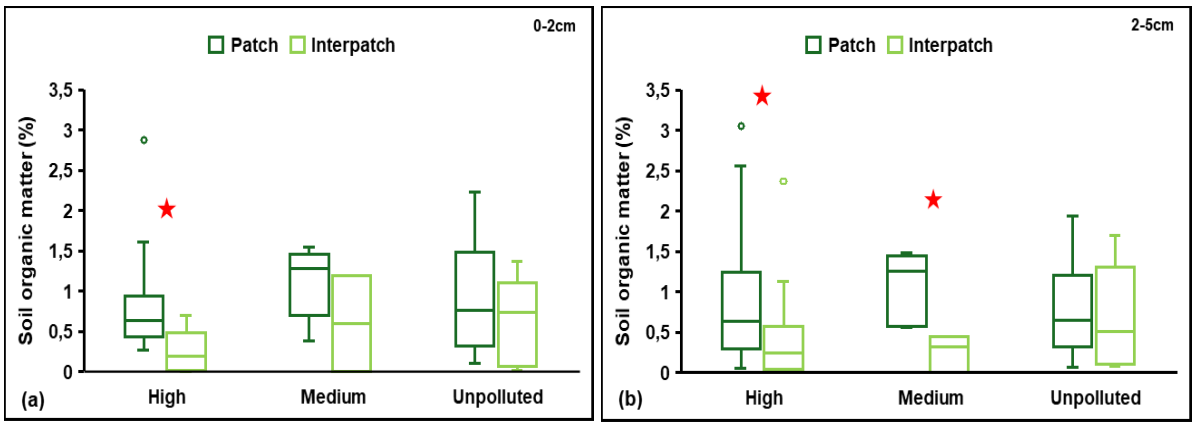


Figure 2.16: Soil organic matter at (**a**) 0-2 cm and (**b**) 2-5 cm of patches and interpatches from the high, medium, and unpolluted zones. A red star indicates a significant difference between he patches and interpatches.

2.3.4. Relationship between Landscape Functional Indices and soil properties

A multiple regression analysis was conducted to understand the relation between the measured soil properties (pH, electrical conductivity, soil organic matter) and the soil surface indices, in terms of stability, nutrient cycling and infiltration. Only significant regression models are presented in this analysis (See **Appendix A, Table A6** for full results of the multiple regression model).

For the high pollution zone, significant regression models were found with infiltration and nutrient cycling at a depth of 0-2 cm. Soil organic matter was a significant predictor of both infiltration ($B = 12.79$; $p = 0.02$) and nutrient cycling ($B = 14.01$; $p = 0.01$). This indicates that soil organic matter positively influences infiltration and nutrient cycling.

For the medium pollution zone, soil properties at the 2-5cm depth are strong predictors of stability and nutrient cycling. For stability, pH (FLP: $B = 35.05$; $p = 0.03$ and SPLP: -32.23; $p = 0.04$), electrical conductivity (FLP: $B = 0.04$; $p = 0.09$ and SPLP: $B = -0.39$; $p = 0.06$) and soil organic matter ($B = 44.04$; $p < 0.01$). This may imply that electrical conductivity and pH at a depth of 2-5 cm contribute to stability and nutrient cycling.

For the unpolluted zone, at 0-2 cm, FLP pH appears to positively influence infiltration ($B = 28.10$; $p = 0.08$) and nutrient cycling ($B = 21.54$; $p = 0.01$). At the 2-5cm depth, pH and electrical conductivity appear to be significant predictors of all three indices: stability, nutrient cycling, and stability.

Table 2.7 is found on the next page.

Table 2.7: Summary of multiple regression analyses between soil surface indices (in terms of stability, nutrient cycling, and infiltration) and soil properties (pH, electrical conductivity, and soil organic matter). P-values in bold and with an asterisk (*) indicate a significant individual association ($p < 0.05$). Soil properties were measured at depths of 0-2 cm and 2-5 cm.

	Soil surface index (R Square)	Soil physicochemical property	Coefficient	Standard error	t-statistic	p-value
High pollution zone (N = 24)						
	Infiltration	Intercept	86,7	30.3	2,86	**0,01 ***
	(r^2 = 0.56)	FLP pH (0-2 cm)	2,05	7.11	0,28	0,77
		SPLP pH (0-2 cm)	-11,3	9.00	-1,25	0,22
		FLP EC (0-2 cm)	0,01	0.01	0,38	0,70
		SPLP EC (0-2 cm)	-0,01	0.01	-0,57	0,57
		SOM (0-2 cm)	12,8	3.55	3,59	**0,02 ***
	Nutrient cycling	Intercept	48,5	30,0	1,61	0,12
	(r^2 = 0.57)	FLP pH (0-2 cm)	-1,29	7,05	-0,18	0,85
		SPLP pH (0-2 cm)	-3,51	8,93	-0,39	0,69
		FLP EC (0-2 cm)	0,01	0,01	0,65	0,52
		SPLP EC (0-2 cm)	-0,01	0,01	-0,80	0,43
		SOM (0-2 cm)	14,1	3,52	3,96	**0,01 ***
Medium pollution zone (N = 9)						
	Stability	Intercept	36,2	34,5	1,04	0,37
	(r^2 = 0.99)	FLP pH (2-5 cm)	35,5	4,92	7,12	**0,03 ***
		SPLP pH (2-5 cm)	-32,3	3,88	-8,29	**0,04 ***
		FLP EC (2-5 cm)	0,34	0,05	6,18	**0,02 ***
		SPLP EC (2-5 cm)	-0,39	0,05	-6,82	**0,01 ***
		SOM (2-5 cm)	44,4	2,63	16,72	**<0.01 ***
	Nutrient cycling	Intercept	3,82	40,7	0,09	0,93
	(r^2 = 0.92)	FLP pH (2-5 cm)	30,7	5,79	5,31	**0,01 ***
		SPLP pH (2-5 cm)	-27,3	4,57	-5,96	**0,03 ***
		FLP EC (2-5 cm)	0,41	0,06	6,26	**0,04 ***
		SPLP EC (2-5 cm)	-0,45	0,06	-6,65	**0,05 ***
		SOM (2-5 cm)	51,2	3,10	16,5	**<0.01 ***

Unpolluted zone (N = 27)						
	Infiltration	Intercept	-95,6	84,81	-1,12	0,27
	(r^2 = 0.43)	FLP pH (0-2 cm)	28,10	9,66	2,91	**0,03 ***
		SPLP pH (0-2 cm)	-13,2	10,4	-1,21	0,28
		FLP EC (0-2 cm)	0,02	0,04	0,46	0,67
		SPLP EC (0-2 cm)	0,12	0,07	1,54	0,13
		SOM (0-2 cm)	0,76	2,40	0,31	0,75
	Nutrient cycling	Intercept	-12	72,3	-1,69	0,10
	(r^2 = 0.41)	FLP pH (0-2 cm)	21,5	8,24	2,61	**0,01 ***
		SPLP pH (0-2 cm)	-2,32	8,88	-0,26	0,79
		FLP EC (0-2 cm)	0,06	0,03	0,16	0,87
		SPLP EC (0-2 cm)	0,04	0,06	0,69	0,49
		SOM (0-2 cm)	-2,37	2,05	-1,15	0,26
	Stability	Intercept	-24,5	62,3	-0,39	0,69
	(r^2 = 0.62)	FLP pH (2-5 cm)	18,4	7,65	2,40	**0,02 ***
		SPLP pH (2-5 cm)	-8,46	6,55	-1,29	0,21
		FLP EC (2-5 cm)	0,09	0,03	2,57	0,01
		SPLP EC (2-5 cm)	-0,06	0,05	-0,11	0,91
		SOM (2-5 cm)	1,45	1,43	1,00	0,32
	Infiltration	Intercept (2-5 cm)	-261	86,4	-3,03	**0,01 ***
	(r^2 = 0.64)	FLP pH (2-5 cm)	26,3	10,6	2,48	**0,02 ***
		SPLP pH (2-5 cm)	13,4	9,07	1,48	0,15
		FLP EC (2-5 cm)	0,11	0,05	2,17	**0,04 ***
		SPLP EC (2-5 cm)	-0,06	0,07	-0,86	0,39
		SOM (2-5 cm)	-1,65	1,99	-0,82	0,41
	Nutrient cycling	Intercept (2-5 cm)	-203	70,01	-2,90	**0,01 ***
	(r^2 = 0.65)	FLP pH (2-5 cm)	28,8	8,59	3,35	**0,03 ***
		SPLP pH (2-5 cm)	1,34	7,35	0,18	0,85
		FLP EC (2-5 cm)	0,07	0,04	1,81	0,08
		SPLP EC (2-5 cm)	-0,01	0,06	-0,02	0,98
		SOM (2-5 cm)	-0,88	1,61	-0,54	0,59

2.4 Discussion

The soil surface indices (SSIs) (stability, infiltration, and nutrient cycling) along each pollution zone were assessed at different time points: the high and medium pollution zones were assessed in August 2021, while the unpolluted site was assessed in December 2023. August is the dry season along the Phalaborwa region, and experiences little to no rain with minimum temperatures going below 15° C, while December typically experiences more rain with minimum temperatures around 21° C. Therefore, the SSIs are not directly comparable across each pollution zone since the indices are a product of the abiotic and biotic features of the landscape (Tongway and Hindley, 2004). However, this chapter aimed to delineate each pollution zone into patches and interpatches and assess the robustness of the LFA protocol in the presence of heavy metal contamination by relating patch and interpatch SSIs to direct measurements of soil contamination.

Under heavy metal contamination, the LFA indices no longer reliably reflect soil function. The usual relationships between patch type, soil properties, and functional indices weaken, suggesting that the LFA model mainly captures nutrient dynamics rather than true ecosystem function. Therefore, in polluted landscapes, the LFA protocol alone is a poor predictor of landscape functionality and should be complemented with chemical and ecotoxicological assessments.

Significant, albeit weak relationships were mostly found between SSIs and soil properties measured along the unpolluted zone when testing for the effect of contamination and patch type. The surface pH of FLP extractions from the unpolluted zone was found to be a significant predictor of stability, soil infiltration, and nutrient cycling indices. These pH values ranged between 7 and 7.5, indicating a neutral soil pH. The electrical conductivity of the FLP extractions at 2-5 cm was also found to be significantly correlated with stability and infiltration indices, with values ranging between 100 – 300 µS/cm. Soil pH and electrical conductivity are intimately related to maintaining soil stability by promoting microorganism soil aggregates, supporting vegetation, and improving infiltration (Murphy, 2015). Accordingly, the infiltration index was also positively correlated to the pH and electrical conductivity of FLP extracts along both surface depths (0-2 cm and 2-5 cm) as well as the SPLP extracts, but not at the 0-2 cm depth. The nutrient cycling index followed a similar pattern of response along the unpolluted zone. These indicate that the flow of water along the

soil between interpatches and the subsequent percolation along patch areas of the control site follows a predictable model as determined by the LFA framework.

A significant regression model was also found between stability, infiltration, and nutrient cycling with SOM along the high and medium pollution zones, but not along the unpolluted zone, which was unexpected. Although there was quite a large variation in the result, the SOM along the unpolluted zone were observed to be higher than the high and medium pollution zones (an observation but not a significant result). Additionally, there were significant differences between the patches and interpatches along the high and medium pollution zones than in the unpolluted zone. Soil decomposition is adversely affected by the presence of heavy metals in soil (Bååth, 1989). This reduction in the rate of decomposition leads to a reduction in soil organic matter along the surface. Similar results were also found by Zhang and Wang (2007). However, in the case of long-term contamination, this result could also imply an adaptation by the microbial community to withstand the effects of contamination, as found by Zamulina *et al*. (2022). In both cases, the correlation between the LFA indices with SOM implies that the LFA model picks up on the visual assessment of increased litter on site, and interprets it as a functional attribute, however, physicochemical analysis of the soils reveals the opposite.

It is worth noting that the pH and electrical conductivity on the high pollution zone were highly variable, indicating a range of acidic to neutral soils with a high salinity, promoted by the presence of Cu, Pb, Br, NI, and SO4 in solution. The pH readings for the high pollution zone were low in both the FLP and SPLP extracts at both depths, indicating that the soil has a low buffering capacity and an acidic composition (Villen-Guzman et al., 2015). Similarly, the electrical conductivity of both the FLP and SPLP extracts from the high pollution zone is equally high, indicating that salts are readily leachable in both acidic and neutral conditions (Wuana and Okieimen, 2011). Although not directly comparable, the lack of relationship between any soil surface index and the soil properties along the high pollution zone from the high pollution zone indicates the inability of the LFA protocol to capture a wide variability in pH and measurements of salinity.

The observed significant regression models on the unpolluted zone, for the vast majority, may also be due to the greater occurrence of patches over interpatches. Combined with the influence of heavy metal contaminants altering the observed soil physicochemical properties, the proportion of patches to interpatches, and greater interpatch distance in the high pollution

zone may result in an increased loss of water and vital resources from the system. Various studies have confirmed that a greater occurrence of patches results in higher index scores (Holm et al., 2002; Maestre and Puche, 2009). The medium pollution zone cannot be included in this observation due to the identification of just a single interpatch and the segmentation of the gradsect due to wildlife and time constraints during fieldwork.

Potential limitations to this study design include:

- The modification of the gradsect along the medium pollution zone had obvious implications for this study. Statistics derived from this pollution zone were not robust enough to be included in the interpretation of this chapter.
- This design could not compare the SSIs between the three pollution zones because one site was surveyed in 2023. This hindered the ability to directly compare the indices between pollution zones, thereby reducing a further dimension of interpretation.

Recommendations for future work can include long-term LFA data and soil analysis to ensure that short-term monitoring data is relevant from a time scale perspective. Additionally, more soil properties should be investigated in relation to the SSIs, such as cation exchange capacity and soil respiration to further investigate the effects of SOM.

2.5 Conclusion

The research question this chapter aimed to answer was: What is the relationship between LFA indices (stability, infiltration, and nutrient cycling) and soil properties of patches and interpatches across a gradient of soil pollution? This question was answered by using the LFA protocol to organise three gradsects along a pollution gradient into its respective patches and interpatches and assess the LFA indices along each patch and interpatch. Soil samples were then collected at each patch and interpatch and assessed for soil properties (in terms of pH, electrical conductivity, soil organic matter, and soluble concentrations of Cu, Pb, Br, Ni, and SO_4). The relationship between key soil properties and the LFA indices was then determined with a multiple regression analysis.

Soil function was found to be adequately captured along the unpolluted zone that contained a negligible amount of heavy metal contamination, as seen by significant but weak multiple regressions between soil physicochemical properties and soil surface indices. However, along a heavy metal-contaminated site, the presence of contaminants can indirectly alter the transfer

of water and nutrients in the system (through increased salinity affecting water availability, soil acidification and reduction in infiltration capacity, and a reduction in biological activity), thereby resulting in the system not conforming to the LFA predictive model of functionality. Therefore, to be confidently used as a monitoring tool along a heavy metal contaminated site, the LFA protocol should be accompanied by more direct measurements of soil surface function, as included in this paper and the recommendations for future work given above.

Chapter three – The influence of patch type on soil leachate ecotoxicity to *Daphnia magna* and *Aliivibrio fischeri*

3.1 Introduction

Heavy metal pollution due to industrial sources is of environmental concern due to its ability to remain in terrestrial and aquatic ecosystems (Park *et al*. 2023). Soils polluted with heavy metals can impact water quality through leaching, resulting in the entry of contaminants into the water system, impacting landscape quality and function. Aquatic organisms can absorb and adsorb contaminants and transfer these pollutants to other organisms through predation, resulting in a transfer of contaminants through the food web (Barron, 1995). Understanding the ecotoxicological effects of soil pollution on aquatic organisms provides insights into the environmental risk of rainwater runoff from the polluted site. To understand the various biological pathways of pollutants, it is important to use different aquatic organisms as biological indicators (Bae *et al*., 2014).

Daphnia magna and *Aliivbrio fischeri* are commonly used in ecotoxicological tests because of their sensitivity to a wide range of pollutants (Bandarra *et al.*, 2023). *Daphnia magna* is a freshwater cladoceran and reflects the impact of pollutants through an immobilisation response. *Aliivibrio fischeri* is a marine bacterium and indicates sensitivity to pollutants through inhibition of its natural luminescence. Since *D. magna* is a freshwater flea and *A. fischeri* is a marine bacterium, using them together allows for different trophic levels of the aquatic system to be investigated (Müller *et al*., 2019).

A realistic approach to assessing the leachable ability of pollutants through the soil would consider the movement and distribution of pollutants through the landscape system. The distribution of pollutants along a landscape is dependent on the spatial distribution of the abiotic and biotic components of the environment, referred to as spatial heterogeneity (Ludwig and Tongway, 1997). The various types of land units along a landscape function to capture or promote the flow of water and vital nutrients. According to Tongway and Ludwig (2004), patches are areas that accumulate resources, and interpatches are areas of resource loss. This can result in an unequal variation of pollutants across the landscape, leading to a differential impact on water quality. Soil leachates derived through *ex-situ* laboratory

conditions can simulate the resulting water quality in rainwater runoff and puddles or under acidified rainfall conditions. Therefore, investigating the ecotoxicological influence of soil leachates from the various patches and interpatches along a landscape can allow for a more nuanced approach to monitoring landscape function. In this project, the aim of Chapter 2 was to characterise gradsects, from three zones of varying pollution levels, into patches and interpatches, and describe the soil properties of each patch and interpatch type. This chapter follows on from that approach in assessing the ecotoxicological impact of soil leachates on *D. magna* and *A. fischeri.*

The key research question posed in this chapter is: "How does the ecotoxicity of soil leachates to *D. magna* and *A. fischeri* change between patches and interpatches across a gradient of soil pollution, and how is this related to the properties of each solution?" These leachate solutions simulate stream water and rainfall runoff conditions experienced on-site to test the toxicity of water available along the landscape.

To answer this question, the specific objectives of this chapter are:

1. To assess the *D. magna* immobilisation in response to the effect of patch type and pollution level. Patch type and pollution level were described in Chapter 2.
2. To assess if *D. magna* immobilisation varies due to the presence or absence of food, during exposure to patch and interpatch soil leachates from differing pollution levels.
3. To assess *A. fischeri* bioluminescence in response to patch type and pollution level.
4. To determine which soil properties (described in Chapter 2) may be responsible for the observed responses of *A. fischeri* and fed/unfed *D. magna*.

3.2 Methods

3.2.1. Soil leachate solutions

Ecotoxicity tests with *D. magna* and *A. fischeri* were done using two soil leachate extractions: a Field Leach Procedure (FLP) (Hageman, 2005) and a Synthetic Precipitate Leach Procedure (SPLP) (US EPA, 1994). Leachate solutions were derived from soils collected at 0-2 cm in patches and interpatches of three pollution zones on catenal slopes draining into a stream. Positioning of the pollution zones and identification of patches and interpatches were done following the Landscape Function Analysis (LFA) protocol (Tongway and Hindley, 2004: Chapter 2). This was done to document the quality of water flowing through interpatches, accumulating along patches, and eventually released into the nearby stream.

The FLP extracts allow an insight into the water quality in clean stream water or after normal rainfall conditions, whereas the SPLP extracts look into water quality after industrial acidified rainfall conditions. A pilot test involving the exposure of *D. magna* to FLP solutions resulted in the immediate death of the neonates (which had hatched less than 24 hours before). From previous studies on the sensitivity of *A. fischeri* (Erzinger *et al*., 2018), it was assumed that a similar pattern of immediate inhibition would occur. Therefore, the FLP and SPLP solutions had to be further diluted from a 1:20 range (as explained in Chapter 2) to a range of 1:100 as recommended by the USEPA (US Environmental Protection Agency, 2002) when dealing with leachates derived from mine waste. This was done to ensure valid parameters for assessing a time-response relationship for each test. Methods are structured around the standard protocols specific to working with D. *magna* and *A. fischeri*, however, modifications were necessary since this was a field experiment.

3.2.2. Acute toxicity with *Daphnia magna*

Daphnia magna ephippia (batch number: DM140923) were sourced from a Daphtoxkit F magna, from MicroBioTests. Contents of the Daphtoxkit (product number: TK33 Daphtoxkit F) included concentrated salt solutions (**Table 3.1**), Petri dishes, six 30-well polycarbonate test plates, parafilm strips, and vials of *Spirulina* powder for pre-feeding.

3.2.2.1 Control solutions

Standard protocols indicate the use of negative and positive controls (ISO 6341:2012). A reconstituted standard freshwater solution, made with concentrated salt solutions included in the Daphtoxkit (**Table 3.1**), served as a negative control to provide a natural baseline for the test since *D. magna* is an euryhaline freshwater crustacean. The four vials of concentrated salts were enough to make up 2ℓ of standard freshwater solution. Each vial of salt concentrate was added to a 2ℓ volumetric flask. Deionised water was added, and the solution was shaken to homogenise. After use for each *D. magna* test, the solution was stored in a refrigerator at 4° C. Before use, the solution was brought to room temperature by placing it in a controlled environment incubator at 25° C (New Brunswick G25 Controlled Environment Shaker).

Positive controls consisted of three solutions per test: a standardised reference toxicant and solutions containing the highest and lowest detectable amount of Cu^{2+} in the FLP and SPLP solutions (**Table 3.1**). Soluble concentrations of contaminants were semi-quantitatively detected with the use of test strips, and Cu^{2+} was found to occur in elevated concentrations in the soil (Chapter 2). Potassium dichromate is used as a standard reference toxicant in

laboratory experiments (ISO 6341:2012), however, this was not relevant to this field study. Since an appropriate reference toxicant needs to be relevant and have a known toxicity to the study species, copper sulphate ($CuSO_4$) was used as recommended by Lilius *et al*. (1994). This also further informed the selection of Cu^{2+} in the FLP and SPLP solutions. The use of positive controls establishes a baseline when determining the effective concentration of test solutions that cause a 50% mortality for dilution experiments using a test organism. Since this was a field experiment, and the objective was to test the sensitivity of each test organism after exposure to soil extracts from various points in the field (i.e., the ecosystem-level effects), the determination of EC50 values was not an objective of this chapter. However, the presence of positive controls served as a validation step and was included in these tests.

Table 3.1: Negative and positive controls used for the *Daphnia magna* test.

	Formula	**Chemical concentration**
Negative control		
Standard freshwater	$NaHCO_3$	67.8 mg/ℓ
	$CaCl_2.2H_2O$	294 mg/ℓ
	$MgSO_4.7H_2O$	123 mg/ℓ
	KCl	5.75 mg/ℓ
Positive controls [a]		
Reference toxicant	$CuSO_4$	1.16 x 10^{-6} M
High pollution zone FLP [b]	Cu^{2+}	0.0477 mg/ℓ
Medium pollution zone FLP	Cu^{2+}	0 mg/ℓ
High pollution zone SPLP [c]	Cu^{2+}	0.261 mg/ℓ
Medium pollution zone SPLP [d]	Cu^{2+}	0.01 mg/ℓ

[a] the unpolluted zone did not have any detectable level of Cu^{2+} in FLP or SPLP solutions; therefore, only the reference toxicant was used as a positive control
[b] diluted to 1:100 of original solution (4.77 mg/l)
[c] diluted to 1:100 of original solution (26.1 mg/l)
[d] diluted to 1:100 of original solution (1 mg/l)

3.2.2.2 Hatching of Daphnia magna ephippia

Before the start of each test, a vial of *D. magna* ephippia had to be prepped for hatching. Ephippia were rinsed with tap water to remove the storage medium and then transferred to a 15 cm petri dish with 15mℓ standard freshwater (**Figure 3.1a**). The petri dish was then covered and placed into a controlled environment incubator at 25° ± 3° C with a continuous

illumination of 138 µmol/s/m^2 (**Figure 3.1b and c**). Each batch of *D. magna* neonates hatched after 72 ± 12 hours. Neonates were fed an algal suspension made from mixing standard freshwater into a vial of *Spirulina* powder two hours before the start of each test (**Figure 3.1d**).

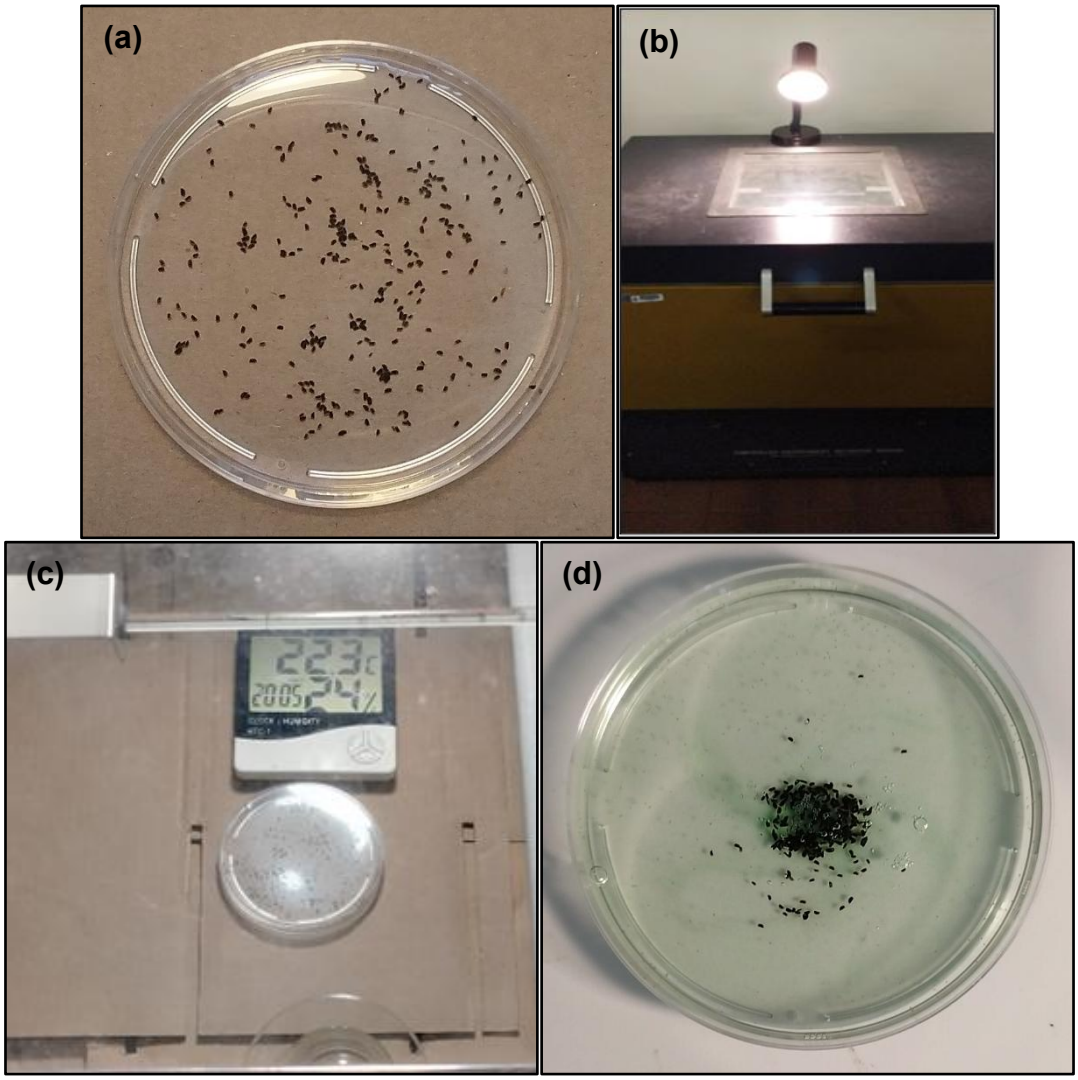


Figure 3.1: (a) *Daphnia magna* ephippia in petri dishes before hatching. **(b)** and **(c)** Incubator set-up to facilitate an environment conducive to the hatching of ephippia. **(d)** *Daphnia magna* neonates that were recently hatched and received a feeding of algae two hours before the start of the test.

Standard protocols recommend that, for the acute toxicity test, feeding only occurs two hours before the start of the test, and starvation thereafter for the duration of the test (OECD, 2004; test number 202). To account for the presence of food, which may also occur in a freshwater ecosystem along with the presence of contaminants, a subset of wells along each test plate was dedicated to fed *D. magna*. This added a level of comparison into how *D. magna* food status and the presence of food may affect the bioavailability of contaminants.

3.2.2.3 Assessment of toxicity

Each test was conducted in 30-well polycarbonate test plates that were cleaned and sterilised (**Figure 3.2**). Each well across the length of the test plate was denoted by a patch/interpatch. The first three wells going down the plate contained unfed *D. magna*, while the next three wells contained fed *D. magna*. Therefore, the three wells per feeding scheme for each patch/interpatch contained five *D. magna* neonates per triplicate sample of leachate extracts. The amount of *D. magna* added into each well is further explained below.



Figure 3.2: An example of the layout of each 30-well test plate used for the *Daphnia magna* test. The different patches/interpatches for each pollution zone are along the length of the plate, while fed and unfed wells occur along the width of each plate. The three wells per fed and unfed sections comprised the triplicate leachate solutions for each patch/interpatch.

Each well of the test plate received five random active neonates each. Therefore, each feeding scheme per patch/interpatch type contained 15 neonates in total. Neonates were transferred using a micropipette. A parafilm strip was then placed onto the test plate and covered with a lid to ensure that no test media was evaporated. The test plate was then left along the bench top at 25° ± 5 ° C with an artificial photoperiod of 16 hours light and 8 hours dark. The artificial photoperiod was created with the use of an incandescent desk lamp (60 W), which produced a light source of 138 µmol/s/m^2, that was switched off after 16 hours for 8 hours of darkness. *Daphnia magna* neonates were recorded as immobilised (dead) if no swimming/movement occurred after 15 seconds of gentle agitation by hand. The fed *D. magna* wells received a suspension of 15 µℓ algal cells (standard freshwater and spirulina powder) every 24 hours.

The high pollution zone was tested first, followed by the medium pollution zone and then the unpolluted zone, all under the same controlled conditions. In each case, FLP solutions were tested first, and followed by SPLP solutions. The positive and negative controls were tested in parallel to each test. Immobilisation readings of each well were recorded every two hours for the first 12 hours, then every six hours for the next 12 hours, then every 12 hours for the next 108 hours, and finally, every 24 hours until the death of all daphnids. A longer exposure period was used than recommended by the OECD guidelines, to observe any additional effects of contaminants. Dead *D. magna* neonates from each well were removed for the duration of the test. It should be noted that during periods of greater time between readings, dead neonates were only removed upon the start of the next reading.

3.2.3. Inhibition of *Aliivibrio fischeri* bioluminescence

Freeze-dried *A. fischeri* was sourced from Biolight, Aqua Science (BIO2007, Lot: BL11510523) and stored in a deep freezer at -20° C until each test. Each batch of freeze-dried *A. fischeri* was accompanied by a control saline solution of 2% NaCl and a diluent solution containing 20% NaCl.

3.2.3.1 Control solutions

Each test was carried out in triplicate, along with a negative and positive control that ran parallel to the test. Negative controls were a 2% NaCl solution since *A. fischeri* is a marine bacterium species and used for assessment of wastewater. Fulladosa *et al.* (2005) recommended 0.35 mg/ℓ of $CuSO_4$ as a reference toxicant, which served as a positive control. Additional positive controls were like those used during the *D. magna* test, as shown in **Table 3.1**.

3.2.3.2 Assessment of toxicity

Since *A. fischeri* is a marine bacterium, all samples that were less than 21.89 psu had to be adjusted with a 20% NaCl diluent solution to reach a salinity reading of 21.89 psu (± 1 psu) (ISO 11348 – 3, 2007). Forty minutes before the start of each test, vials of freeze-dried *A. fischeri* were reconstituted by adding the diluent solution of 20% NaCl and then incubated in a refrigerator at 4° C for 30 minutes in darkness. The vial of *A. fischeri* was then allowed to stabilise for 5 minutes on the benchtop with ice packs to maintain a constant temperature of 15° C.

The tests were conducted in glass cuvettes that were compatible with an Aboatox 1253 Luminometer. Luminometer readings were taken upon the start of each test (T0), at 5 minutes (T_{5mins}), 15 minutes (T_{15mins}), 30 minutes (T_{30mins}) and 1 hour (T_{60mins}). Initial luminometer readings (T0) just contained 0.5 mℓ of A. fischeri in each cuvette. These allowed for baseline readings of *A. fischeri* luminescence before the addition of any test solution. Thereafter, 0.5 mℓ of each sample was added to each cuvette which was done in 30-second intervals. Luminometer readings for each cuvette continued until 1 hour (T_{60mins}). Readings were expressed in Relative Light Units (RLU).

3.2.4. Data and statistical analysis

For *D. magna*, percentage immobilisation was determined as the total number of *D. magna* immobilised within each well relative to the initial number of mobile *D. magna* (5); and as the total number of *D. magna* immobilised within each feeding scheme relative to the initial number of alive *D. magna* (15). Results for the time taken to reach 1-2%, 25%, 50%, 75%, and 100% immobilisation were subset for this analysis. In each case, the mean (± standard deviation) of the triplicate response of each test solution was determined.

For *A. fischeri*, percentage inhibition at each time point (5, 15, 30, and 60 minutes) was determined first by calculating the control correction factor (KF) (Kahru, 1993):

$$KF = \frac{Luminescence\ of\ control\ after\ contact\ time}{Initial\ luminescence\ of\ control}$$

Then by correcting the raw inhibition values against the control, and calculating the percentage inhibition (% inhibition):

$$\%\ inhibition = 100 - \left(\frac{Luminescence\ of\ test\ sample\ after\ contact\ time}{KF * Initial\ luminescence\ of\ test\ sample}\right) \times 100$$

The mean and standard deviation of percentage inhibition were determined among the triplicate samples.

For *D. magna* and *A. fischeri*, differences between pollution zones were tested with a Kruskal-Wallis test. Differences because of patch type were determined with a Mann-Whitney U test. Non-parametric tests were used since the data did not follow a normal

distribution. All raw data was analysed and processed in Microsoft Excel. Statistical analysis was done in RStudio. A result was considered significant at $p < 0.05$.

A Pearson correlation analysis was then conducted to understand which soil properties (pH, electrical conductivity, soil organic matter, and concentrations of Cu and Pb) may be responsible for the observed outcomes of *A. fischeri* and fed/unfed *D. magna* after exposure to patch and interpatch leachates from differing pollution levels.

3.3 Results

3.3.1. *Daphnia magna* immobilisation

Time-response curves (**Figures 3.3 and 3.4**) were plotted to understand the sensitivity of *D. magna* immobilisation in response to the effect of patch type and pollution level. Full statistical results are shown in **Appendix B.**

During the test, *D. magna* was rarely found to immobilise individually; therefore, the time at which 1-2 immobilisations **($t_{1\text{-}2\%}$)** occurred was noted. For the FLP solutions, fed *D. magna* from patches of the high pollution zone immobilised at a faster rate compared to the fed *D. magna* from patches of the unpolluted zone ($p < 0.001$) (**Figure 3.3 g** and **k**). For the effect of food, a significant difference between the fed and unfed *D. magna* was only observed for the unpolluted FLP patches: fed *D. magna* immobilised quickly compared to the unfed *D. magna* ($p = 0.026$). Overall, the high and medium pollution zones had a quicker onset of death compared to the uncontaminated zone.

The time taken to reach 25% immobilisation (**$t_{25\%}$**) indicates how long it took for *D. magna* to enter an exponential phase of mortality. Unfed *D. magna* from the FLP solutions in patches of the high and medium pollution zones reached a 25% immobilisation in less time than the unpolluted zone ($p = 0.021$ and $p = 0.002$, respectively) (**Figures 3.3a, c**, and **e**). There were no differences in patch type at 25% for either fed or unfed FLP tests. For the effect of food, the unfed *D. magna* from patches of the unpolluted zone immobilised in less time than the fed *D. magna* ($p = 0.001$) (**Figure 3.3 e** and **k**).

The time taken for 50% (**$t_{50\%}$**) of *D. magna* to immobilise represents the mid-exponential phase. The unfed *D. magna* in FLP solutions of patches from the high and medium-polluted zones reached a 50% immobilisation in less time than the unpolluted zone ($p = 0.0002$ and $p = 0.002$; respectively) (**Figure 3.3a, c,** and **e**). A similar pattern occurred for the fed *D. magna* of FLP solutions in patches from the high and medium pollution zone compared to the

unpolluted zone ($p < 0.001$ and $p = 0.001$, respectively) (**Figure 3.4 g, i**, and **k**). Immobilisation of unfed *D. magna* in FLP solutions of interpatches from the high pollution zone was also significantly quicker than the unpolluted zone ($p = 0.001$) (**Figure 3.3 b** and **f**). For the effect of patch type, the unfed *D. magna* in FLP solutions of patches from the medium pollution zone were found to reach a 50% immobilisation in less time than the interpatch of the same zone ($p = 0.035$) (**Figure 3.3 c** and **d**). For the effect of feeding, the unfed *D. magna* from FLP solutions of patches from the unpolluted zone had a significantly quicker 50% immobilisation compared to the fed *D. magna* FLP patches of this zone ($p = 0.003$) (**Figure 3.3 e** and **k**).

The time taken to reach 75% (**$t_{75\%}$**) immobilisation represents the inflection phase, where the exponential rate of immobilisation starts to plateau (at 100%). There were no significant differences in the fed and unfed *D. magna* immobilisation for the time taken to reach 70% in FLP solutions. There were no significant differences in the effect of patch type. For the effect of feeding, the fed *D. magna* from FLP solutions of patches and interpatches both took longer to reach a 75% immobilisation compared to the unfed *D. magna* from these zones ($p < 0.001$ and $p = 0.037$; respectively) (**Figure 3.3 e. f, k,** and **l**),

The time taken for 100% immobilisation (**$t_{100\%}$**) represents the time to reach the plateau phase. The time to 100% immobilisation for the unfed *D. magna* in FLP solutions from patches of the high and medium zones was significantly lower compared to the unpolluted zone ($p < 0.001$ and $p = 0.007$; respectively) (**Figure 3.4a, c,** and **e**). Similarly, the unfed *D. magna* from FLP solutions of interpatches from the high pollution zone immobilised to 100% far quicker than that in the unpolluted zone ($p < 0.001$). The were no other significant differences regarding the effect of patch type or feeding.

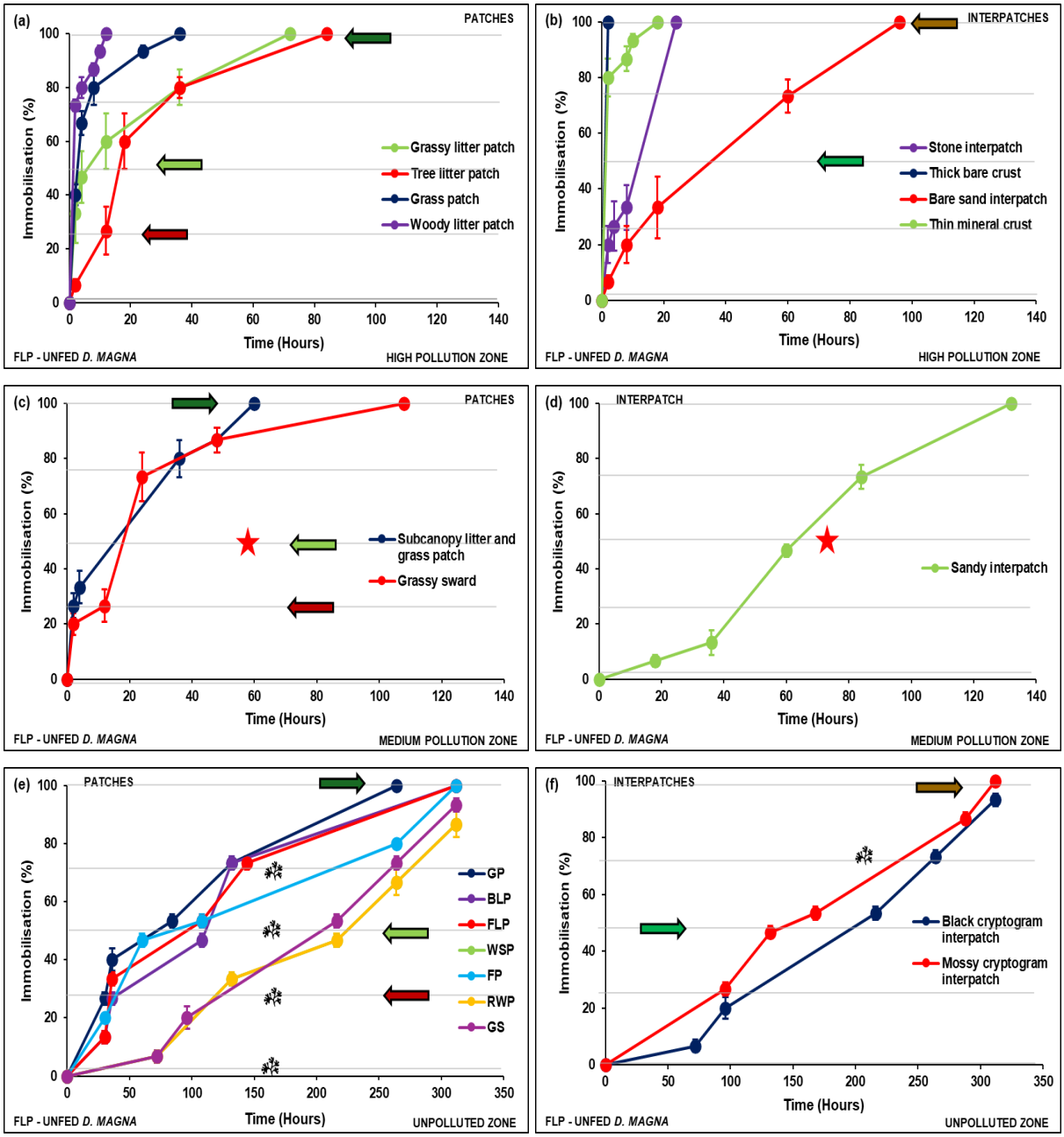


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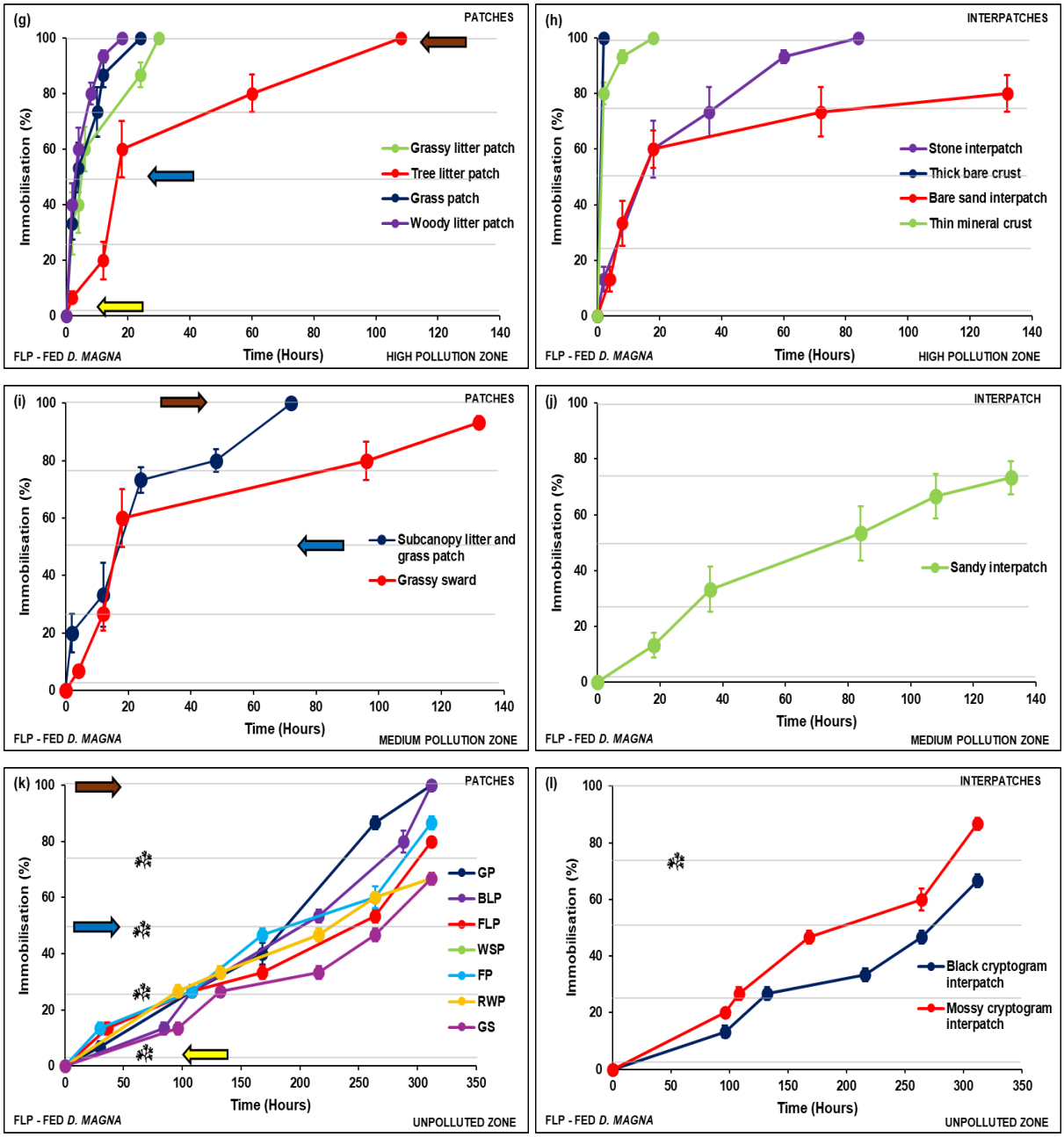


Figure 3.3: Mean (± standard error) time-response curves for *Daphnia magna* immobilisation after exposure to **FLP** solutions of patch and interpatch soils from the high, medium, and unpolluted zones. Tests without feeding (**a–f**) were compared to tests with feeding (**g–l**). Lines across each graph represent the time points used to mark when **1-2%**, **25%**, **50%**, **75%**, and **100%** immobilisation occurred. Graphs that share arrows of a similar colour are significantly different to each other at that time point. A red star indicates a significant difference between patch and interpatch response at that time point. An algae icon represents a significant difference between the fed and unfed response at that time point. Means were determined from triplicate samples (N = 5 per sample) for each patch and interpatch type.

For the time taken for 1-2% immobilisation **($t_{1\text{-}2\%}$)** of *D. magna* in SPLP solutions, unfed *D. magna* from the high pollution zone immobilised at a faster rate compared to the unpolluted zone ($p = 0.003$) (**Figure 3.4 a** and **e**). The unfed patches of the high pollution zone also started to immobilise faster than the interpatches compared to the unfed interpatches from the same zone ($p = 0.005$) (**Figure 3.4 a** and **b**). Similarly concerning patch type, the unfed patches of the unpolluted zone immobilised faster than the unfed interpatches of the same zone ($p = 0.031$). For the fed *D. magna*, SPLP solutions of the sandy interpatch from the medium pollution zone had a quicker immobilisation compared to the black and mossy cryptogram patch of the unpolluted zone ($p = 0.003$) (**Figure 3.4 j** and **l**). For the effect of food, no difference was found in the fed and unfed SPLP solutions for any zone. Overall, the high and medium pollution zones had a quicker onset of death compared to the uncontaminated zone.

For the time taken to reach 25% immobilisation (**$t_{25\%}$**) of unfed *D. magna* in SPLP solutions, patches of the high and medium pollution zones achieved 25% immobilisation quicker compared to the unpolluted zone ($p = 0.002$, and $p = 0.007$; respectively) (**Figures 3.4 a, c**, and **e**). For the fed *D. magna*, only the high pollution zone had 25% immobilisation significantly quicker than the unpolluted zone ($p < 0.001$) (**Figure 3.4g** and **k**). For the fed *D. magna* from interpatches, the high and medium pollution zones achieved a 25% immobilisation quicker than the unpolluted zone ($p = 0.006$ and $p = 0.006$, respectively) (**Figures 3.4h, j**, and **l**). For the effect of patch type, fed *D. magna* from patches of the unpolluted zone, achieved a 25% immobilisation in a longer time than the interpatches ($p = 0.012$). (**Figure 3.4k** and **l**). For the effect of food, no difference was found for the fed or unfed SPLP tests at 25% immobilisation.

For the time taken to reach 50% immobilisation (**$t_{50\%}$**), the unfed *D. magna* in SPLP solutions of patches from the high and medium pollution zone immobilised faster than the unpolluted zone ($p < 0.001$ and $p = 0.008$; respectively) (**Figure 3.4a, c**, and **e**). Similarly, for the fed *D. magna,* 50% immobilisation was significantly quicker in the high and medium pollution zones compared to the unpolluted zone ($p < 0.001$ and $p = 0.005$; respectively) (**Figure 3.4 g, i,** and **k**). For the interpatches, the fed *D. magna* from the high pollution zone had a 50% immobilisation significantly quicker than the interpatches of the unpolluted zone ($p = 0.001$) (**Figure 3.4 h** and **l**). For the effect of patch type, unfed *D. magna* from SPLP solutions of interpatches reached a 50% immobilisation much quicker compared to the patches of this zone ($p = 0.001$) (**Figure 3.4 e** and **f**) and similarly for fed *D. magna*, interpatches reached

50% immobilisation quicker than the patches ($p = 0.035$). For the effect of feeding, the unfed *D. magna* in interpatches of the high pollution zone had a 50% immobilisation significantly quicker than the fed D. magna along the similar zone ($p = 0.001$) (**Figure 3.4 b** and **h**), zone and similarly for the fed *D. magna* from interpatches of the unpolluted zone (**Figure 3.4 f** and **l**).

For the time taken to reach 75% immobilisation ($\mathbf{t_{75\%}}$), the unfed *D. magna* in SPLP solutions of patches from the high and medium pollution zones reached immobilisation quicker than the unpolluted zone ($p < 0.001$, and $p = 0.007$; respectively) (**Figure 3.4 a, c**, and **e**). For the fed D. magna in SPLP solutions of patches, the high pollution zone had a significantly quicker 70% immobilisation compared to the patches of the unpolluted zone ($p < 0.001$) (**Figure 3.4 g** and **k**). For the effect of patch type at 70% immobilisation, only the unfed *D. magna* in SPLP solutions from patches of the unpolluted zone were significantly quicker to immobilise compared to interpatches along this zone ($p = 0.011$). (**Figure 3.4 e** and **f**). For the effect of feeding, fed *D. magna* in SPLP solutions of interpatches from the unpolluted zone took a longer time to reach 75% immobilisation compared to the unfed *D. magna* in SPLP solutions from this zone ($p = 0.023$).

For the time taken to reach 100% immobilisation ($\mathbf{t_{100\%}}$) the unfed *D. magna* in SPLP solutions of patches from the high and medium pollution zones reached 100% immobilisation far quicker than the patches of the unpolluted zone ($p < 0.001$, and $p = 0.014$; respectively) (**Figure 3.4 a, c**, and **e**). Immobilisation to 100% of unfed *D. magna* within the interpatches of the SPLP solutions occurred at a faster rate in the high pollution zone compared to the unpolluted zone ($p < 0.001$) (**Figure 3.4 b** and **f**). Similarly, 100% of fed D. magna within the patches of the SPLP solutions occurred at a faster rate in the high pollution zone compared to the unpolluted zone ($p < 0.001$) (**Figure 3.4 g** and **k**). There were no significant differences in patch type along these zones. For the effect of feeding, the unfed *D. magna* in SPLP solutions of the unpolluted zone reached 100% immobilisation quicker than the fed D. magna from this zone ($p = 0.015$) (**Figure 3.4 f** and **l**).

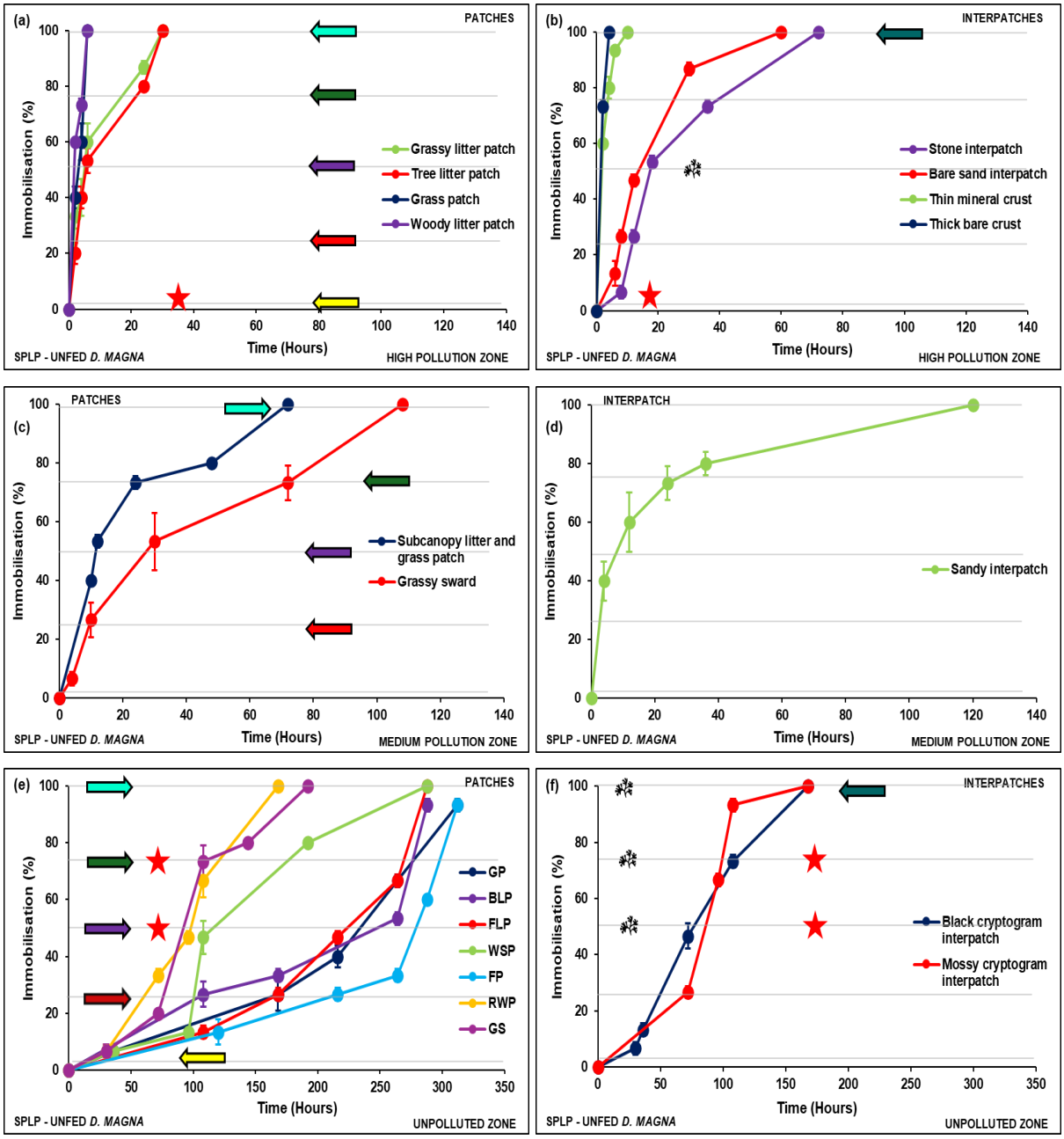


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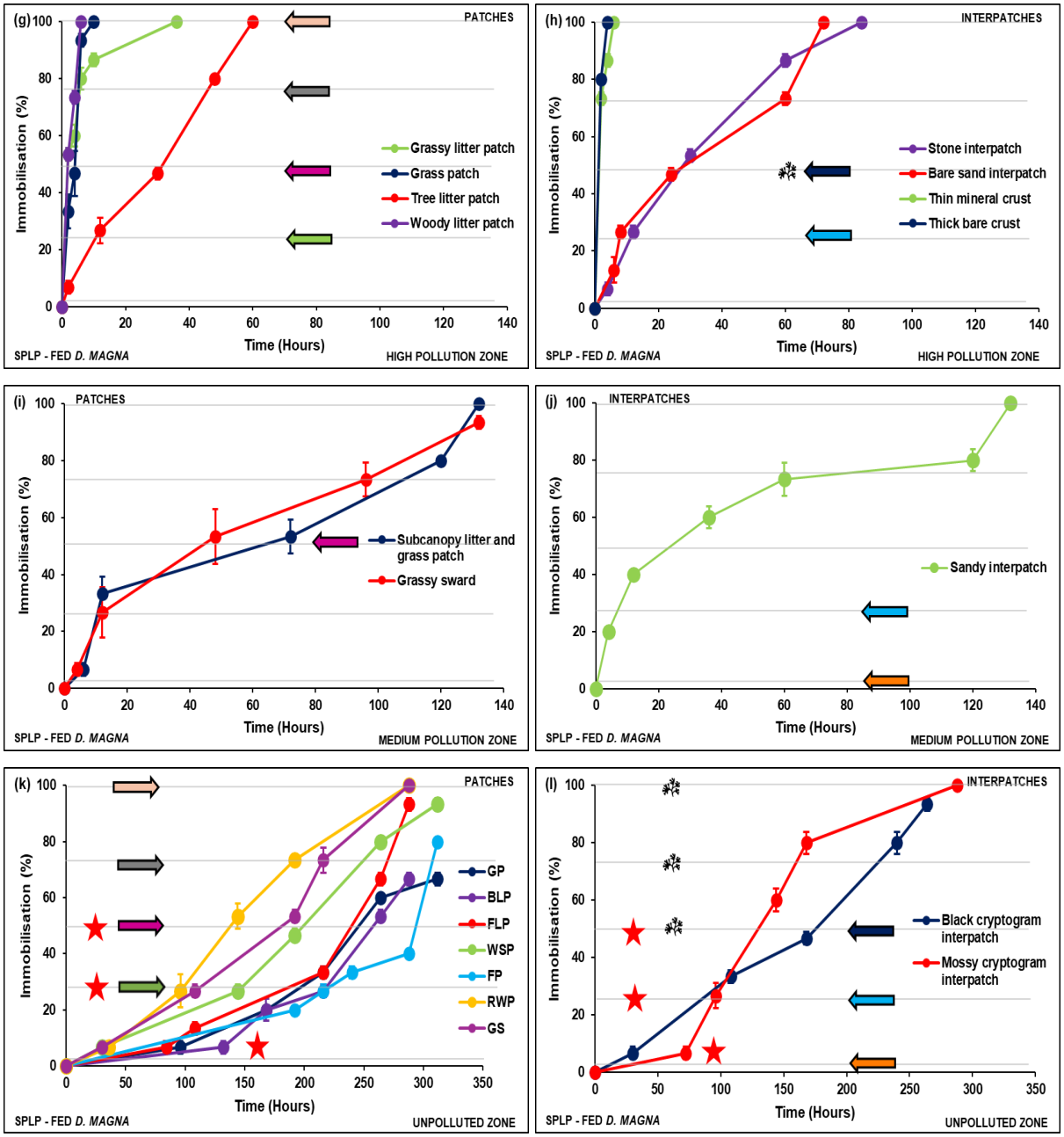


Figure 3.4*:* Mean time-response curves for *Daphnia magna* immobilisation after exposure to **SPLP** solutions of patch and interpatch soils from the high, medium, and unpolluted zones. Tests without feeding (**a–f**) were compared to tests with feeding (**g–l**). Lines across each graph represent the time points used to mark when **1-2%**, **25%**, **50%**, **75%**, and **100%** immobilisation occurred. Graphs that share arrows of a similar colour are significantly different to each other at that time point. A red star indicates a significant difference between patch and interpatch response at that time point. An algae icon represents a significant difference between the fed and unfed response at that time point. Means were determined from triplicate samples (N = 5 per sample) for each patch and interpatch type.

3.3.2. *Aliivibrio fischeri* inhibition of bioluminescence

In terms of the effect of pollution level and patch type, positive values indicate an inhibitory effect of the test solution on *A. fischeri* bioluminescence, and negative values indicate a stimulation of bioluminescence.

Five minutes into the test, results from the FLP tests indicate that patches from the high pollution zone (**Figure 3.5a**) experienced an overall stimulation of bioluminescence, compared to patches from the unpolluted zone (**Figure 3.5e**), which had an overall inhibition in bioluminescence ($p < 0.001$). A similar pattern was found for patches of the medium pollution zone (**Figure 3.5c**) when compared to patches of the unpolluted zone (**Figure e**) ($p < 0.001$).

For the SPLP tests, there was stimulation of bioluminescence in patches of the high pollution zone compared to inhibition of bioluminescence in patches from the unpolluted zone ($p < 0.001$) (**Figure 3.6a**) and similarly for the medium pollution zone and the unpolluted zone ($p < 0.001$) (**Figure 3.6c**). The interpatch response for the SPLP solutions from the high pollution zone (**Figure 3.6b**) was also found to be an overall inhibitory response compared to the interpatches of the unpolluted zone ($p = 0.005$) (**Figure 3.6f**) The patch responses for the medium pollution zone (**Figure 3.6 c**) were found to have a lower stimulative response relative to the interpatch responses ($p = 0.027$).

At 15 minutes into the test, SPLP solutions from the patches of the high pollution zone were again found to have a stimulative effect compared to the patches from the unpolluted zone ($p < 0.001$) (**Figure 3.6e**), similarly with patches from the medium pollution zone compared to the unpolluted zone ($p = 0.002$) The interpatches from the high pollution zone had an overall inhibitory effect, compared to the interpatches from the unpolluted zone ($p = 0.004$). The interpatch from the medium pollution zone was found to have a greater stimulative effect on *A. fischeri* bioluminescence compared to patches ($p = 0.048$).

Inhibitory effects for FLP and SPLP solutions from the high pollution zone only started to show 30 minutes into the test. For the FLP solutions, site 1 showed an inhibitory effect, however, this was much lower compared to the inhibition experienced for the reference site ($p < 0.001$), and similarly along the medium pollution zone and the reference site ($p = 0.004$). No significant differences were found between patches and interpatches from any of the three sites at 30 minutes.

At the end of the test (60 minutes), the high pollution zone was found to have a lower inhibition compared to the unpolluted zone ($p < 0.001$), and similarly for the medium pollution zone and the reference site ($p < 0.001$). No significant differences were found between patches and interpatches at the end of the test. Overall, the contaminated patches and interpatches from sites 1 and 2 seemed to have a stimulatory effect on *A. fischeri* for the first 30 minutes of the test and thereafter began to inhibit bioluminescence, relative to the overall results from the uncontaminated patches and interpatches of the reference site.

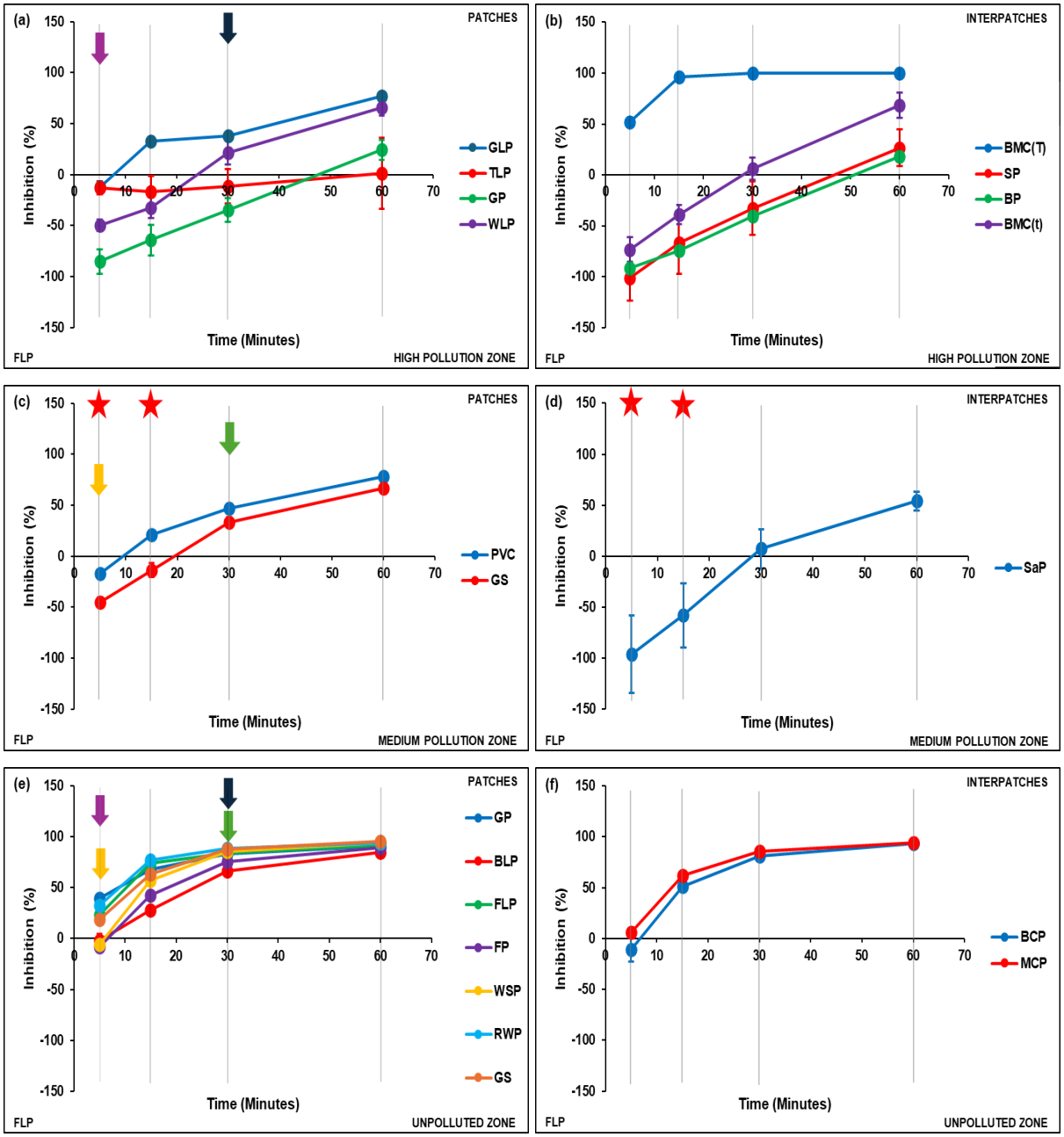


Figure 3.5: Mean (± standard error) percentage inhibition of *Aliivibrio fischeri* bioluminescence after exposure to **FLP** solutions of patch and interpatch soils from the high, medium, and unpolluted zones. Lines along each graph represent the time points when bioluminescence readings were taken: **5, 15, 30,** and **60 minutes**. Graphs that share arrows of a similar colour are significantly different to each other at that time point. A red star indicates a significant difference between patch and interpatch response at that time point. Means were determined from triplicate samples for each patch and interpatch type.

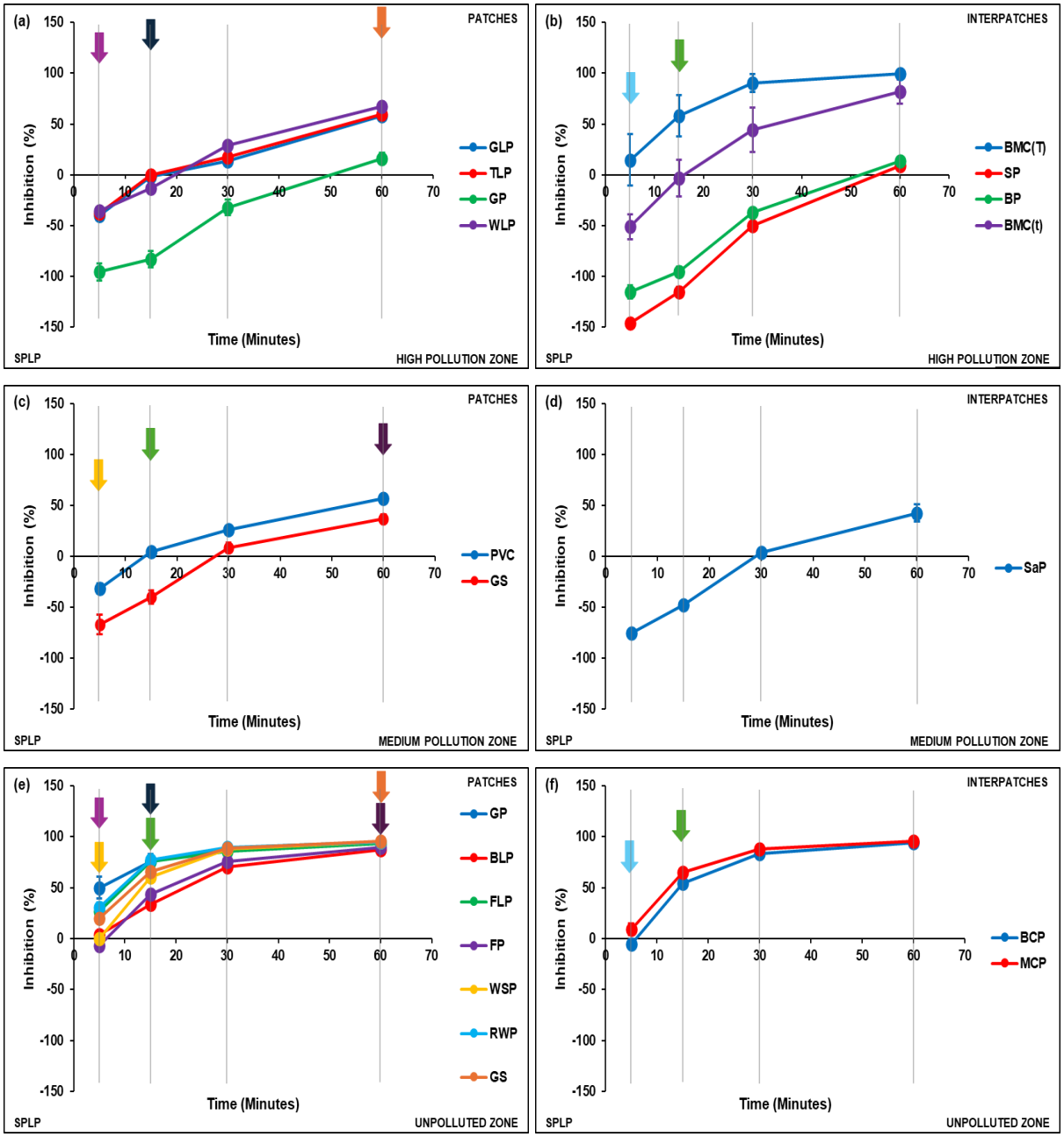


Figure 3.6: Mean (± standard error) percentage inhibition of *Aliivibrio fischeri* bioluminescence after exposure to **SPLP** solutions of patch and interpatch soils from the high, medium, and unpolluted zones. Lines along each graph represent the time points when bioluminescence readings were taken: **5, 15, 30,** and **60 minutes**. Graphs that share arrows of a similar colour are significantly different to each other at that time point. A red star indicates a significant difference between patch and interpatch response at that time point. Means were determined from triplicate samples for each patch and interpatch type.

3.3.3. Correlation between soil properties and *Daphnia magna* immobilisation and *Aliivibrio fischeri* inhibition of bioluminescence

Correlation coefficients between test solution properties (FLP and SPLP) and the time taken for 100% immobilisation ($\mathbf{t_{100\%}}$) of *D. magna* are shown in **Figure 3.7.** Test properties examined were pH, electrical conductivity, and soil organic matter; similarly, for the SPLP solutions, but also adding concentrations of Cu and Pb due to the appropriate sample size in these tests.

For the patches from the high pollution zone, unfed *D. magna* immobilisation did not show any strong correlations with the FLP solutions. Fed *D. magna* immobilisation was negatively related to soil organic matter (weak). For the SPLP solutions, only soil organic matter and Pb concentrations appear to negatively influence *D. magna* survival. For interpatches in this zone, soil organic matter was positively correlated with $\mathbf{t_{100\%}}$ in the FLP solutions. pH seems to have influenced the unfed and fed *D. magna* results. Electrical conductivity and concentrations of Cu and Pb have negative correlations with the results of fed and unfed *D. magna* $\mathbf{t_{100\%}}$ as well.

For the medium pollution zone, pH is negatively related to the unfed *D. magna* in the FLP tests. Electrical conductivity has mixed results in this analysis. For the unpolluted zone, soil organic matter appears to slightly correlate with the unfed *D. magna* $\mathbf{t_{100\%}}$, and similarly for the fed *D. magna* from FLP tests in patches. The interpatches appear to be contradictorily related to soil organic matter and negatively related to pH and electrical conductivity.

Daphnia magna		High pollution zone				Medium pollution zone				Unpolluted zone			
		Patch		Interpatch		Patch		Interpatch		Patch		Interpatch	
		Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed
		n = 12	n = 11	n = 12	n = 11	n = 5	n = 6	n = 3	n = 1	n = 18	n = 7	n = 5	n = 1
FLP	pH	-0,12	0,13	0,43	0,36	0,58	0,06	-	-	0,10	0,46	-0,65	-
	Electrical conductivity	-0,30	-0,40	-0,54	-0,34	0,41	-0,76	-	-	-0,38	-0,34	-0,78	-
	Soil organic matter	-0,45	-0,57	0,62	0,17	0,11	-0,19	-	-	-0,34	0,60	0,57	-
		n = 12	n = 12	n = 12	n = 12	n = 6	n = 6	n = 3	n = 1	n = 16	n = 10	n = 6	n = 5
SPLP	pH	0,16	0,09	0,64	0,68	-0,35	0,43	-	-	0,29	-0,15	-0,12	-0,79
	Electrical conductivity	-0,43	-0,45	-0,62	-0,68	0,26	0,65	-	-	0,24	-0,37	-0,36	-0,42
	Soil organic matter	-0,48	-0,50	0,22	0,33	-0,14	-0,20	-	-	0,51	0,21	-0,58	-0,10
	Cu	-0,31	-0,34	-0,72	-0,76	-	-0,48	-	-	-	-	-	-
	Pb	-0,63	-0,66	-0,80	-0,84	-	-	-	-	-	-	-	-

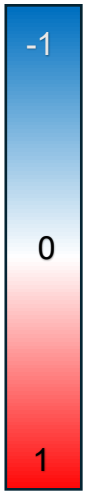


Figure 3.7*:* Heat map of Pearson correlation coefficients between the time taken for 100% *Daphnia magna* immobilisation and properties (pH, electrical conductivity, soil organic matter, and concentrations of Cu and Pb for the FLP and SPLP tests from the high, medium, and unpolluted zones. Correlation results are presented with fed and unfed tests from patches and interpatches of each zone. Positive correlations are highlighted in red, and negative correlations are highlighted in blue. A dash (-) indicates that it was not applicable to test for a correlation. Interpatches of the medium pollution zone were excluded since a sample size of three is not a reliable estimate of any relationship.

Correlation coefficients between test solution properties (FLP and SPLP) and bioluminescence inhibition at 60 minutes are shown in **Figure 3.8.** Test properties examined were pH, electrical conductivity, and soil organic matter; similarly, for the SPLP solutions, but also adding concentrations of Cu and Pb due to the appropriate sample size in these tests.

Correlations within patches were only present in the medium pollution zone, with a positive relationship with pH and electrical conductivity in FLP solutions, and a slight negative relationship with the concentration of Pb. Interpatches of the medium pollution zone are not included in this discussion due to their small sample size. The inhibition of bioluminescence of *A. fischeri* from interpatches of the unpolluted zone appears to be positively correlated with electrical conductivity (both FLP and SPLP tests) and pH.

Aliivibrio fischeri		High pollution zone		Medium pollution zone		Unpolluted zone	
		Patch	Interpatch	Patch	Interpatch	Patch	Interpatch
FLP		n = 12	n = 12	n = 6	n = 3	n = 21	n = 6
	pH	-0,34	-0,68	0,22	-	-0,15	-0,12
	Electrical conductivity	0,16	0,83	0,62	-	-0,03	0,65
	Soil organic matter	0,25	-0,54	0,04	-	-0,34	0,07
SPLP		n = 12	n = 12	n = 6	n = 3	n = 21	n = 6
	pH	0,02	-0,83	0,82	-	-0,08	0,65
	Electrical conductivity	-0,21	0,82	0,40	-	-0,11	0,66
	Soil organic matter	0,27	-0,49	-0,05	-	-0,06	0,07
	Cu	0,09	0,90	-	-	-	-
	Pb	-0,12	0,93	-0,53	-	-	-

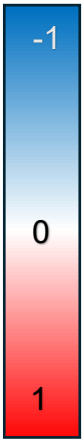


Figure 3.8: Heat map of Pearson correlation coefficients between the inhibition of bioluminescence at 6omins and properties (pH, electrical conductivity, soil organic matter, and concentrations of Cu and Pb for the FLP and SPLP tests from the high, medium, and unpolluted zones. Correlation results are presented from patches and interpatches of each zone. Positive correlations are highlighted in red, and negative correlations are highlighted in blue. A dash (-) indicates that it was not applicable to test for a correlation. Interpatches of the medium pollution zone were excluded since a sample size of three is not a reliable estimate of any relationship.

3.4 Discussion

The research question for this chapter is: How does the ecotoxicity of soil leachates to *D. magna* and *A. fischeri* change between patches and interpatches across a pollution gradient, and how is this related to the properties of each solution?

3.4.1. *Daphnia magna* immobilisation and the effect of food

In polluted soils, correlations between soil properties and toxicity responses were generally weak, indicating that contamination masked normal ecological relationships. Patch type did not strongly influence Daphnia magna immobilisation, except in the unpolluted zone where some patch effects remained evident. Overall, under heavy pollution, patch structure lost its ecological meaning, showing that the LFA framework alone cannot predict biological impairment in contaminated systems.

Daphnia magna was found to be sensitive to the effect of pollution levels, with a threefold increase in the time to immobilise in the unpolluted zone compared to the high and medium-polluted zones. The soil analysis from Chapter 2 indicates that the high and medium pollution zones contained elevated concentrations of Cu and Pb (and Br and SO_4), and an increased salinity compared to the unpolluted zone. The presence of heavy metal contaminants and increased salinity is well known to have a toxic effect on *D. magna* (Deleebeeck *et al*., 2008), and according to Sarkar *et al*. (2006), even low concentrations of Cu and Pb are highly toxic to *D. magna*. This level of toxicity can cause immobilisation through oxidative stress and cell damage (Dorchin and Shanas, 2013).

There was no difference in the effect of patches and interpatches; the high and medium-pollution zones had a similar effect on *D. magna*, with immobilisation starting within the first two hours of exposure to the FLP and SPLP solutions. This was expected considering that soil properties (pH and electrical conductivity) did not significantly differ between patches and interpatches on these zones for the FLP and SPLP solutions. This pattern was consistent with the fed and unfed *D. magna*, which was unexpected considering the adsorption of metal ions onto the surface of negatively charged algal cells serving as another route of contaminant exposure (Taylor *et al.,* 1998). However, contrasting research has found that the toxicity of contaminants decreases after adsorbing onto organic molecules (Long *et al*., 2004). The commonality in these contrasting points is that toxicity depends on the interplay between the contaminant's ionic nature and the solution's chemical composition.

Significant differences between the effects of patches and interpatches on *D. magna* immobilisation were observed along the unpolluted zone, with patches having a mitigating effect on *D. magna* immobilisation. This may be due to the higher SOM in patches compared to interpatches. This pattern may also be seen in the high and medium-polluted zones.

3.4.2. Inhibition of bioluminescence in *Aliivibrio fischeri*

The high and medium polluted zones initially stimulated *A. fischeri* bioluminescence and then inhibited the production of bioluminescence as the test progressed. The initial stimulation and resulting inhibition in response to exposure to each contaminated solution can be attributed to the stress-hormesis effect, which mostly happens at low levels of contamination (Agathokleous *et al*., 2020). Certain interpatches (such as the thick and thin mineral crusts) were observed to have a higher degree of salinity and heavy metal contaminants, such as Pb and Cu, compared to other patches. This results in a strong inhibitory response compared to the pattern of stimulation followed by inhibition observed in the other interpatches and patches overall. Strong negative correlations with pH and positive correlations between SPLP concentrations of Cu and Pb, and salinity may have driven this pattern.

The bioluminescence of *A. fischeri* is dependent on luciferase activity; therefore, contaminants in solution may have decreased bioluminescence by decreasing luciferase activity directly, or by the production of reactive oxygen species (Erzinger *et al*., 2018) – caused by increased salinity, increased Pb and Cu concentrations, and a decreased pH. As mentioned above, pH and electrical conductivity drive the bioluminescence results; an acidic solution with a high salinity can increase the inhibition of *A. fischeri* bioluminescence. This pattern is consistent for both patches and interpatches, and there did not seem to be any mitigating effect from either patch type.

3.4.3. Limitations of study design

Soil texture was not included in this analysis, so information about how particle size proportions could have affected *D. magna* and *A. fischeri* was not understood. The patch categories could not be standardised for either patch or interpatch soils, as seen in the differences within the specific patch and interpatch results. This may have introduced a degree of variability to the results. Soil characterisation could be improved by assessing additional soil properties, such as cation exchange capacity. Temperature and dissolved oxygen were not fully controlled in this experimental design. This may have impacted the *D.*

magna and *A. fischeri* responses. The sample size used for the correlation analysis was too small to derive any realistic conclusions.

3.5 Conclusion

The aim of this chapter is to determine if patches and interpatches affect the ecotoxicity of leachates extracted from soils from a pollution gradient. *Daphnia magna* displayed extreme sensitivity to the effects of pollution levels. Patch type was not a good indicator of *D. magna* immobilisation, with mostly the unpolluted zones showing a difference. Similarly, for the effect of food, these effects were mostly prevalent in the unpolluted zones and not the polluted zones. *Aliivibrio fischeri* appeared to have been stimulated by low concentrations of pollutants, thereby limiting its usefulness as a bioindicator in this study. Patch type did not have a significant effect on the bioluminescence of *A. fischeri*.

Chapter Four – Soil leachate ecotoxicity to six species of vascular plants in response to patch type and contamination level in a semi-arid lowveld savanna

4.1 Introduction

Industrial and agricultural activities are on the rise, leading to an increase in pollutants introduced into the surrounding environment. Monitoring how various species of plants respond to soil pollution is essential when assessing environmental risk and resilience. However, this is not a straightforward task, since plant responses are dependent on their growth stage position within the landscape (spatial heterogeneity) and the degree of pollution (Gomez *et al*., 2014).

Monitoring the response of vascular plants typically involves assessing growth parameters such as germination response and root development after exposure to polluted media (Alengebawy *et al*., 2021). These serve as indicators of the phytotoxicological effects of the polluted media. Germination and radicle development are useful monitoring metrics since they reflect the initial stages of plant growth, and when the plant is at its most sensitive stage (Bareke, 2018).

Examining germination and radicle development when considering factors such as pollution zones and spatial heterogeneity can allow for a more refined monitoring procedure. In this project, Chapter 2 described three pollution zones and delineated each gradsect along the zone into its respective patch and interpatch type. Identifying patch types considered the spatial heterogeneity of the pollution zone, with patches serving as areas of resource (water and vital nutrients) accumulation and interpatches serving as areas of resource loss (Tongway and Hindley, 2004).

The key research question posed in this chapter is: “How does patch/interpatch type influence the phytotoxicity of soils and leachate solutions to plants, and how is this related to the properties of each test substrate?” In this context, test substrates refer to the soil or leachates used to germinate each species. The six species of vascular plants used were: *Amaranthus*

hybridus L., *Dactyloctenium australe* Steud. *Lepidum sativum* L., *Medicago sativa* L., *Brassica nigra* L., and *Sorghum bicolor* (L) Moench.

To answer this question, the specific objectives of this chapter are:

1. To assess the sensitivity of germination of six vascular plants, from different test substrates, in response to the effect of pollution level and patch type. Patch type and pollution level were identified in Chapter 2.
2. To assess how the radicle development of six vascular plants, from different test substrates, may be inhibited (relative to a control) in response to patch type and pollution level. Patch type and pollution level were identified in Chapter 2.
3. To determine which soil properties (identified in Chapter 2) may be responsible for the peak germination and radicle development of six vascular plants after exposure to patch and interpatch soil and aqueous extracts.

4.2 Methods

4.2.1. Study species

To account for variation in sensitivity between species, six species of plants were chosen from four families (**Table 4.1**). Two African grass species were chosen (an Indigenous lawn grass *D. australe,* and a leaf and grain crop *S. bicolor*), along with a mustard and cabbage species (*L. sativum* and *B. nigra*), and finally, a leaf and grain crop species (*A. hybridus*) and a legume leaf crop species (*M. sativa*). Two monocotyledon (monocot) plants and four dicotyledon (dicot) plants were chosen. The OECD (Organisation for Economic Cooperation and Development) guidelines recommend the use of at least one monocot and one dicot plant to be used to phytotoxicity testing since the differing physiology and morphology of these species will result in different responses to contaminant exposure (OECD, 2006; Test number 208). Each plant species chosen is generally used in phytotoxicity tests and, in some cases, phytoremediation (*A. hybridus*: Tőzsér *et al*., 2023; *D. australe*: Pandey *et al*., 2020; *L. sativum*: Bafeel, 2010; *M. sativa*: Aydinalp and Marinova, 2009; *B. nigra*: Angelova and Ivanov, 2009; *S. bicolor*: Hashemi *et al*., 2023). Seeds were sourced from verified seed suppliers in Johannesburg, South Africa.

Table 4.1: Information on species used for phytotoxicity testing.

Family	Species	Common name	Monocotyledon (Monocot)/ Dicotyledon (Dicot)	Seed supplier	Batch number	Regulatory status/ Compliance
Amaranthaceae	*Amaranthus hybridus* L.	Amaranth	Dicot	Starke Ayres	V2532201KBA	Genetically Modified Organisms Act of 1997 Plant Improvement Act, 1976
Poaceae	*Dactyloctenium australe* Steud.	Berea grass	Monocot	Sakata seed Southern Africa	Z0022442	Plant Improvement Act, 1976 Plant Breeders Rights Act 12 of 2018
Brassicaceae	*Lepidum sativum L.*	Garden cress	Dicot	Kirchhoffs	10254155	Plant Improvement Act, 1976 Plant Breeders Rights Act 12 of 2018
Fabaceae	*Medicago sativa L.*	Lucerne	Dicot	AGT Foods Africa	670/IE	Plant Breeders Rights Act 12 of 2018
Brassicaceae	*Brassica nigra L.*	Mustard greens	Dicot	Kirchhoffs	10248457	Plant Improvement Act, 1976 Plant Breeders Rights Act 12 of 2018
Poaceae	*Sorghum bicolor (L)* Moench (Sweet Betty)	Sweet sorghum	Monocot	AGT Foods Africa	811/1	Plant Breeders Rights Act 12 of 2018

4.2.2. Aqueous extracts and soils

Seedling responses were assessed in three tests: two liquid tests using soil leachate solutions, and a solid test using the actual soil sample. Leachate solutions were derived from soil extracts using the Field Leach Procedure (FLP) (Hageman, 2005) and the Synthetic Precipitation Leach Procedure (SPLP) (Hageman, 2004; US EPA, 1994). Solid tests were simply direct contact with the soil by placement on the surface. Three tests were done to understand the seed response after exposure to soil leaching by clean stream water or rainfall (FLP), or industrially acidified rainfall (SPLP), and the fraction of contaminants readily available in the soil. This was done to get a better understanding of the effect of salinity and bioavailable metal contaminants, and nutrients during each test that could have affected seedling germination in the site soils, under controlled laboratory conditions. Note that leachate extractions alone cannot fully capture the toxicity of the test sample since soil tests relate to the total contaminants available (Barral and Paradelo, 2011).

Soil samples were collected from patches and interpatches identified after conducting the Landscape Function Analysis protocol (Tongway and Hindley, 2004) along three pollution zones: a high pollution zone, a medium pollution zone and an unpolluted zone. Leachate solutions and soils were not further modified or diluted for this test. Soil samples and subsequent leachate solutions were derived from four patches and four interpatches collected from the high pollution zone; two patches and a single interpatch from the medium pollution zone; and seven patches and two interpatches from the unpolluted zone (**Table 4.2**). Each sample was collected in triplicate.

Table 4.2: Soil samples collected from patches and interpatches from the high pollution zone, medium pollution zone, and unpolluted zone. Each soil sample was collected in triplicate. Detailed descriptions of each patch and interpatch, and physicochemical properties can be found in Chapter 2.

		Soil code	Name
High pollution zone	Patches	GLP	Grassy litter patch
		TLP	Tree litter patch
		GP	Grass patch
		WLP	Wood litter patch
	Interpatches	BMC(T)	Thick bare mineral crust interpatch
		SP	Stone interpatch
		BP	Bare interpatch
		BMC(t)	Thin bare mineral crust interpatch
Medium pollution zone	Patches	PVC	Subcanopy grass and litter patch
		GS	Grassy sward
	Interpatch	SaP	Sandy interpatch
Unpolluted zone	Patches	GP	Grass patch
		BLP	Broadleaf patch
		FLP	Fine leaf patch
		FP	Forb patch
		WSP	Woody shrub patch
		RWP	Riparian weed patch
		GS	Grassy sward
	Interpatches	BCP	Bare cryptogram interpatch
		MCP	Mossy cryptogram interpatch

4.2.3. Experimental design

This assessment of phytotoxicity, using percentage germination and root length, is based on standard protocols (US EPA Ecological Effects Test Guidelines OPPTS 850.4200 Seed germination/ root elongation), but modified using a standard ecological approach to measuring seed germination parameters (ISO 18763, 2016 and OECD, 2006; test number 208). The phytotoxicity tests were carried out in 24-well polycarbonate plates (hereafter referred to as “repli-dishes”). The six seed species were placed in wells along the length of the repli-dish and each species was replicated four times across the breadth of the repli-dish (**Figure 4.1**). Seeds were commercial packets with a viability of > 98% and therefore not surface sterilized before each test. Four randomly selected seeds per species were

individually placed into each well of the repli-dish. Each repli-dish represented a patch/interpatch type (three dishes per patch/interpatch to accommodate triplicate wells).

Soils and leachate samples from the unpolluted zone served as field control samples. Where possible, comparisons were drawn between matched patches and interpatches between the unpolluted zone and the high and medium pollution zones.

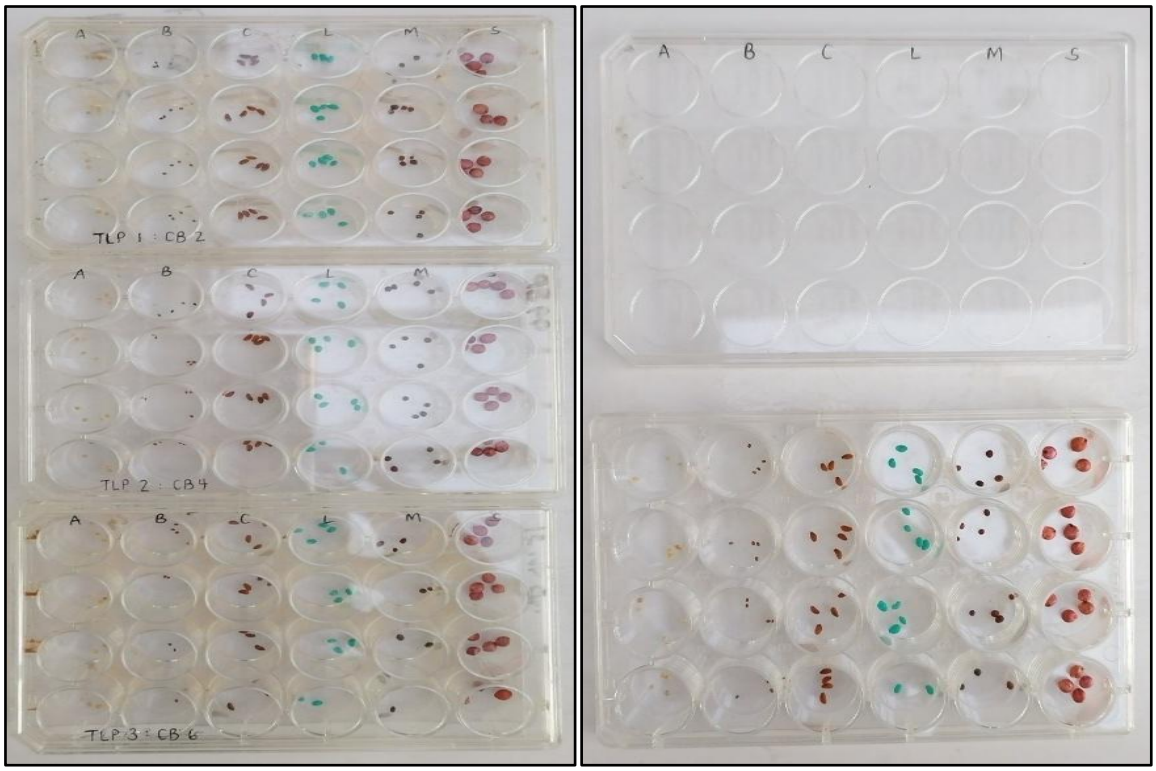


Figure 4.1: Twenty-four well repli-dishes (with three replicate dishes) containing four seeds per species in each well. A represents *A. hybridus,* B represents *D. australe*, C represents *L. sativum*, L represents *M. sativa*, M represents *B. nigra*, and S represents *S. bicolor*. Each well of four seeds was replicated four times down each repli-dish for all species.

4.2.4. Assessment of ecotoxicity

For the liquid-phase tests, soil leachate extracts, which were stored in a cold room at 5° C, were brought to room temperature by being placed into a controlled environment incubator (New Brunswick G25 Controlled Environment Shaker) set at 25° C ± 3° C. Before starting each test, 1 ml ± 0.5 ml was poured into each well of the repli-dish already containing the four seeds. This was done using a 3 ml plastic syringe; the solution was just enough to cover each seed. Each dish took approximately three minutes to fill.

For the solid phase test, 1 g of each soil sample (sieved to the 2 mm fraction) was transferred to the corresponding repli-dish, using a plastic weigh boat. Seeds were added after the addition of each soil sample so that the seeds were not buried in the soil, making the

subsequent scoring much easier. Thereafter, 1 ml of deionised water was added to each well that contained the soil and seeds. Each dish took approximately ten minutes to fill.

The filled repli-dishes (**Figure 4.2**) were then placed into an incubator set at 25° C ± 3° C. Since the objective was to investigate germination, the seeds were left to germinate in low light conditions. The use of an incubator controlled for temperature, carbon dioxide, humidity, and light. It should be noted that load-shedding resulted in dips in incubation temperature when the building generator did not switch on, however, this was not observed for most of the duration of these tests. For each site, the FLP test was conducted first, followed by the SPLP test, and then the direct soil test (**Table 4.2**).



Figure 4.2: Filled repli-dishes containing seeds with (**a**) FLP solutions, (**b**) SPLP solutions, and (**c**) soil samples.

Table 4.3: Start dates of each phytotoxicity test. Samples from the high and medium pollution zones were tested at the same time and with the same test cohort.

	Phytotoxicity test	Start date of test
High and medium pollution zone	FLP	23 June 2023
	SPLP	17 July 2023
	SOIL	26 July 2023
Unpolluted zone	FLP	5 June 2024
	SPLP	24 June 2024
	SOIL	23 July 2024

Each test was carried out for 14 days from day 1 of the initial score. Seeds were scored daily for signs of imbibition and radicle emergence. Pictures of each repli-dish were also taken for scoring (**Figure 4.3**). A germination score would be the emergence of a radicle that is greater than 1 mm. The number of infected seeds was also noted – this was scored by any visible fungus present (**Figure 4.4**). The infected seeds were first noted down and then removed from the repli-dish. Only germination data are presented in this chapter. Incubator temperature, time and date of scoring were also noted down for each day. Lids were removed for approximately one hour each day for gaseous exchange during scoring and then replaced afterwards to minimise water loss. The reduction of water through absorption by the seed was addressed by removing the leftover water and refilling each well. This amount was standardized for each species, and refilling was noted by 1 mℓ incremental additions of the respective solution. Repli-dishes were reused for each test after acid washing with a 5% acetic acid solution and then soaked overnight in 3% hydrogen peroxide to remove any organic residue.

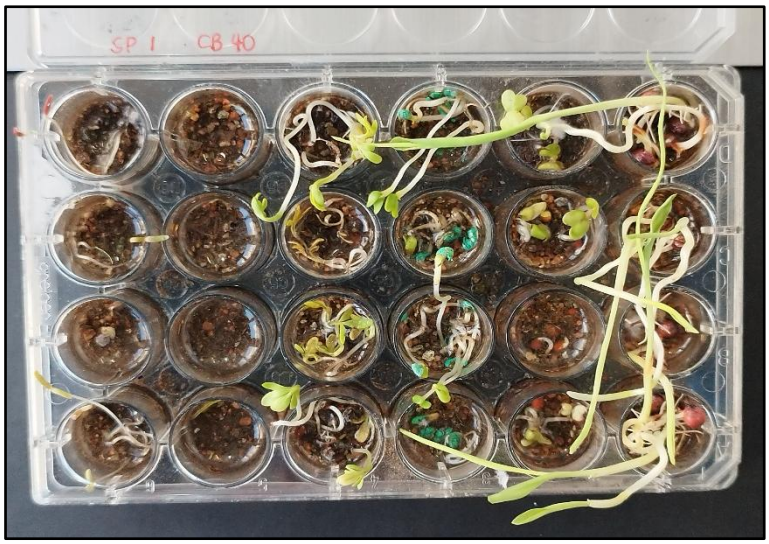


Figure 4.3: Example of a repli-dish containing seeds placed directly on the soil test sample.



Figure 4.4: Example of a seed infected by fungi (red circle).

After day 14, each seedling was harvested, preserved in 70% ethanol (**Figure 4.5a**) and stored in a cold room at 5° C. The preserved seedlings were then taken to the Moss Herbarium at the University of the Witwatersrand for scanning of root lengths. Images for the root length of each seedling were taken with an Epson Expression 1640 XL scanner and saved as JPEG files, making sure to include a ruler each time to set a scale specific to the number of pixels in each image (**Figure 4.5b).** The scanned images were then processed with Image J software (Schneider *et al*., 2012), using the measure tool to trace along the root of each seedling, and recorded in millimetres.

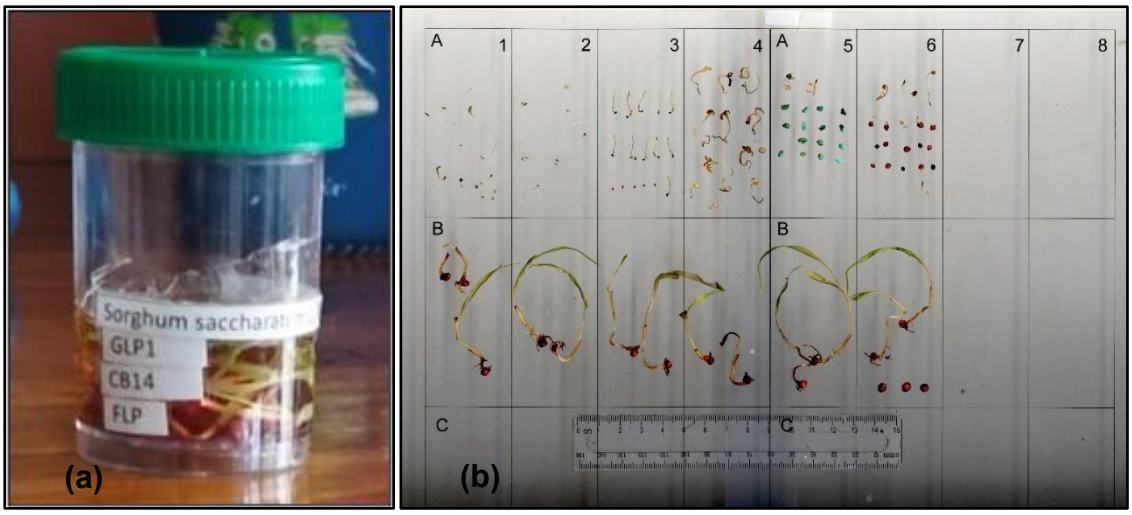


Figure 4.5: (a) Seedlings harvested and stored in 70% ethanol before storage in a cold room at 5° C. **(b)** Example of scanned seedlings to measure root length.

4.2.5. Data and statistical analysis

Cumulative germination was recorded as the total number of seeds germinated up to and including the date of observation. Percentage germination was then recorded as the cumulative germination per day relative to the initial number of seeds. This data was used to produce time-germination graphs for the 14 days. From this, peak germination values, which is the highest observed percentage of germination, were compared between pollution zones and between patches and interpatches. Differences between pollution zones were tested with a Kruskal-Wallis test, and differences between patches and interpatches were determined with Mann-Whitney U tests.

Inhibition of root elongation data was assessed under the effect of patch type and contamination level using the following formula (Hickey and McNelly, 1975):

$$\frac{Root\ length\ in\ treatment\ (High\ or\ medium\ pollution\ zone)}{Mean\ root\ length\ in\ control\ (Unpolluted\ zone)} \times 100$$

For the effect of patch type, the plainest patch category, in terms of lowest soil organic matter and vegetation occurrence, was used as the control for other patch categories along that pollution zone. The remaining patch and interpatch categories were the treatment samples. For the effect of contamination, the tests conducted on the unpolluted zone served as controls for the contaminated treatment tests from the high and medium pollution zones. In each case, the corresponding patch categories from the unpolluted zone were used as a control for similar kinds of patch categories from the high and medium pollution zones. Only the tree,

grass, and bare patch categories were similar in each pollution zone, the rest were left out of this analysis.

Bar graphs were used to illustrate the inhibitory or stimulatory effect of the treatment root with respect to the control root. Values greater than 100 indicated a stimulatory or hormetic effect and were transformed to positive values within a range of 0 – 100. Values less than 100 indicated an inhibitory effect and were transformed to negative values within a range of 0 – 100.

Pearson correlation coefficients were determined between peak germination values, root length and soil properties, in terms of pH, electrical conductivity and soil organic matter, to understand which properties may have most influenced the ecotoxic response for each species. All raw data were analysed and processed in Microsoft Excel. Statistical analysis was done in RStudio.

4.3 Results

4.3.1. Germination and inhibition of root elongation of *Amaranthus hybridus*

For the FLP solutions along the high pollution zone (**Figure 4.6a and b**), patch and interpatch germination exponentially increased until reaching peak germination of 40 – 60% around day 4. The medium polluted zone (**Figure 4.6 c and d**) experienced exponential germination until a peak of 43 – 61% on day 5. The unpolluted zone (**Figure 4.6 e and f**) experienced two exponential germination phases and plateaued when reaching 36 – 57% on day 5. The peak germination values were significantly higher along the patches of the high pollution zone ($p = 0.002$) and the medium pollution zone ($p = 0.016$) relative to the unpolluted zone. No differences were found concerning the effect of patch type on peak germination values within each pollution zone.

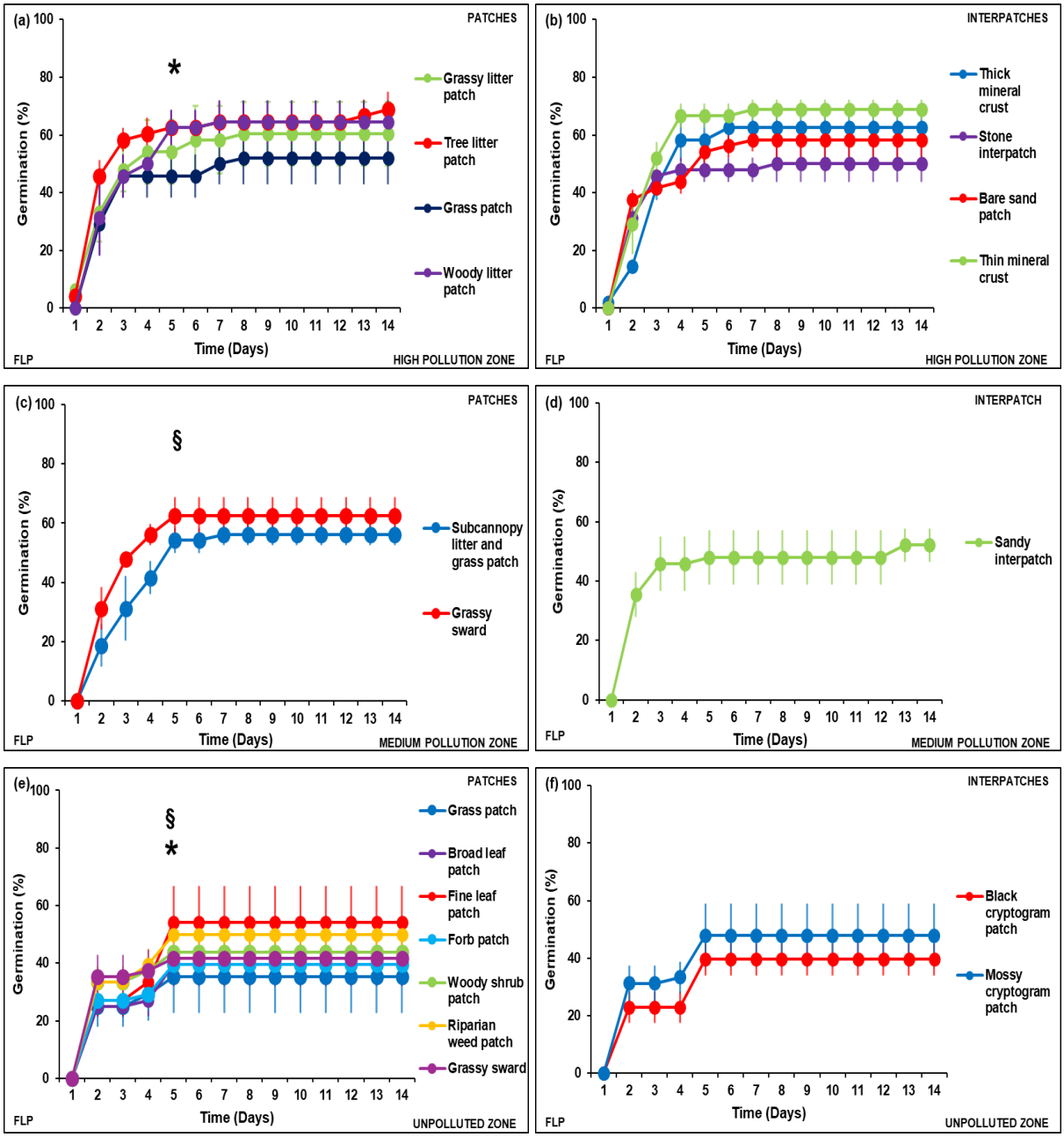


Figure 4.6: Time-germination curves for the mean (± standard error) germination of *Amaranthus hybridus* after exposure to **FLP** solutions from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values were derived from a triplicate response of each patch/interpatch. There were no significant differences in peak germination values between patches and interpatches. Graphs that share similar symbols indicate a significant difference in the peak germination between the patch/interpatch amongst the pollution zones. Please refer to table 4.2 for a description of each patch/interpatch type

When assessing the effect of patch type on root elongation of *A. hybridus* grown in FLP solutions (**Figure 4.7**) the tree litter patch (TLP) along the high pollution zone was 73% of the control, indicating a 27% inhibition of root elongation, in comparison to the thick mineral

crust (BMC[T]), which was inhibited by to 12 % of the control, indicating an 88% inhibition of root elongation. Overall, while all patch categories experienced an inhibition of root elongation, except the grass litter patch (GLP), the interpatches resulted in a greater inhibition of *A. hybridus* compared to the patches. The root length of patches along the medium pollution zone was stimulated in length when compared to the control plainest patch category. Along the unpolluted zone, the root length along all patch categories was inhibited in comparison to the plainest patch category, except the grassy sward, implying that the root length of *A. hybridus* along the plainest patch category was much longer compared to the other patch categories.

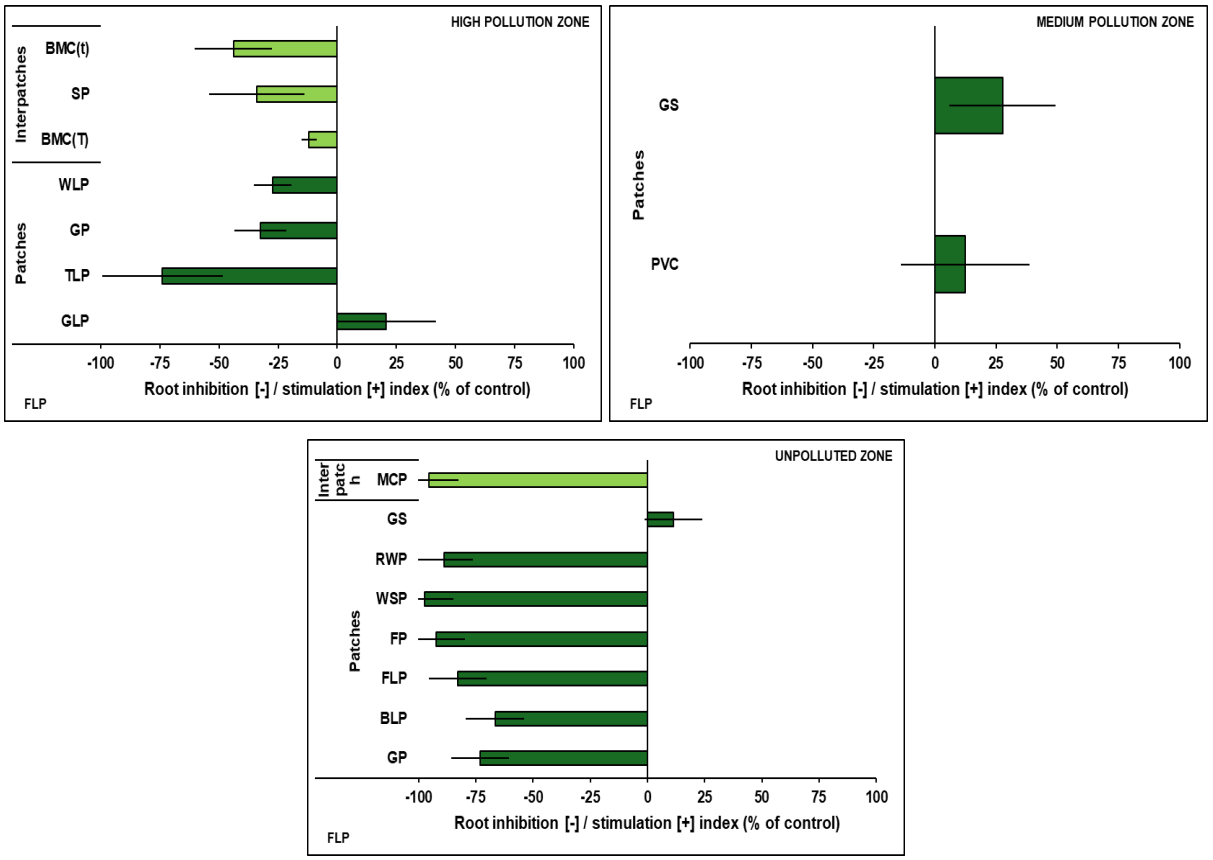


Figure 4.7: The effect of patch type on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in **FLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium-polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of the pollution level of FLP solutions (**Figure 4.8**), the bare and tree patches along the high pollution zone seemed to have longer root lengths when compared to the control bare and tree patches from the unpolluted zone. Only the root length along the grass patch (GP) of this zone was inhibited. For the medium pollution zone, the root length of both patches was stimulated to 52-55% of the length of the control, whereas the bare interpatch was 70% of the unpolluted control length. Conversely, for the medium pollution zone, the grass and tree patch were 52-55% of the control root length, indicating approximately 50% stimulation, while the root length along the bare interpatch was 95% of the control, indicating a 5% inhibition.

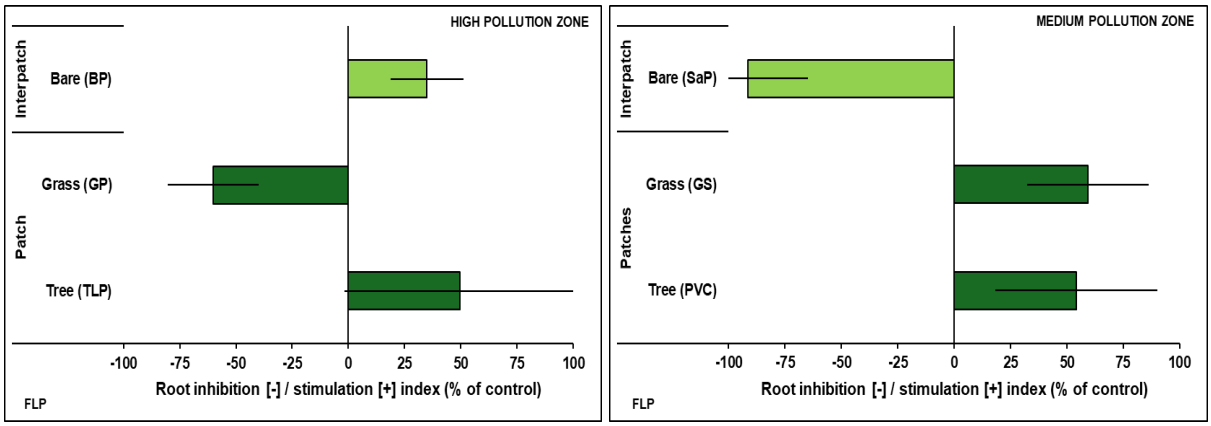


Figure 4.8: The effect of pollution level on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in polluted **FLP** solutions (treatment) relative to unpolluted (control) **FLP** solutions. Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone, Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone, Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sward) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP test (**Figures 4.9a and b**), the high pollution zone experienced an exponential-plateau-exponential growth until a peak at 41 – 631%. The medium pollution zone (**Figures 4.9c and d**) also had an exponential-plateau-exponential growth until a peak at 41 – 65% on day 8. The unpolluted zone followed a similar pattern with an exponential-plateau-exponential growth until a peak on day 5 at 36 – 58%. There was no significant difference in peak germination regarding the effect of the contaminated sites. However, for the effect of

patch type, the interpatch along the medium polluted zone had a significantly higher peak germination value compared to the patches ($p = 0.049$).

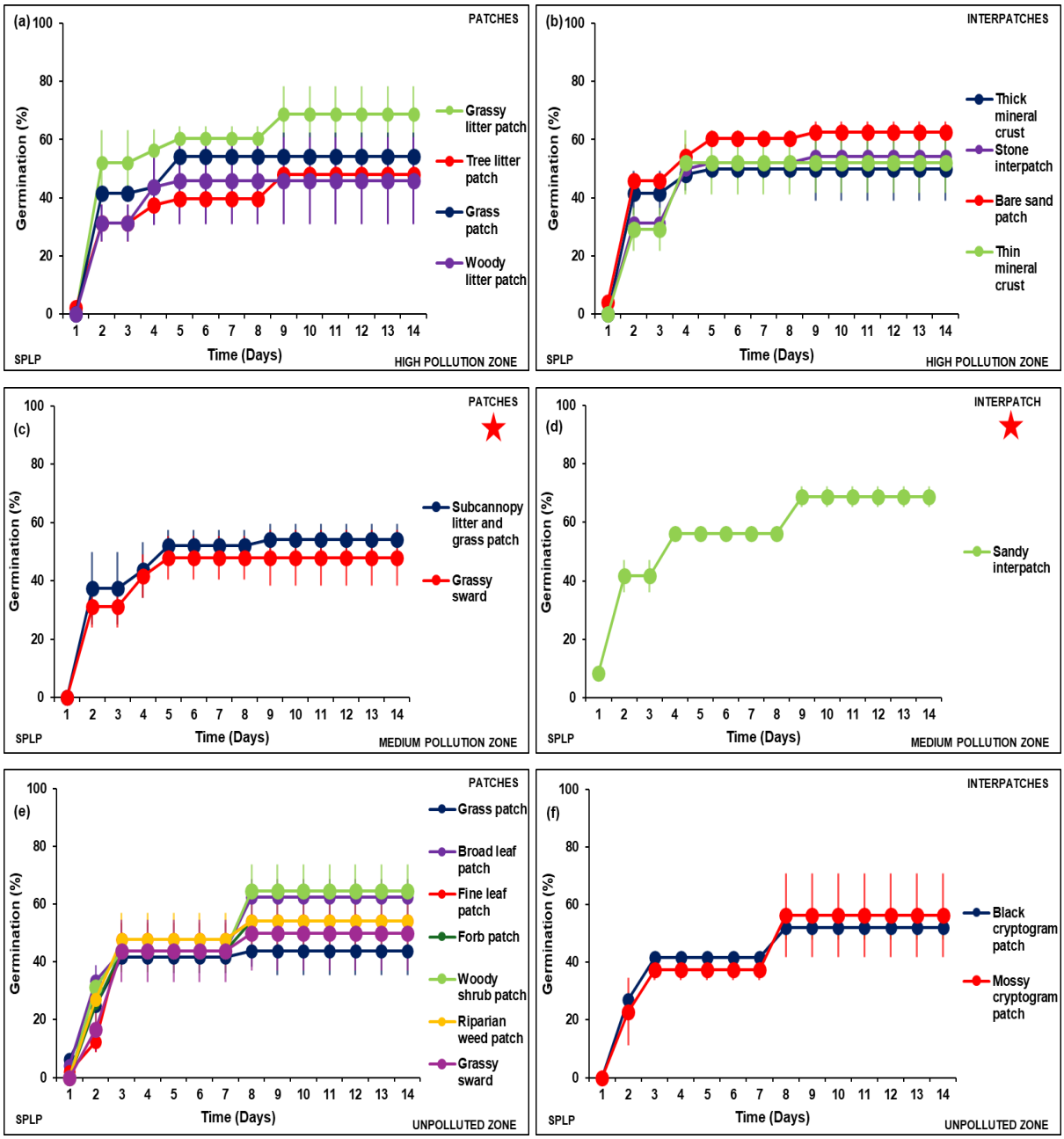


Figure 4.9: Time-germination curves for the mean (± standard error) germination of *Amaranthus hybridus* after exposure to **SPLP** solutions from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones for 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Each pollution zone was assessed according to patch and interpatch distributions. Significant differences between patch and interpatch peak germination values are indicated with a red star. There were no significant differences in peak germination values of the patches/interpatches amongst pollution zones.

For the SPLP (**Figure 4.10**) solutions, the root length from patches and interpatches was inhibited. The interpatches of the high pollution zone were 23-26% of the length of the control, indicating a 77-74% inhibition of root elongation. Whereas the patches were 20-80% of the length of the control. For the medium pollution zone, the subcanopy litter and grass patch (PVC) had a stimulation in root length, compared to the grassy sward (GS), which was inhibited. The patches along the unpolluted zone experienced a greater extent of stimulation in root length compared to the interpatch, implying that the *A. hybridus* roots from the patch SPLP solutions were longer than the control plainest patch category.

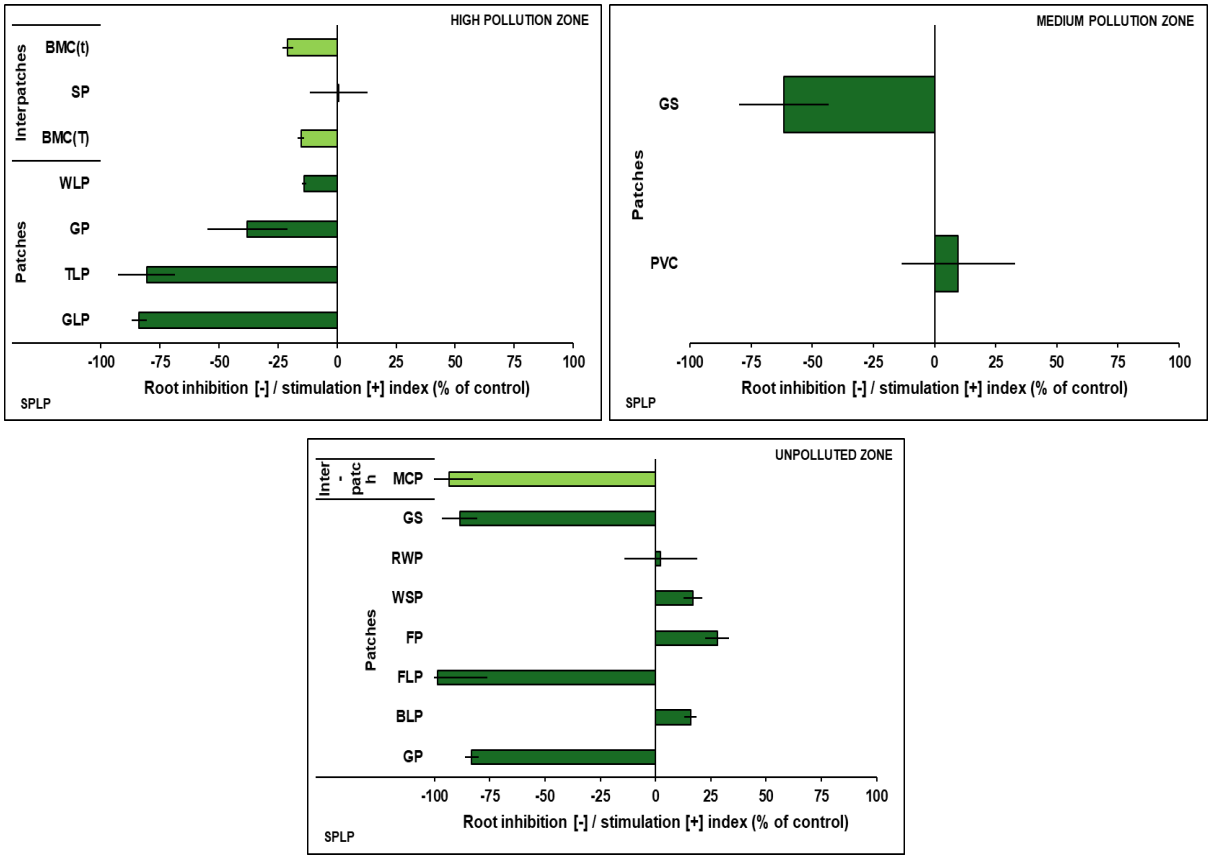


Figure 4.10: The effect of patch type on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in **SPLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on *A. hybridus* grown in SPLP solutions (**Figure 4.11**), the bare patch from the high pollution zone was the only patch category to have stimulation in root length, while the tree and grass patches were 55-97%, respectively, of the unpolluted

control root length. The root lengths from the medium pollution zone were in a range of 51-75% of the control, indicating an inhibition of 49-25%.

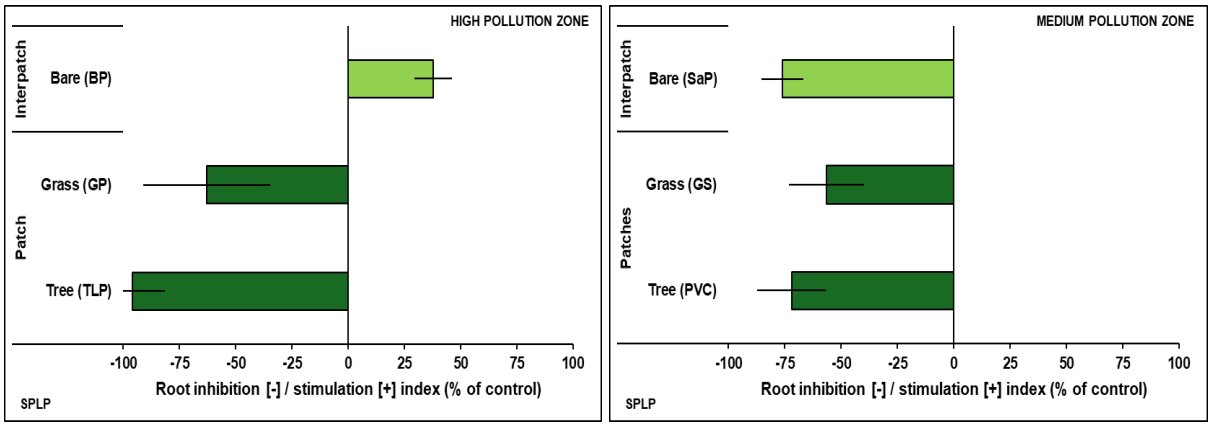


Figure 4.11: The effect of pollution level on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in polluted **SPLP** solutions (treatment) relative to unpolluted (control) **SPLP** solutions. Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sward) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the direct soil test (**Figure 4.12 a and b**), the high pollution zone had a peak germination of 36 – 61% on day 4. The medium pollution zone (**Figure 4.12 c and d**) had a peak germination of around 50% on day 6. Along the unpolluted zone (**Figure 4.12 e and f**), germination increased exponentially until reaching a peak in a range of 35 – 50% on day 3. No significant differences were found regarding the effect of contaminated site or patch type on peak germination values.

Figure 4.12 is found on the next page.

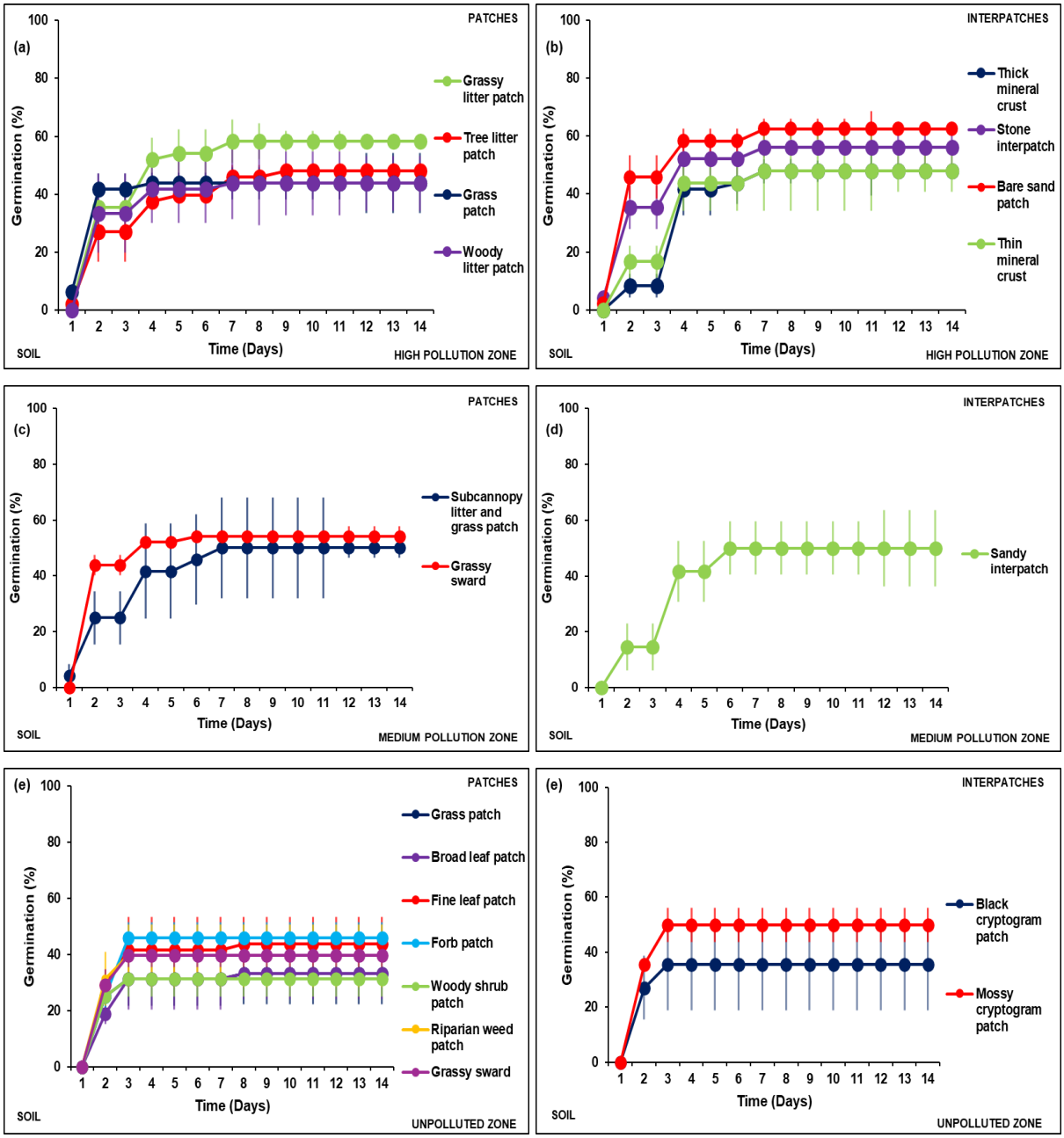


Figure 4.12: Time-germination curves for the mean (± standard error) germination of *Amaranthus hybridus* after exposure to **SOILS** of patches and interpatches from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values were derived from a triplicate response of each patch/interpatch. There were no significant differences in peak germination between patches and interpatches. There were no significant differences in peak germination between pollution zones.

For the effect of patch type on *A. hybridus* grown directly in soils (**Figure 4.13**), the high pollution zone experienced an overall inhibition of root length amongst the patch categories. Only the grassy litter patch (GLP), stone interpatch (SP), and thin mineral crust (BMC[T]) were stimulated relative to the control length of *A. hybridus*. For the medium pollution zone, the sub-canopy grass and litter patch (PVC) did not affect root length, in relation to the

control plainest patch category. The unpolluted zone had an overall stimulation in root length relative to the control, with the mossy cryptogram interpatch having the smallest stimulative response of 30% of the control. Only the broad-leaf patch (BLP), forb patch (FP), and woody shrub patch (WSP) were 70-80% of the control.

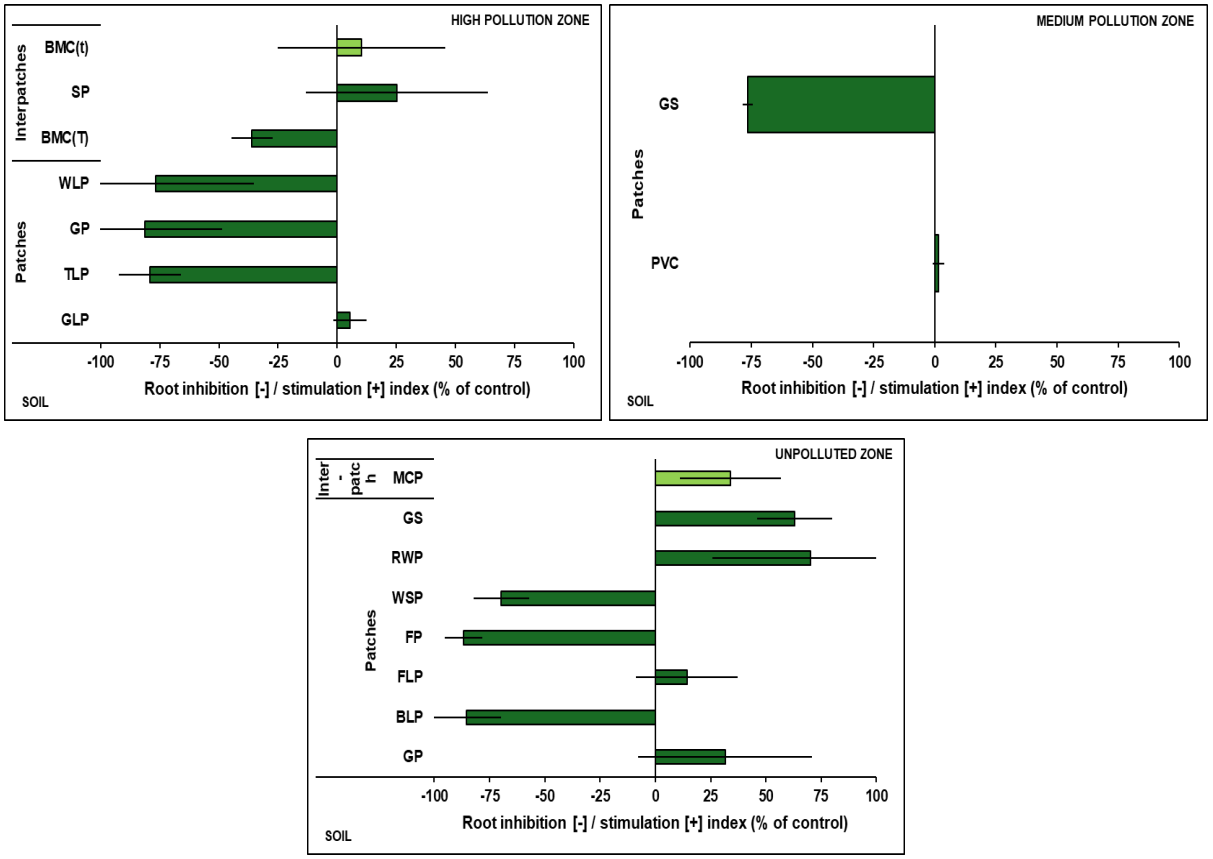


Figure 4.13: The effect of patch type on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in **SOILS** of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on root elongation of *A. hybridus* grown directly in the soil (**Figure 4.14**), the tree, grass and bare patch categories were inhibited compared to the unpolluted control. The root lengths of the medium pollution zone's bare and tree patch categories were stimulated compared to the unpolluted controls, while the grass patch was inhibited.

Figure 4.14 is found on the next page.

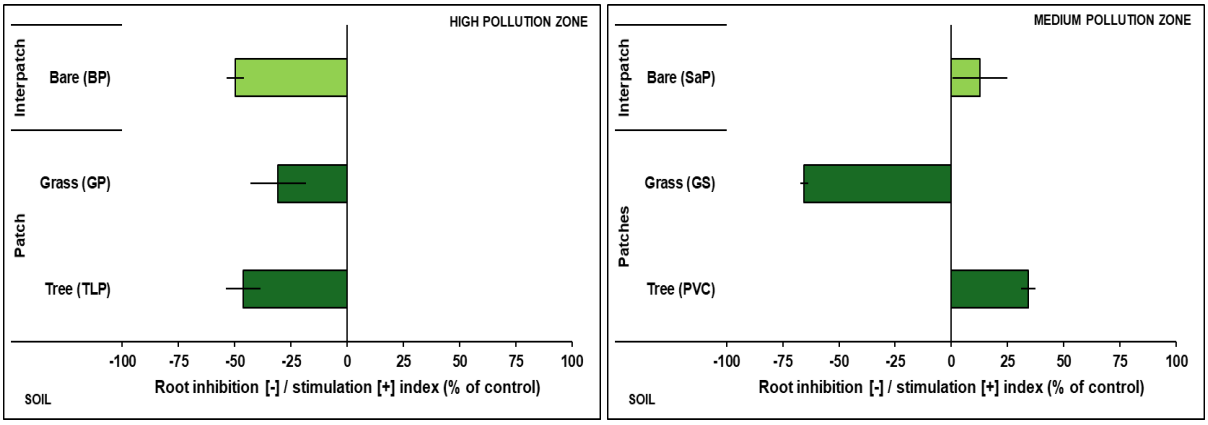


Figure 4.14: The effect of pollution level on the inhibition/stimulation of root length of *Amaranthus hybridus* grown in polluted **SOILS** (treatment) relative to unpolluted (control) **SOILS**. Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.2. Germination and root elongation of *Dactyloctenium australe*

For the FLP tests (**Figure 4.15**) germination started on day 3 for the patches and interpatches along each pollution zone. However, for the high pollution zone (**Figure 4.15 a and b**) the thick mineral crust interpatch was the only interpatch that germinated after day 3. Thereafter, germination increased to a peak range of 15-45% on day 7. The medium pollution zone (**Figure 4.15 c and d**) reached a peak range of 22-26% for patches and 42% for interpatches on days 7-10. The high and medium pollution zones did not reach 50% germination for the duration of the test. Germination along the unpolluted zone exponentially increased to a range of 36 – 61% from days 3 – 8. Peak germination along patches of the unpolluted site was significantly higher compared to the high pollution zone ($p = 0.006$) and the medium pollution zone ($p < 0.001$), and similarly, interpatch peak germination along the unpolluted zone was significantly higher compared to patches along the high pollution zone ($p < 0.001$). For the effect of patch type, patch peak germination along the high pollution zone was significantly higher compared to the interpatches ($p = 0.024$) (A full record of statistical test results are found in **Appendix C**, **Table C1** and **C2**).

Figure 4.15 is found on the next page.

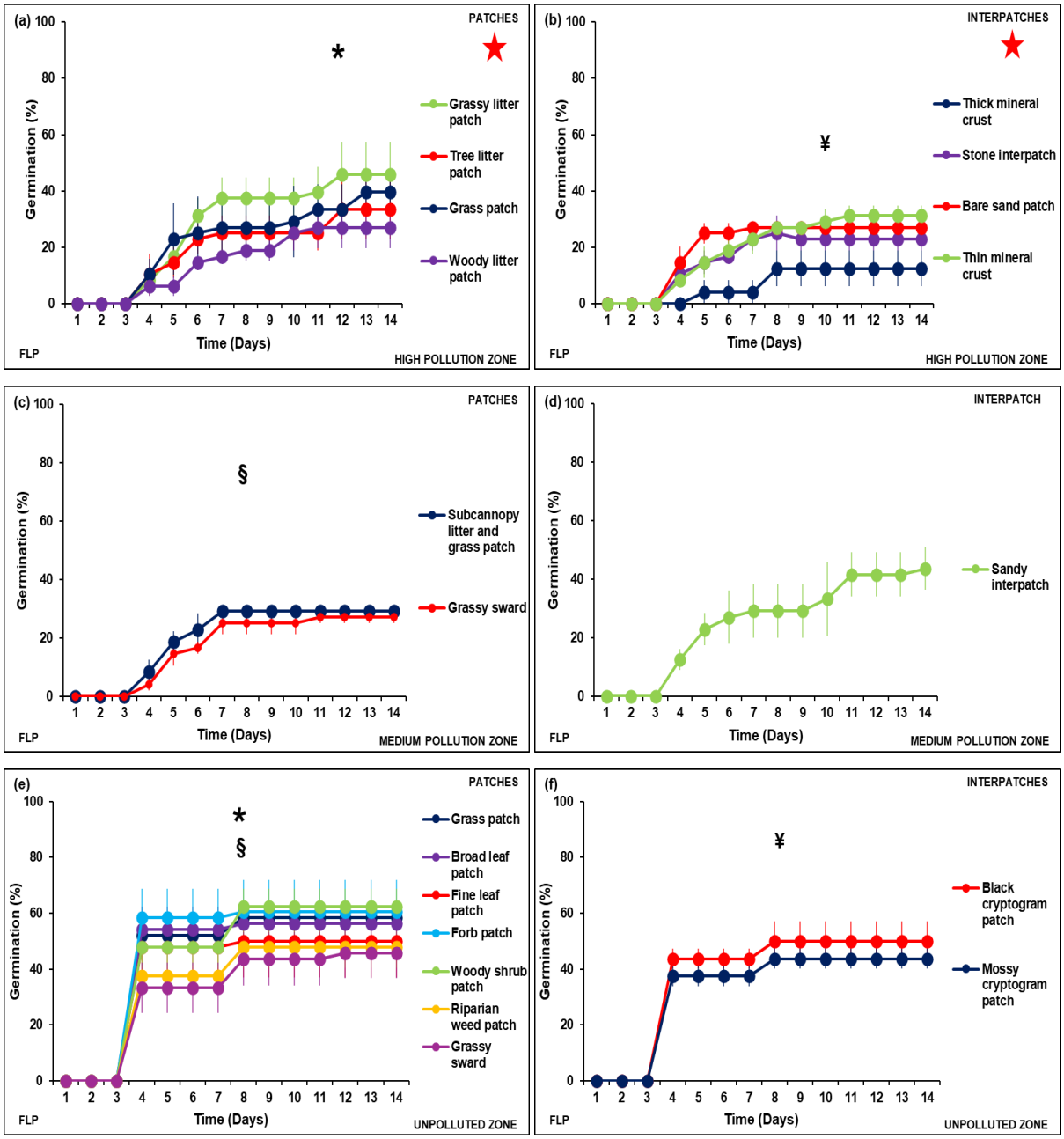


Figure 4.15: Time-germination curves for the mean (± standard error) germination of *Dactyloctenium australe* after exposure to **FLP** solutions of patches and interpatches from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. A red star indicates a significant difference in peak germination between the patches and interpatches of that pollution zone.

For the effect of patch type on the root length of *D. australe* grown in FLP solutions (**Figure 4.16**), the patches and interpatches of the high pollution zone were all inhibited, with an exception for the stone interpatch (SP) which had no effect, albeit a highly variable result. The thick mineral crust interpatch (BMC[T]) was 20% of the control, indicating an 80% inhibition, compared to the grass litter patch (GLP), which was 55% of the control, indicating

a 45% inhibition. Along the medium pollution zone, the sub-canopy litter and grass patch did not affect root length relative to the control, while the grassy sward was 49% of the control – indicating a 51% stimulation. All the patch and interpatch categories of the unpolluted zone had an inhibitory effect on the root length of *D. australe* in comparison to the plainest patch category (control).

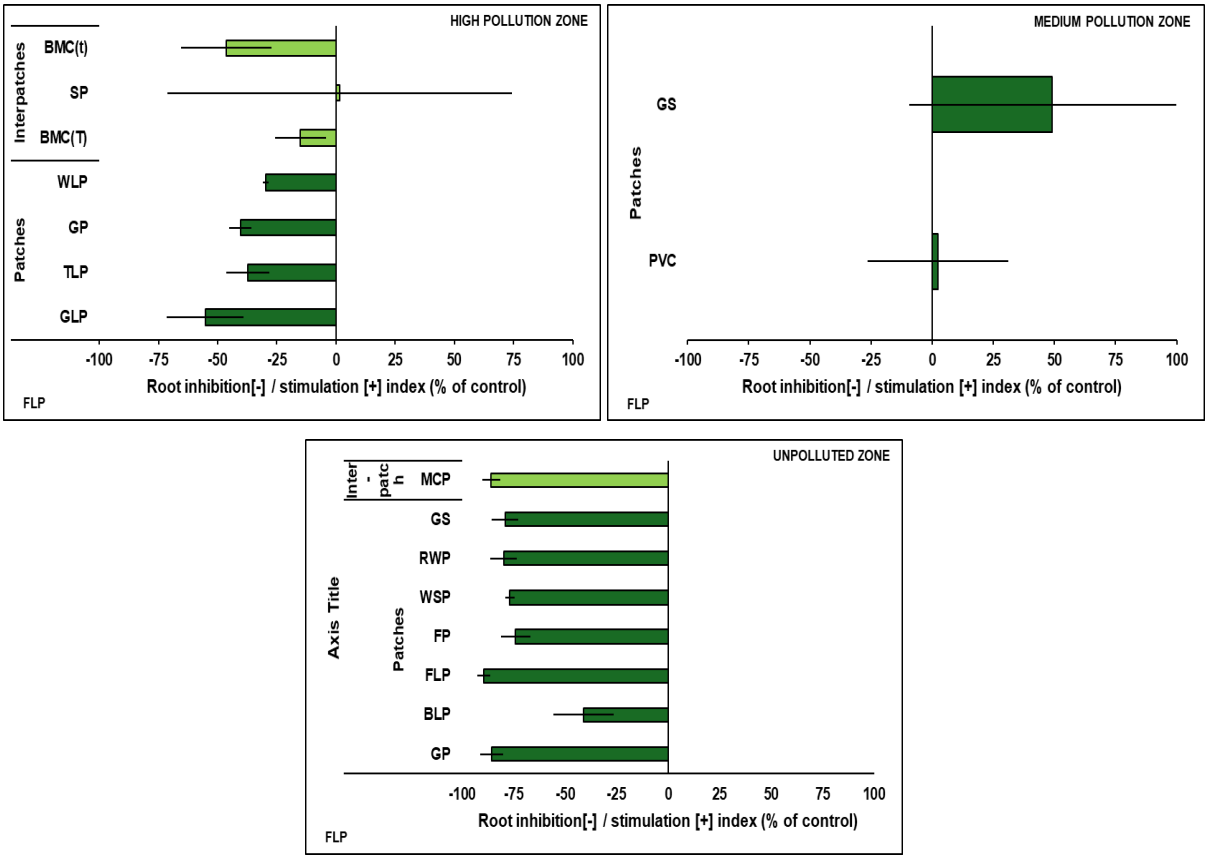


Figure 4.16: The effect of patch type on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in **FLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *D. australe* grown in FLP solutions (**Figure 4.17**), the patch categories of the high and medium pollution zones had an inhibitory effect on the root length in comparison to the unpolluted control patch categories.

Figure 4.17 is found on the next page.

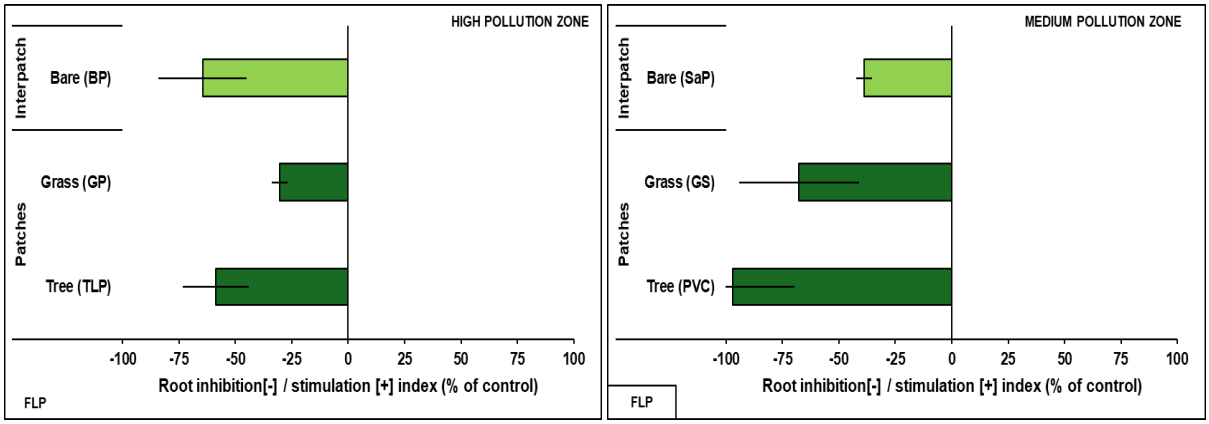


Figure 4.17: The effect of pollution level on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in polluted **FLP** solutions (treatment) relative to unpolluted **FLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP tests, germination of *D. australe* started on day 3 for each pollution zone (**Figure 4.18**). The high pollution zone experienced an exponential increase in germination from day 3 to day 9 until approaching a peak of 16 – 41% (**Figure 4.18 a and b**). Germination along the medium pollution zone increased until a range of 25 – 39% after day 8 (**Figure 4.18 c and d**). Germination did not exceed 50% along the high and medium pollution zones. The unpolluted zone reached a peak range much quicker in comparison to the high and medium polluted zones– after day 3 germination remained constant in a range of 41 – 58% (**Figure 4.18 e and f**). Peak germination was significantly higher along the patches of the unpolluted zone compared to the high pollution zone ($p < 0.001$) and the medium pollution zone ($p = 0.008$), as well as the interpatches of the unpolluted zone compared to the high pollution zone ($p = 0.006$). No significant difference was found regarding the effect of patch type within each pollution zone among the SPLP tests.

Figure 4.18 is found on the next page.

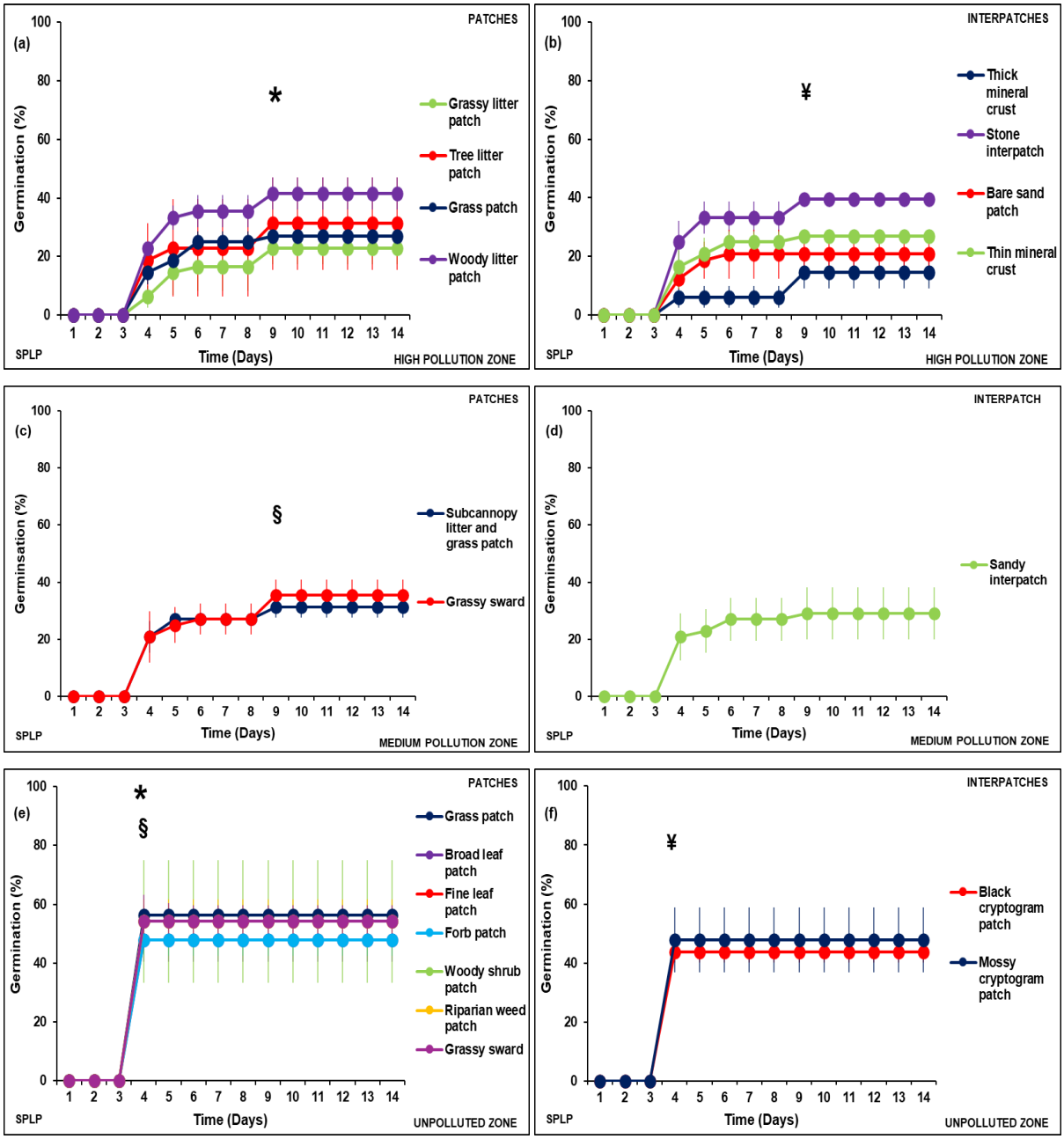


Figure 4.18: Time-germination curves for the mean (± standard error) germination of *Dactyloctenium australe* after exposure to **SPLP** solutions of patches and interpatches from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values were derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. There were no significant differences in peak germination between patches and interpatches of each pollution zone.

For the effect of patch type on the root length of *D. australe* grown in SPLP solutions (**Figure 4.19**), patches and interpatches of the high pollution zone and patches of the medium pollution zone had an inhibitory effect on root length in comparison to the plainest patch category along the respective zone. For the unpolluted zone, all patch categories, except the

woody shrub patch (WSP) and grass patch (GP), had an inhibitory effect on the root length of *D. australe*.

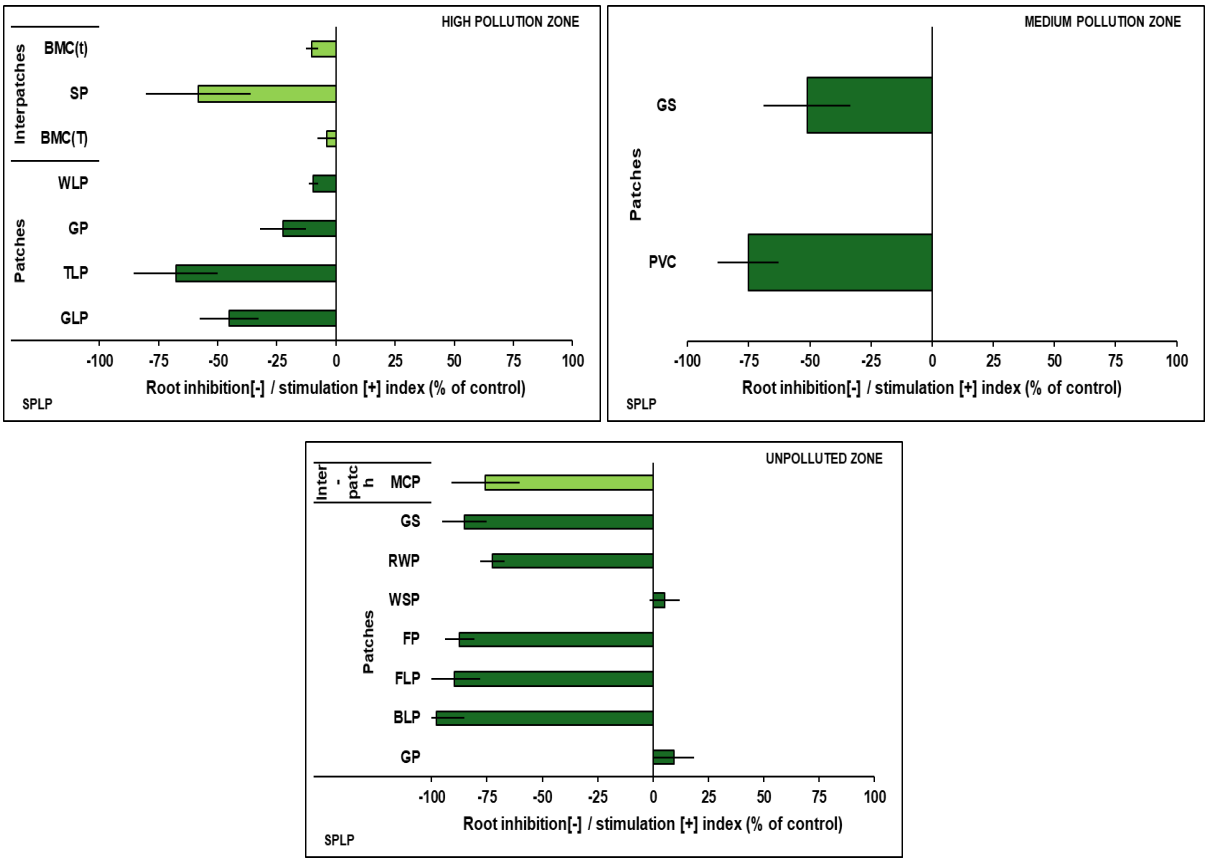


Figure 4.19: The effect of patch type on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in **SPLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *D. australe* grown in SPLP solutions (**Figure 4.20**), the bare interpatch of the high pollution zone had a stimulatory effect on root length and was 48% of the unpolluted control bare interpatch, indicating a 52% stimulation. The grass and tree patch categories had an inhibitory effect on root length, with 26% and 97% of the control patch categories. All patch categories of the medium pollution zone had an inhibitory effect on root length relative to the control patch categories.

Figure 4.20 is found on the next page.

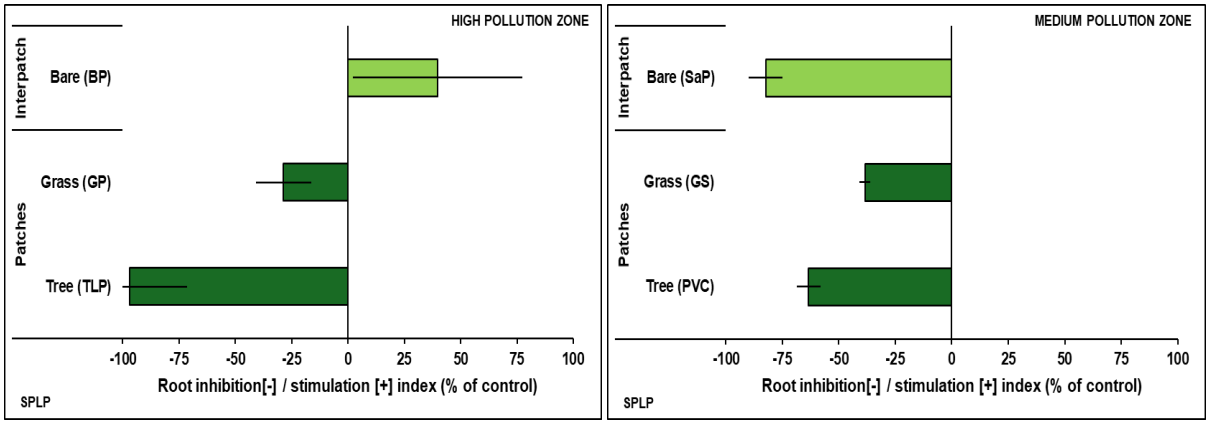


Figure 4.20: The effect of pollution level on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in polluted **SPLP** solutions (treatment) relative to unpolluted **SPLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the soil tests (**Figure 4.21**), germination appeared to stagger until day 5 for the high and medium pollution zones, with a slow increase until an exponential increase on day 10 – resulting in a germination range of 22 – 48% for the high pollution zone and 36 – 61% for the medium pollution zone. Whereas an exponential germination phase started on day 3 for the unpolluted zone and reached a plateau of 22 – 50%. No significant differences were found between the peak germination along either pollution zone or between patches and interpatches along each pollution zone.

Figure 4.21 is found on the next page.

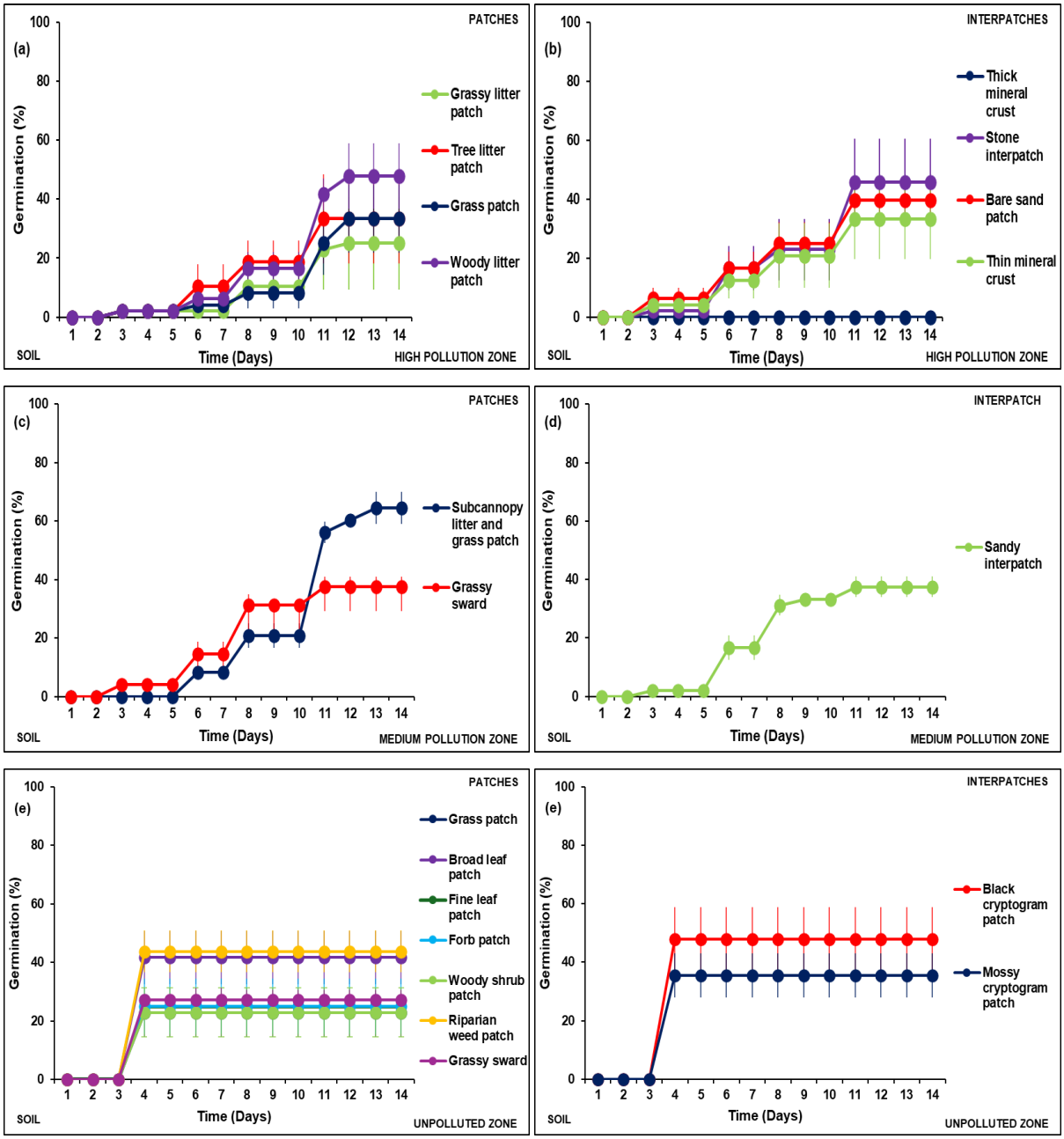


Figure 4.21: Time-germination curves for the mean (± standard error) germination of *Dactyloctenium australe* after exposure to **SOILS** from the **high pollution** (**a** and **b**), **medium pollution** (**c** and **d**) and **unpolluted** (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. There were no significant differences in peak germination between patches and interpatches. There were no significant differences in peak germination between pollution zones.

For the effect of patch type on the root length of *D. australe* grown directly in soil (**Figure 4.22**), all the patches and interpatches of the high pollution zone, and patches of the medium pollution zone had an inhibitory effect on root length relative to the plainest patch category

for each zone. In contrast, the interpatch and patch categories of the unpolluted zone had a stimulatory effect on root length relative to the plainest patch category of that zone.

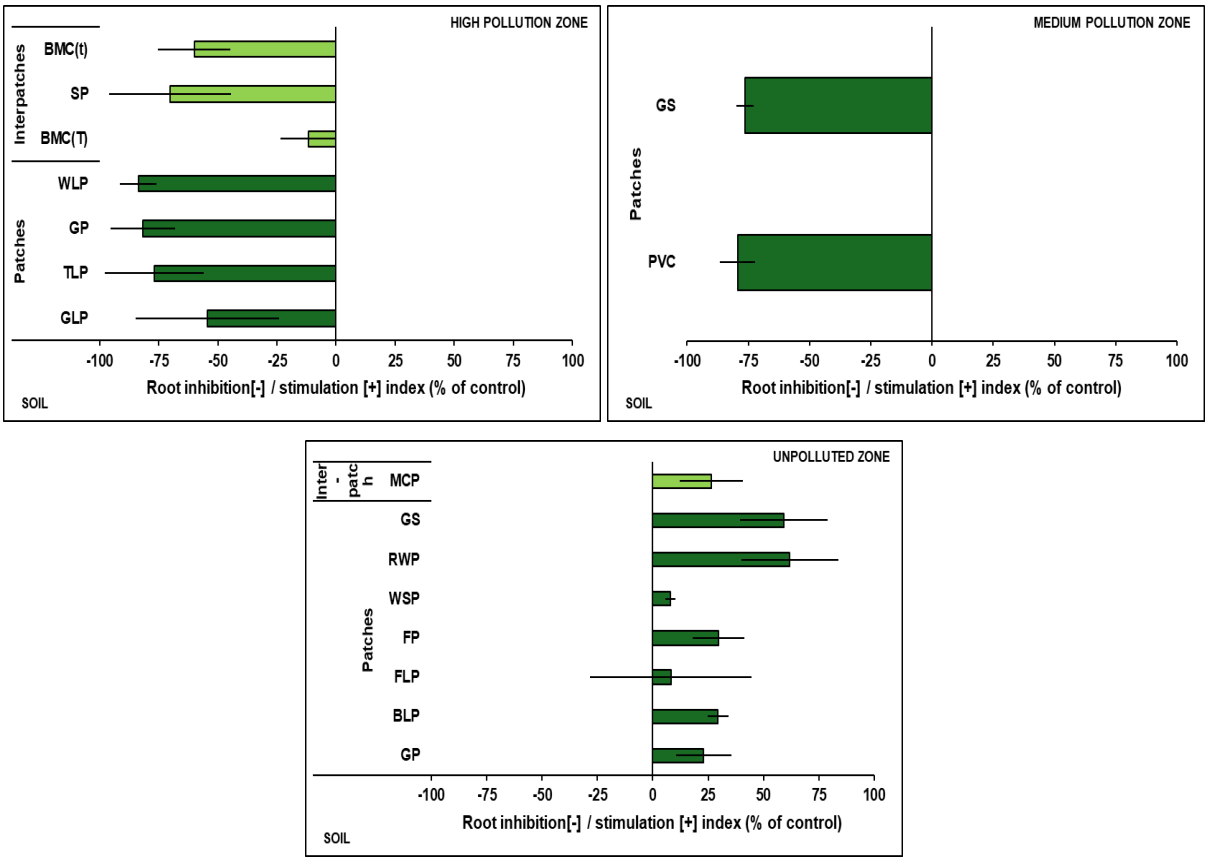


Figure 4.22: The effect of patch type on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in **SOILS** of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *D. australe* grown directly in soils (**Figure 4.23**), all patch categories of the high pollution zone had an inhibitory effect on root length relative to the unpolluted control patch categories. For the medium pollution zone, the tree and grass patch categories had an inhibitory effect on root length, with 76% of the control length, indicating a 24% inhibition. The bare patch category had a stimulatory effect of 24% of the control, indicating a 76% stimulation.

Figure 4.23 is found on the next page.

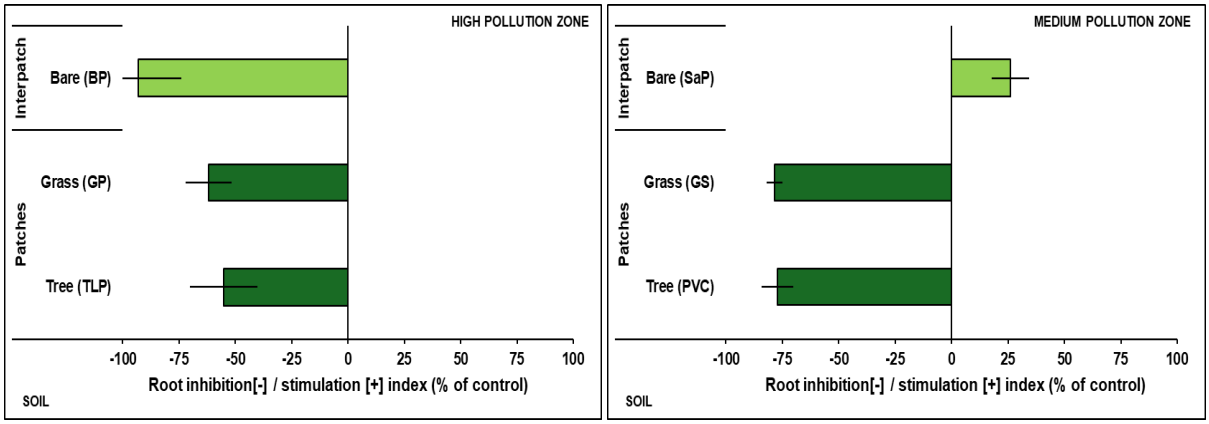


Figure 4.23: The effect of pollution level on the inhibition/stimulation of root length of *Dactyloctenium australe* grown in polluted **SOILS** (treatment) relative to unpolluted **SOILS** (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:

For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.

For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.3. Germination and root elongation of *Lepidium sativum*

For the FLP tests, *L. sativum* seeds positively responded to the high and medium pollution zones – with day 1 germination scores in a range of 35 – 89% for the high pollution zone (**Figure 4.24 a and b**), and 41 – 61% for the medium pollution zone (**Figure 4.24 c and d**), compared to the unpolluted zone (**Figure 4.24 e and f**) which did not exceed 10% germination during day 1, and peak germination was only reached after day 7. Peak germination was reached after day 4 for the high and medium pollution zones – this occurred in a range greater than 75%. However, germination along the unpolluted zone did not exceed 22%. Patches along the high and medium pollution zones were found to have significantly higher peak germination values compared to the unpolluted zone (site 1: $p < 0.001$; site 2: $p < 0.001$), and similarly for the interpatches along the unpolluted zone compared to the high pollution zone ($p = 0.005$) and the medium pollution zone ($p = 0.001$). No significant differences were found regarding the effect of patch type along each pollution zone.

Figure 4.24 is found on the next page.

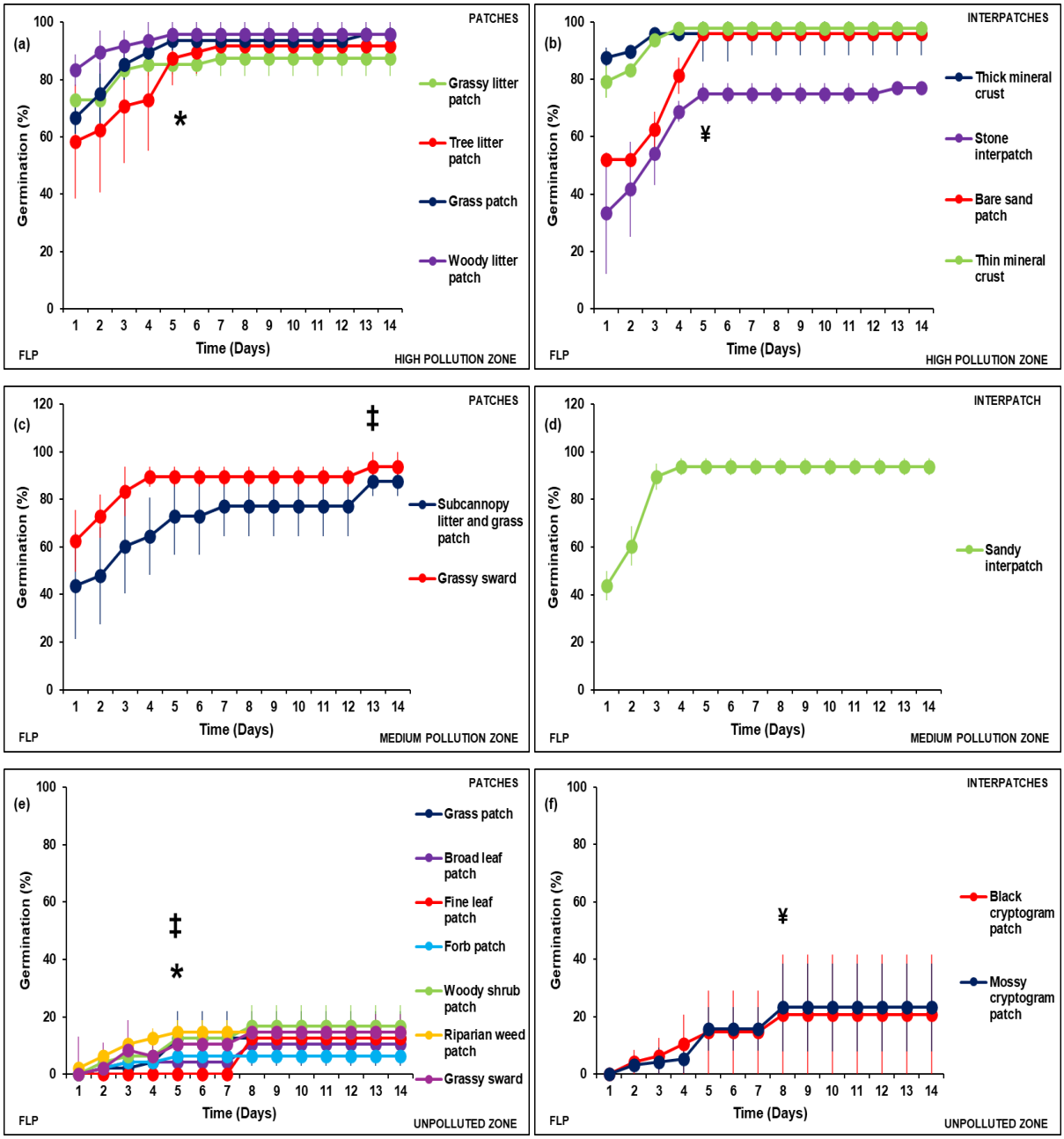


Figure 4.24: Time-germination curves for the mean (± standard error) germination of *Lepidium sativum* after exposure to **FLP** solutions of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *L. sativum* grown in FLP solutions (**Figure 4.25**), both patch and interpatch categories of the high pollution zone were inhibited, except for the stone interpatch (SP) and tree litter patch (TLP), which were stimulated to 10% and 15% of the control, respectively. All patch categories of the medium pollution zone were stimulated relative to the control. For the unpolluted zone, the grassy sward (GS) and riparian

weed patch (RWP) were stimulated to 17% and 78% of the control, respectively. While the other interpatch and patch categories were inhibited.

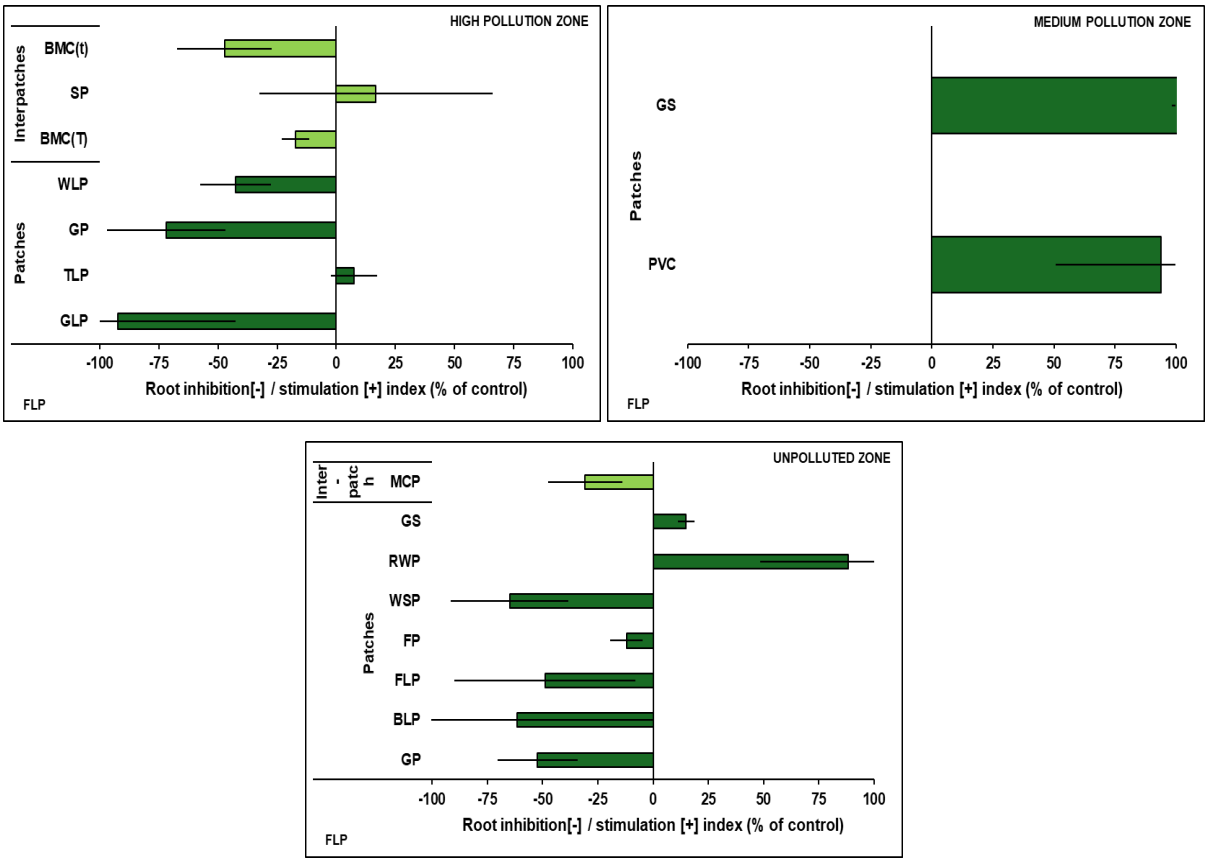


Figure 4.25: The effect of patch type on the inhibition/stimulation of root length of *Lepidium sativum* grown in **FLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *L. sativum* grown in FLP solutions (**Figure 4.26**), patch categories along the high and medium pollution zones (except the bare patch category of the medium zone had no effect) were stimulated to a vast extent in relation to the control patch categories – indicating that the roots grew better in polluted FLP solutions.

Figure 4.26 is found on the next page.

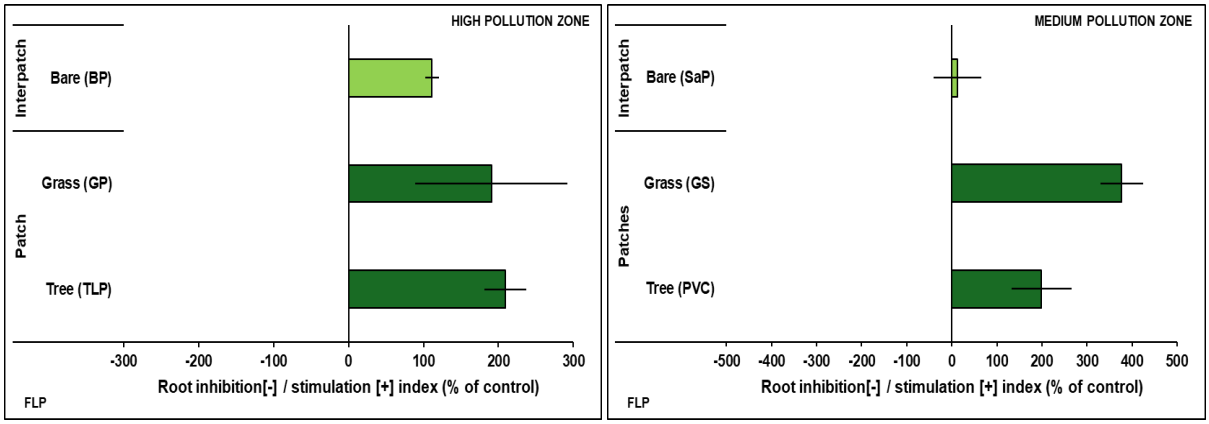


Figure 4.26: The effect of pollution level on the inhibition/stimulation of root length of *Lepidium sativum* grown in polluted **FLP** solutions (treatment) relative to unpolluted **FLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP tests (**Figure 4.27**), *L. sativum* from the high and medium pollution zones started to germinate on day 1, whereas germination only commenced on day 2 for the unpolluted zone. However, the unpolluted zone experienced a steep exponential increase in germination, reaching a peak phase on day 3 within a wide range of 25 – 90%. In comparison, germination from the high pollution zone only peaked after day 8, in a range of 20 – 75%. The medium pollution zone peaked after day 6, within a range of 17 – 30%. Patches along the unpolluted zone were found to have a significantly higher germination peak compared to patches along the medium pollution zone ($p = 0.003$). Similarly, interpatches along the unpolluted zone had higher peak germination values compared to the high pollution zone ($p = 0.004$) and medium pollution zone ($p = 0.003$). No significant differences were found regarding the effect of patch type along each zone.

Figure 4.27 is found on the next page.

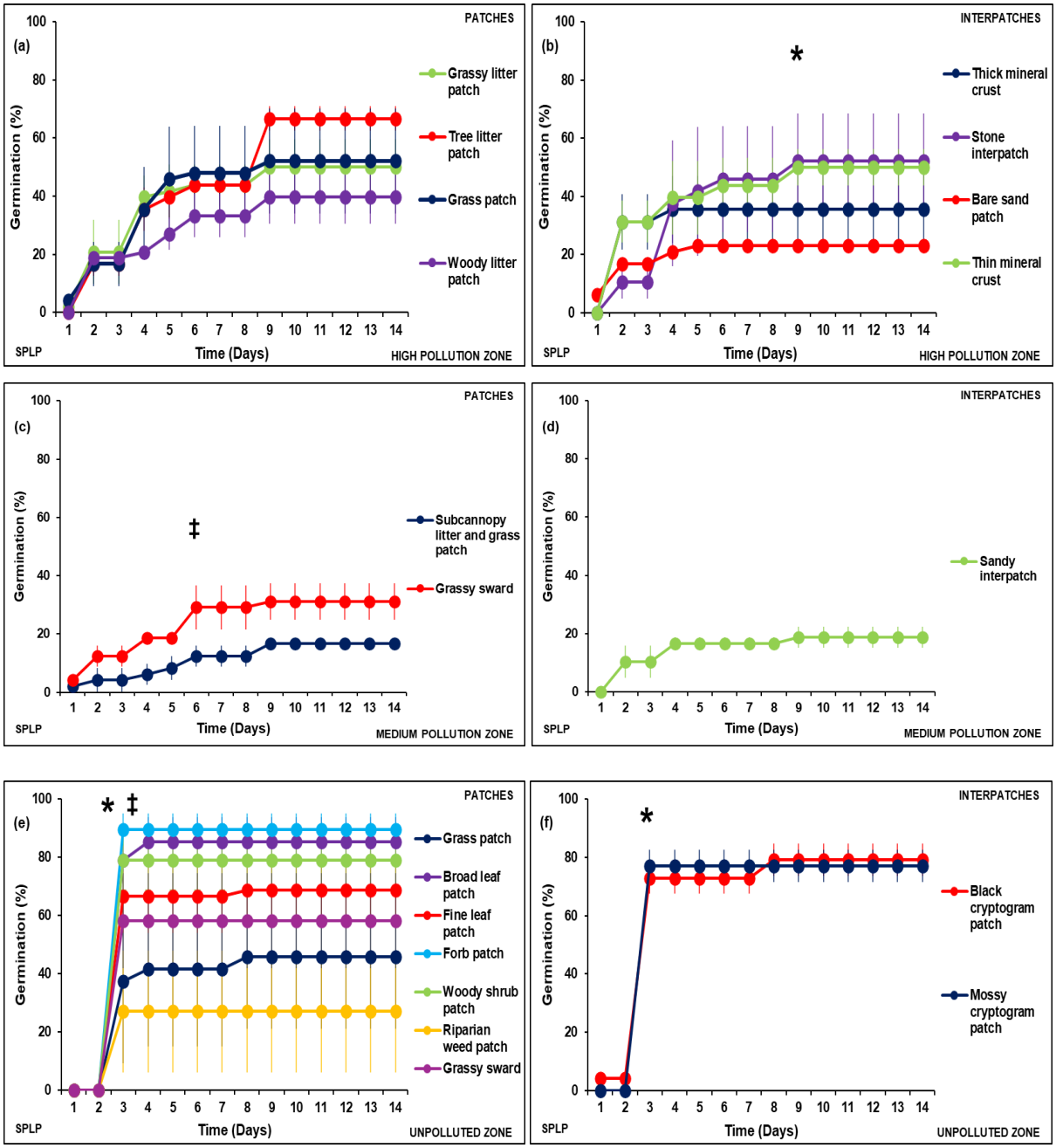


Figure 4.27: Time-germination curves for the mean (± standard error) germination of *Lepidium sativum* after exposure to **SPLP** solutions of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *L. sativum* grown in SPLP solutions (**Figure 4.28**), all patches and interpatches, except the thick mineral crust interpatch (BMC[T]), were stimulated in relation to the root length of the plainest patch. There was no effect on the thin mineral crust interpatch. For the medium pollution zone, the root length along all patches was

inhibited relative to the control. Similarly, for the unpolluted zone, the patches (except the riparian weed patch [RWP] grassy sward [GS]) and interpatch were stimulated in relation to the plainest patch category.

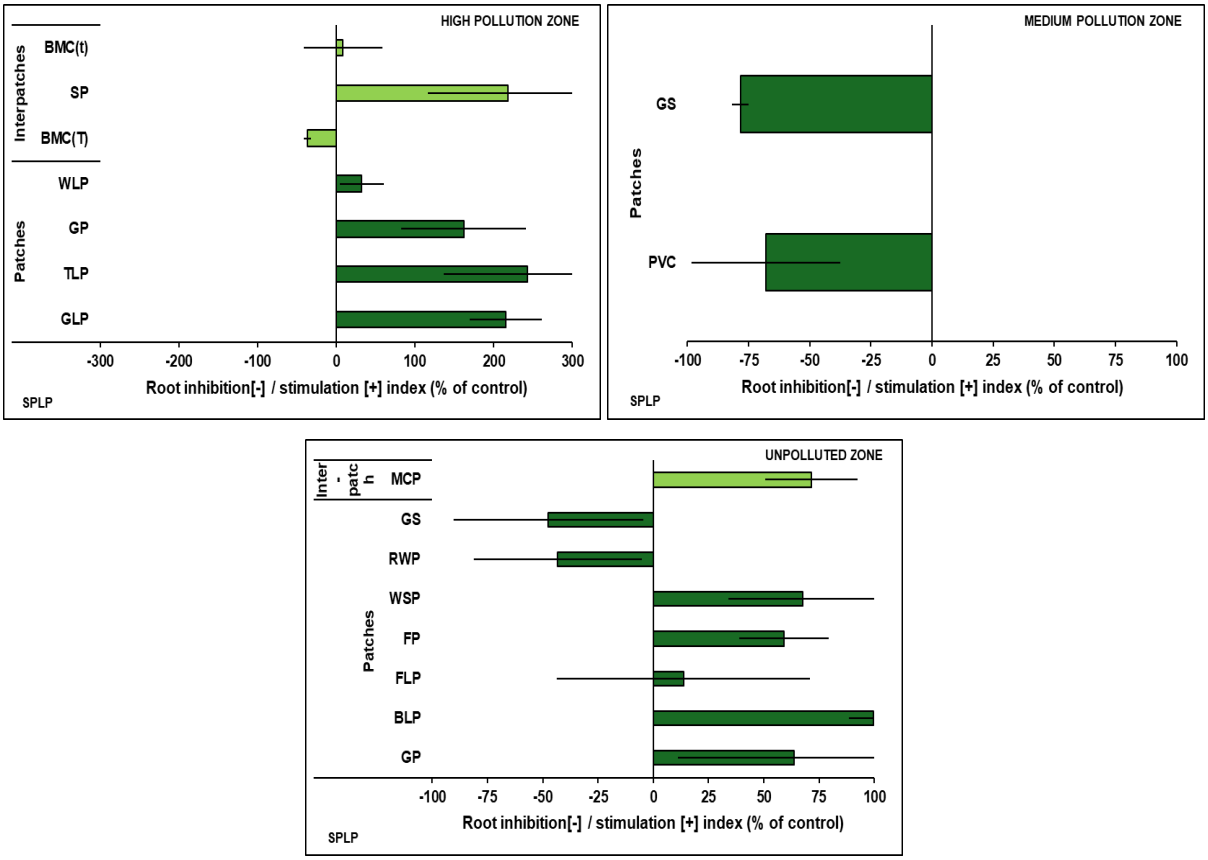


Figure 4.28: The effect of patch type on the inhibition/stimulation of root length of *Lepidium sativum* grown in **SPLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *L. sativum* grown in FLP solutions (**Figure 4.29**), the bare patch category along the high pollution zone was inhibited in relation to the plainest patch category, while the patches were stimulated. All patch and interpatch categories on the medium pollution zone had an inhibitory effect on root length relative to the control.

Figure 4.29 is found on the next page.

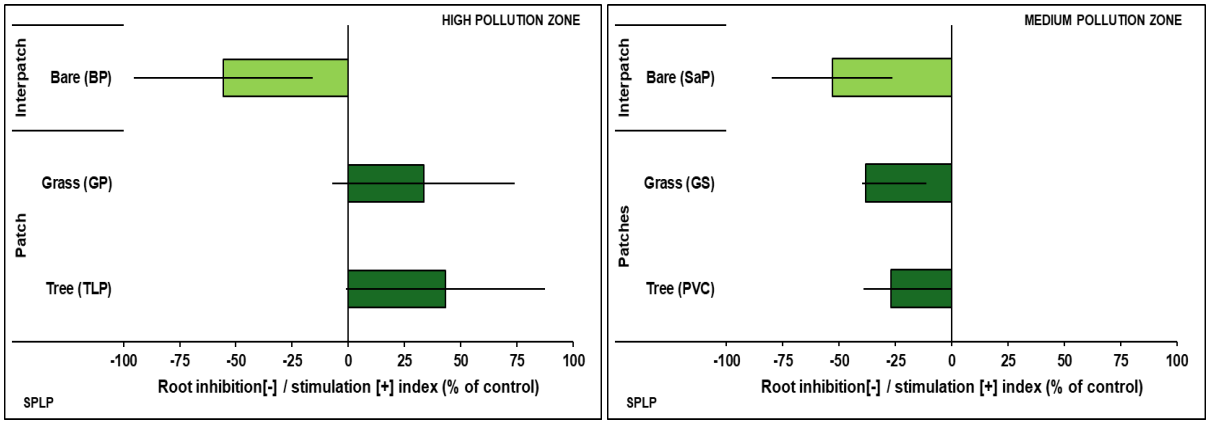


Figure 4.29: The effect of pollution level on the inhibition/stimulation of root length of *Lepidium sativum* grown in polluted **SPLP** solutions (treatment) relative to unpolluted **SPLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:

For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.

For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the direct soil tests, (**Figure 4.30**), germination began on day 1 for the high and medium pollution zones. In contrast, germination for the unpolluted zone started on day 1 and went into day 2. Along the high pollution zone, germination increased until day 8 and reached a peak within a range of 26 – 85%. The thick mineral crust (BMC[T]) had a 72% germination score on day 1. Site 2 also reached a peak state after day 8, within a range of 75 – 81%. The unpolluted zone encountered two plateau states: the first on day 3 within a range of 22 – 76%, and the second on day 8 within a range of 41 – 82%. Regarding the effect of the pollution level on peak germination, patches along the medium pollution zone were found to have greater germination compared to the high pollution zone ($p = 0.003$). Regarding the effect of patch type on peak germination, patches along the unpolluted zone were found to germinate significantly more than interpatches ($p = 0.041$).

Figure 4.30 is found on the next page.

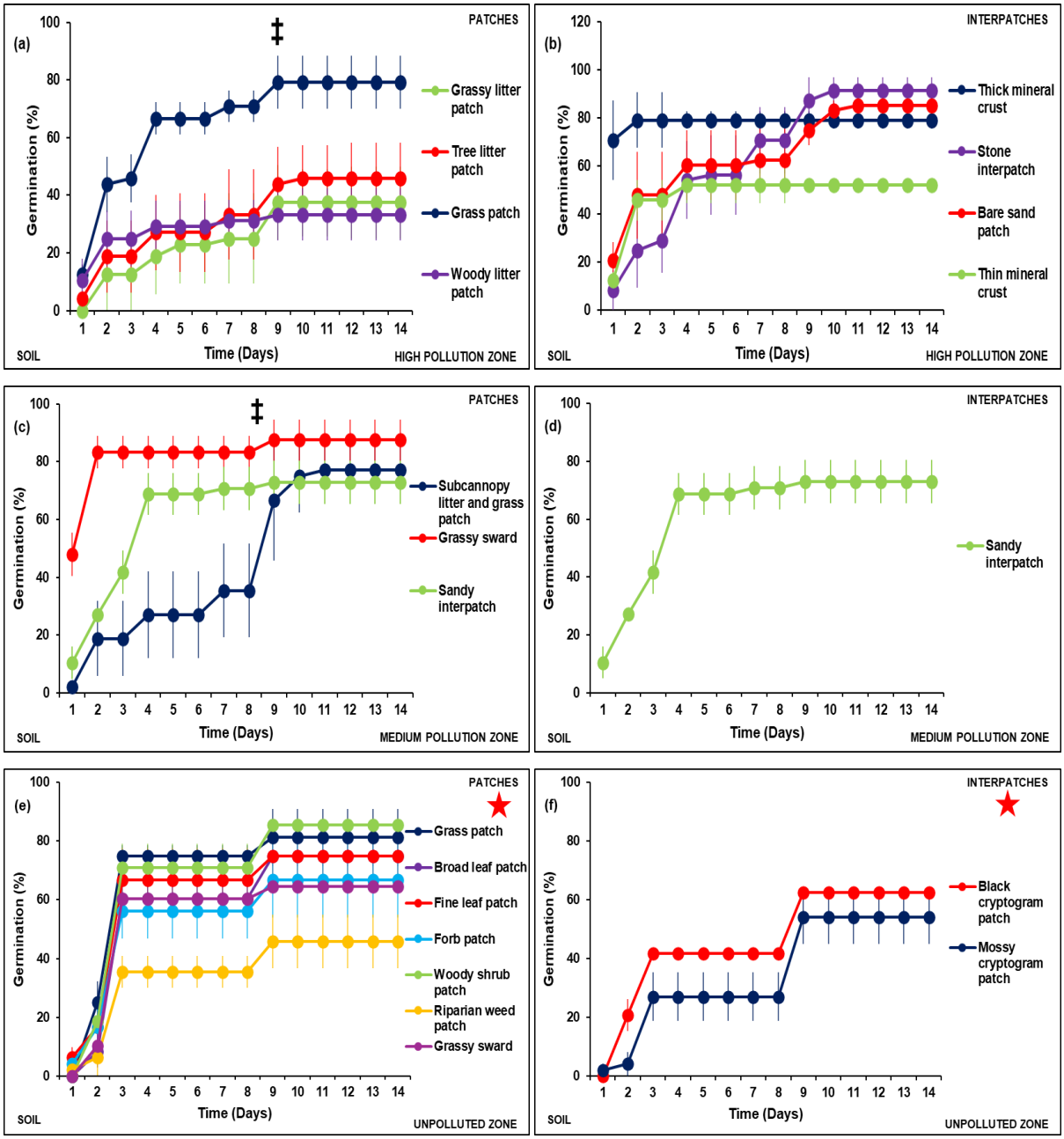


Figure 4.30: Time-germination curves for the mean (± standard error) germination of *Lepidium sativum* after exposure to **SOILS** of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. A red star indicates a significant difference between the patches and interpatches of that pollution zone.

For the effect of patch type on the root length of *L. sativum* grown directly in soils (**Figure 4.31**), all patch and interpatch categories in the high pollution zone were inhibited, except the bare sand interpatch which did not have an effect. For the medium pollution zone, the grassy sward (GS) alone had an inhibitory effect relative to the control.

Figure 4.31 is found on the next page.

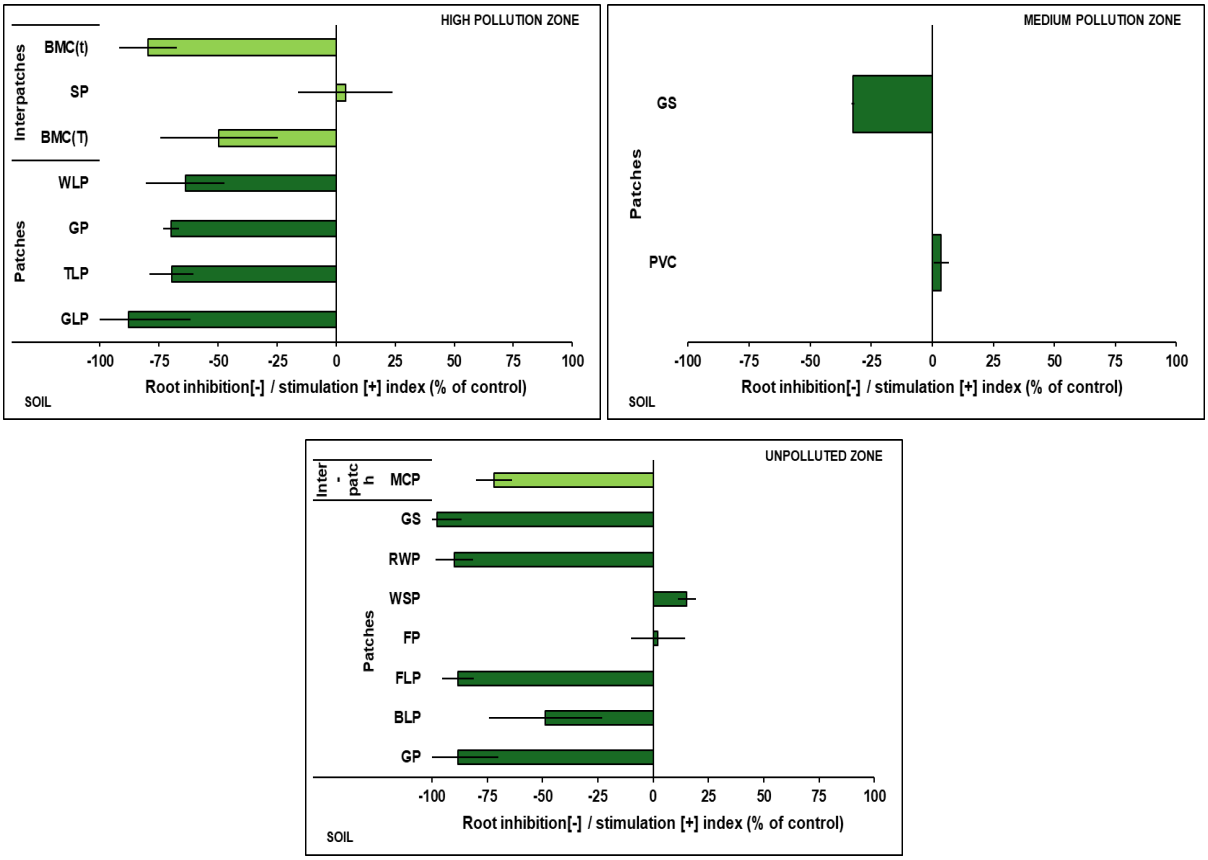


Figure 4.31: The effect of patch type on the inhibition/stimulation of root length of *Lepidium sativum* grown in **SOILS** of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *L. sativum* grown directly in soils (**Figure 4.32**), patch categories along the high and medium pollution zones had a stimulatory effect on root length, relative to the unpolluted patch categories.

Figure 4.32 is found on the next page.

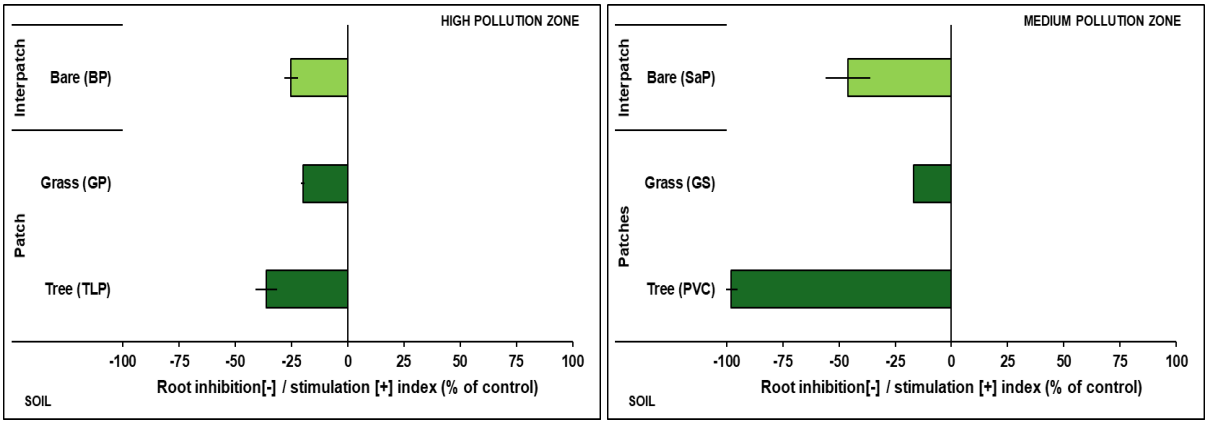


Figure 4.32: The effect of pollution level on the inhibition/stimulation of root length of *Lepidium sativum* grown in polluted **SOILS** (treatment) relative to unpolluted **SOILS** (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.4. Germination and root elongation of *Medicago sativa*

For the FLP tests (**Figure 4.33**), germination in the high pollution zone started on day 1 and continued into day 6, with a slight exponential phase until a first plateau on day 7, then a second slight exponential phase on day 12, and a second plateau on day 13, within a range of 10 – 62%. *M. sativa* responded more along the thick mineral crust than other interpatches along the high pollution zone. Along the medium pollution zone, germination started on day 1 and continued into day 4 before an initial exponential growth and plateau on day 8, and a second on day 10 within a range of 30 – 41%. Germination along the unpolluted zone seemed to only pick up after day 6, albeit with a slight response, until day 11 – 12 with an increase to 21%. Peak germination scores were higher along patches of the high pollution zone compared to the unpolluted zone ($p = 0.004$), and similarly between patches in the medium pollution zone and the unpolluted zone ($p < 0.001$). Regarding the effect of patch type, patches along the unpolluted zone had higher germination scores compared to interpatches ($p = 0.017$).

Figure 4.33 is found on the next page.

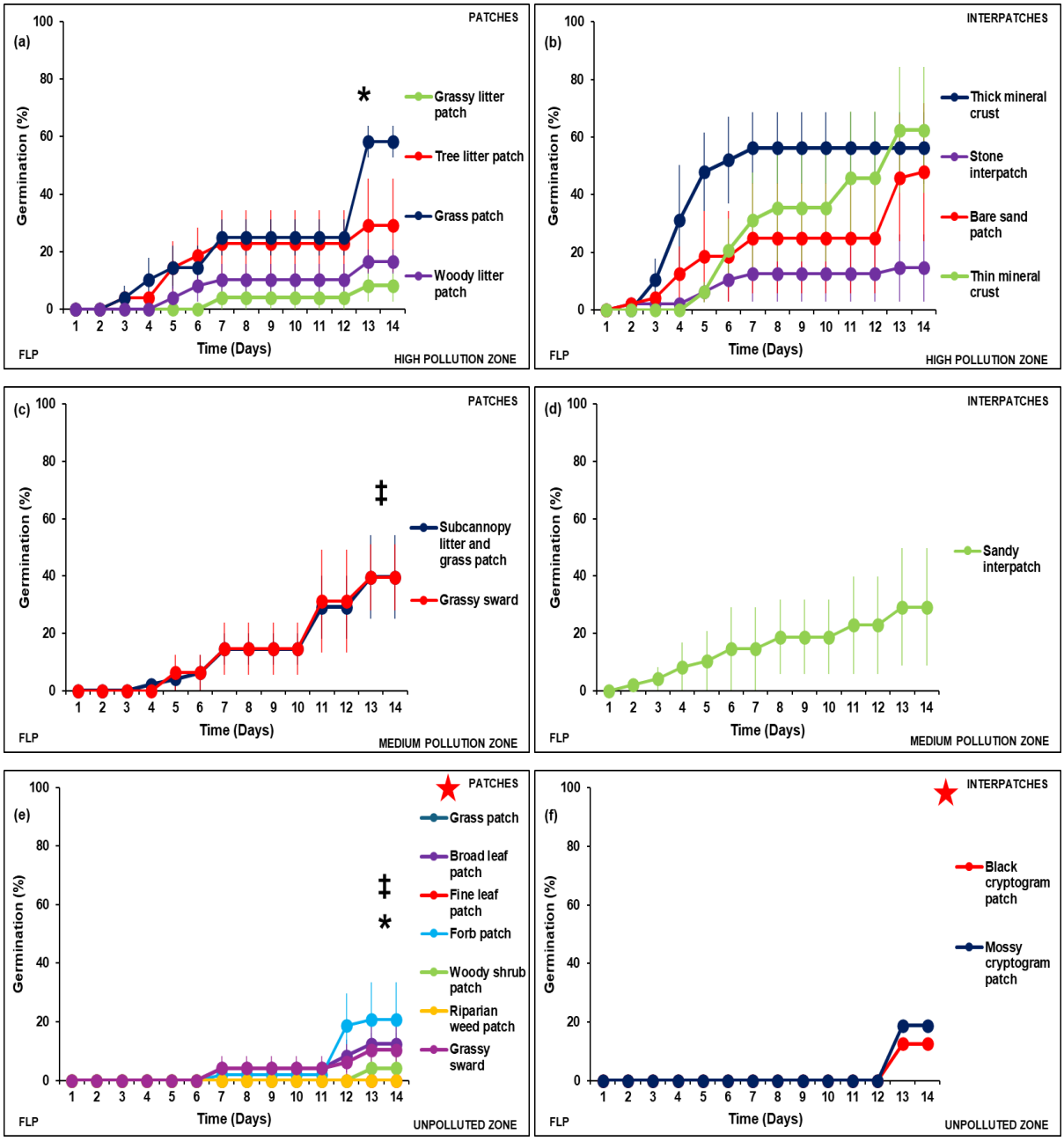


Figure 4.33: Time-germination curves for the mean (± standard error) germination of *Medicago sativa* after exposure to **FLP** solutions of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**), and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. A red star indicates a significant difference between the peak germination in patches and interpatches of that pollution zone.

For the effect of patch type on the root length of M. *sativa* grown in FLP solutions (**Figure 4.34**), all interpatches of the high pollution zone had an inhibitory effect on root length relative to the plainest patch control. Contrasting results for the patches, with the woody litter

patch (WLP) and tree litter patch (TLP) having an inhibitory effect, and the grass patch (GP) and grass litter patch having a stimulatory effect. For the medium polluted zone, the grassy sward (GS) had a stimulatory effect while the sub-canopy litter and grass patch had an inhibitory effect on root length. The interpatch and patches of the unpolluted zone had an overall stimulatory effect on root length, except for the woody shrub patch (WSP) having an inhibitory effect relative to the plainest patch category in that zone.

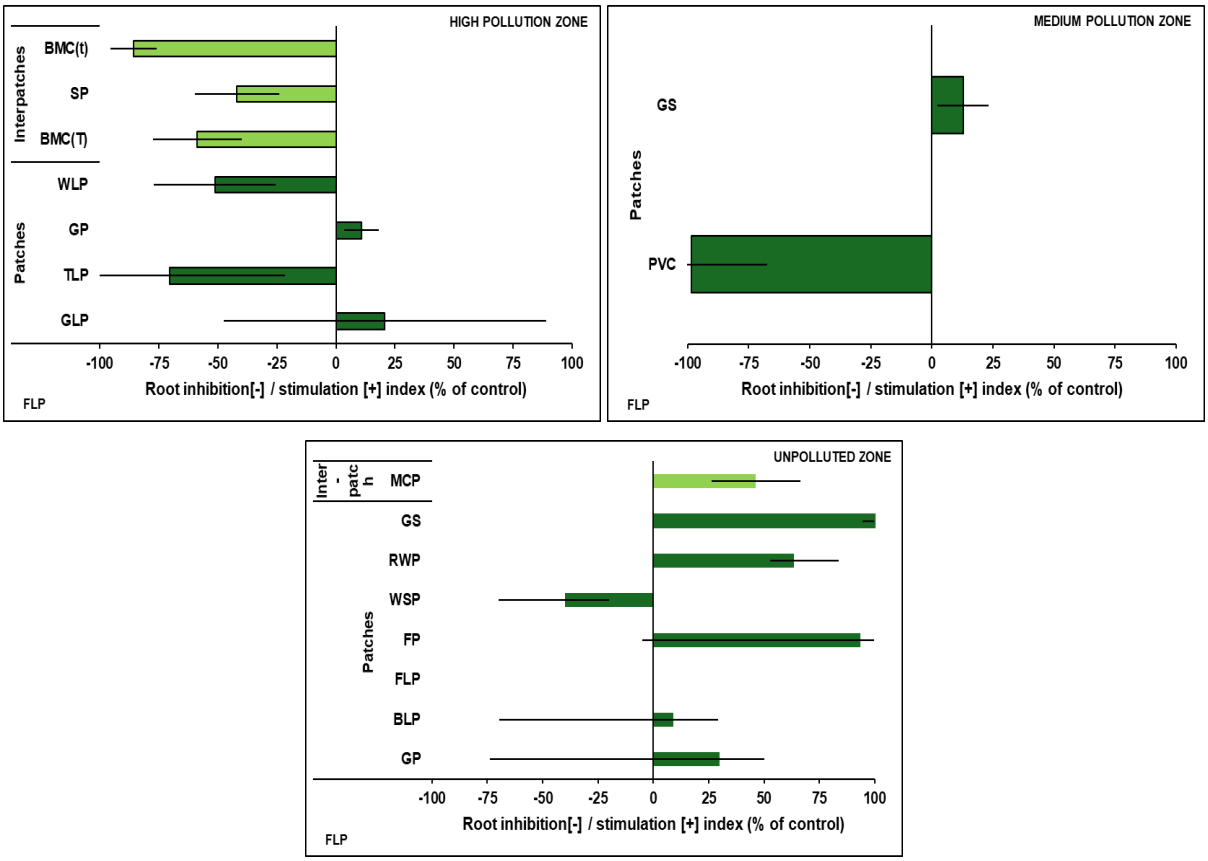


Figure 4.34: The effect of patch type on the inhibition/stimulation of root length of *Medicago sativa* grown in **FLP** solutions **of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *M. sativa* grown in FLP solutions (**Figure 4.35**), the tree patch category had an inhibitory effect on root length for the high pollution zone, while the grass and bare patch had a stimulatory effect relative to the

unpolluted control patch categories. All patch categories on the medium pollution zone had a stimulatory effect relative to the unpolluted controls.

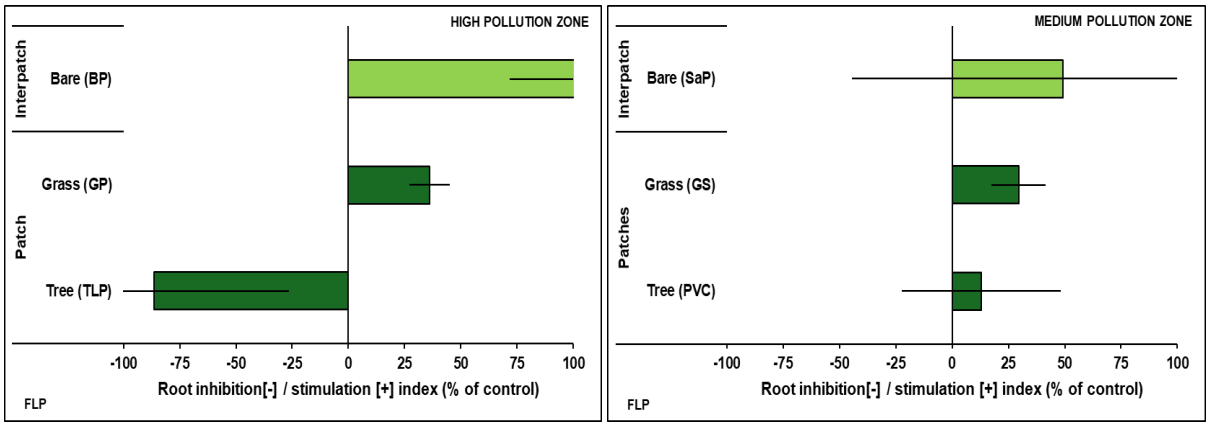


Figure 4.35: The effect of pollution level on the inhibition/stimulation of root length of *Medicago sativa* grown in polluted **FLP** solutions (treatment) relative to unpolluted **FLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP tests (**Figure 4.36**), germination in the high pollution zone started to pick up on day 3 and continued into day 8, with a slight exponential phase before peaking on day 9 at a range of 5 – 36%. Germination along the medium pollution zone started on day 4 for patches and day 8 for the interpatch, with a peak range of 10 – 45% occurring after day 8. For the unpolluted zone, germination only started on day 7 and immediately encountered a peak range of 5 – 12%. A 50% germination was not reached in either test. Peak germination along the patches of the medium pollution zone was significantly higher than in the unpolluted zone ($p = 0.004$). No significant differences were found regarding the effect of patch type within each zone.

Figure 4.36 is found on the next page.

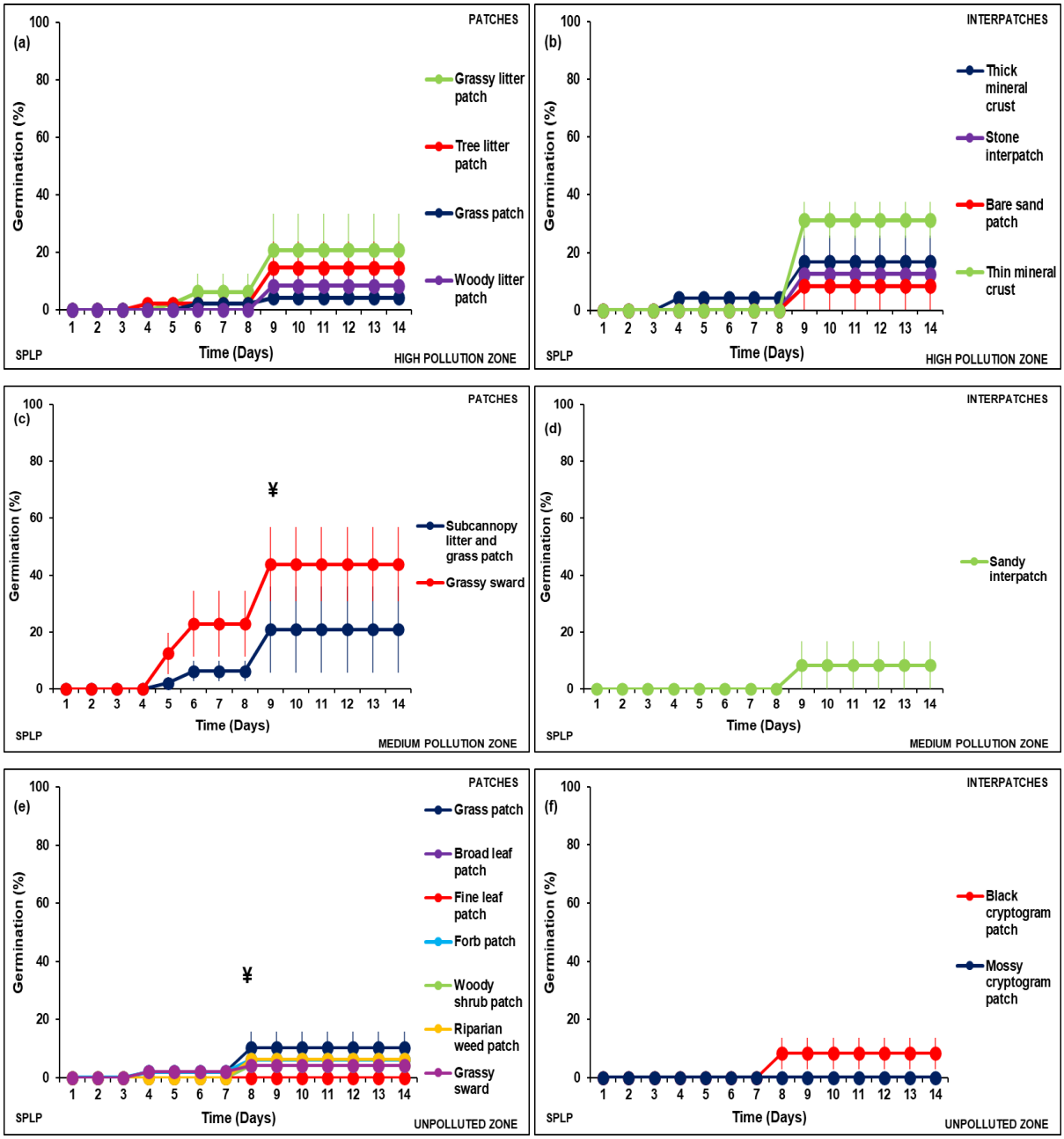


Figure 4.36: Time-germination curves for the mean (± standard error) germination of *Medicago sativa* after exposure to **SPLP** solutions of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *M. sativa* grown in SPLP solutions (**Figure 4.37**), all patches and interpatches on the high, medium, and unpolluted zones experienced an inhibitory effect relative to the plainest patches category found within each zone.

Figure 4.37 is found on the next page.

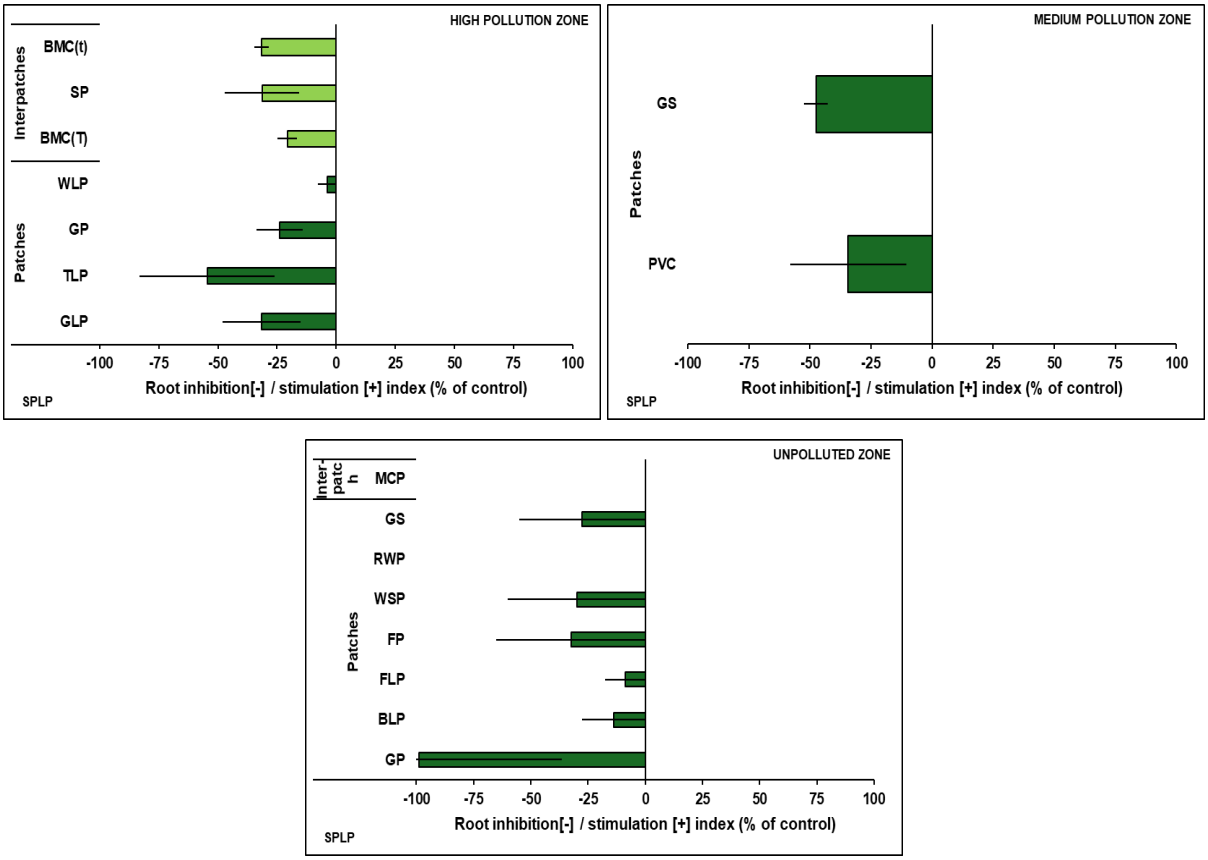


Figure 4.37: The effect of patch type on the inhibition/stimulation of root length of *Medicago sativa* grown in **SPLP** solutions **of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *M. sativa* grown in SPLP solutions (**Figure 4.38**), only the grass patch of the high pollution zone had an inhibitory effect on root length, whereas the bare and grass patch categories had a stimulatory effect, albeit a highly variable result. In comparison, the patch categories in the medium pollution zone had an overall inhibitory effect in relation to the unpolluted control patch categories.

Figure 4.38 is found on the next page.

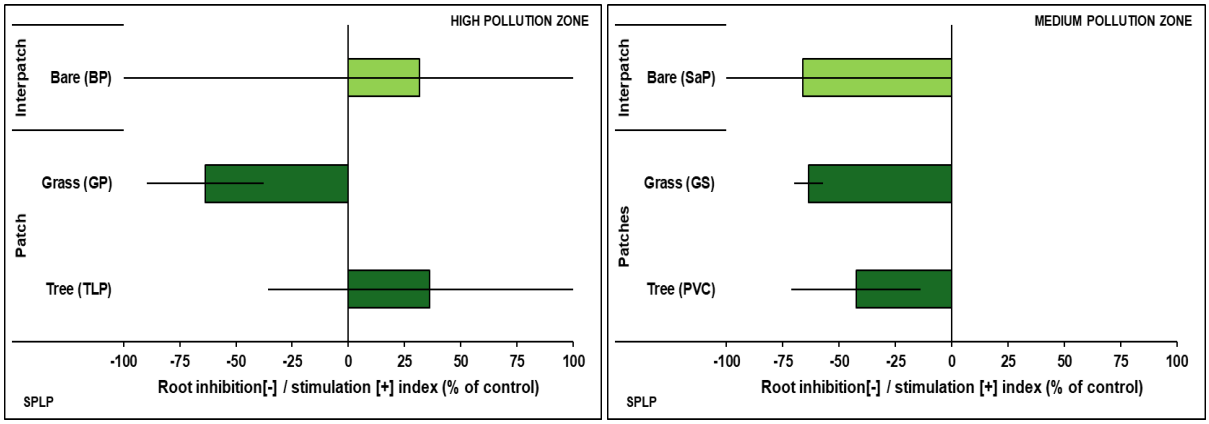


Figure 4.38: The effect of pollution level on the inhibition/stimulation of root length of *Medicago sativa* grown in polluted SPLP solutions (treatment) relative to unpolluted SPLP solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: tree, grass, and bare patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:

For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.

For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the soil test (**Figure 4.39**), the thick mineral crust inhibited almost any germination along the high pollution zone – germination started on day 1 and continued into day 6 with an exponential increase until a peak on day 8 – within a range of 30 – 57%. Germination started on day 3 for the medium pollution zone and reached a peak of 16 – 58% on day 6. Along the unpolluted zone, germination started on day 3 and reached a peak range of 6 – 36% on day 4. Peak germination was found to be higher along patches of the high and medium pollution zones compared to the unpolluted zone (high: $p < 0.001$; medium: $p = 0.008$). No significant differences were found regarding the effect of patch type within each zone.

Figure 4.39 is found on the next page.

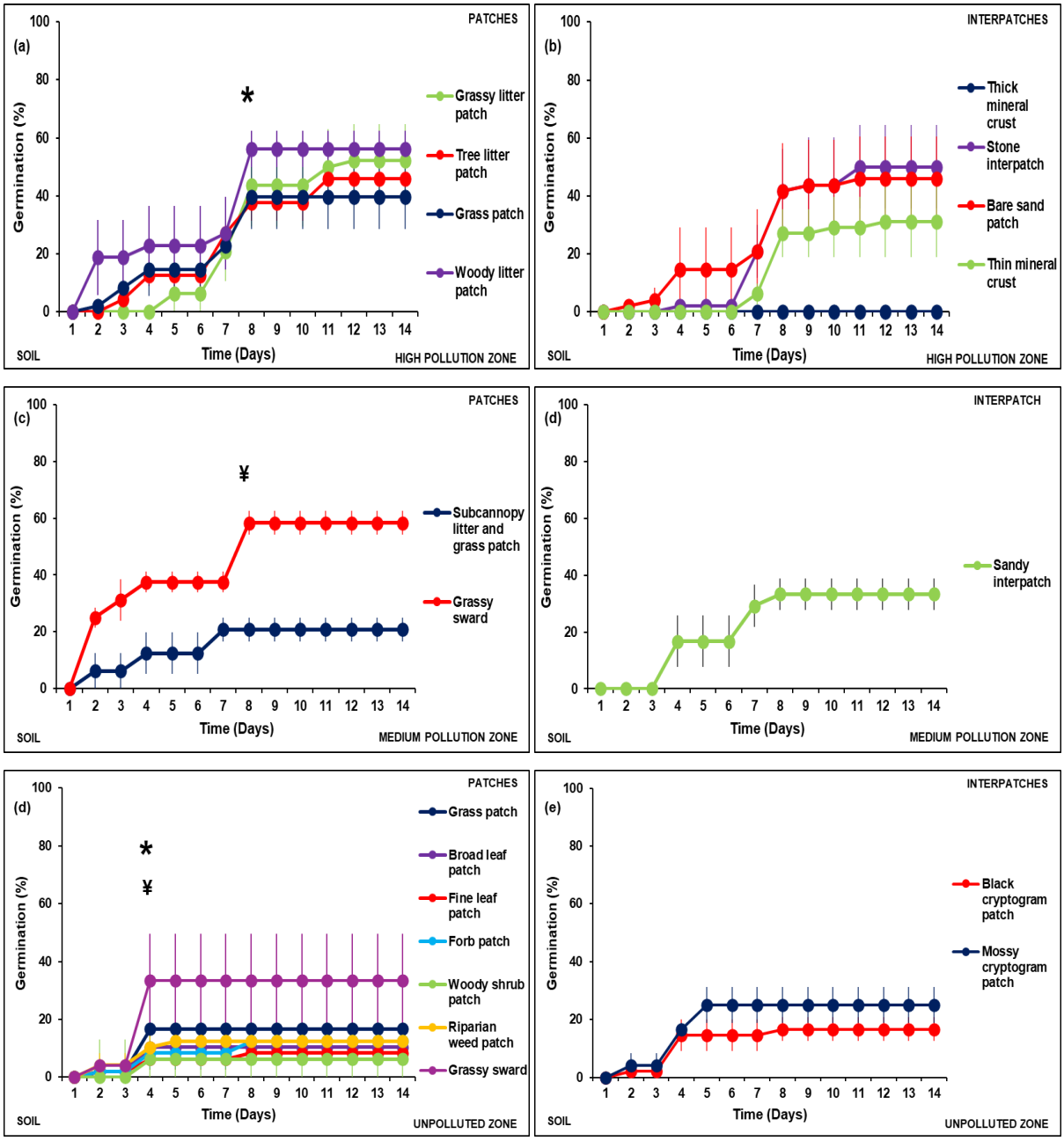


Figure 4.39: Time-germination curves for the mean (± standard error) germination of *Medicago sativa* after exposure to **SOILS** of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**), and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. The were no significant differences in peak germination between patches and interpatches of each pollution zone.

For the effect of patch type on the root length of *M. sativa* grown in soils (**Figure 4.40**), patches and interpatches of the high and medium pollution zones showed an overall inhibitory effect on root length, except the thick mineral crust, which had no effect. The

unpolluted zone had a mixed result of inhibition and stimulation, with patches having a stimulatory effect, except the riparian weed patch (RWP), the grassy sward (GS), and the grass patch with an inhibitory effect. The mossy cryptogram interpatch (MCP) also had an inhibitory effect in relation to the plainest patch category in that zone.

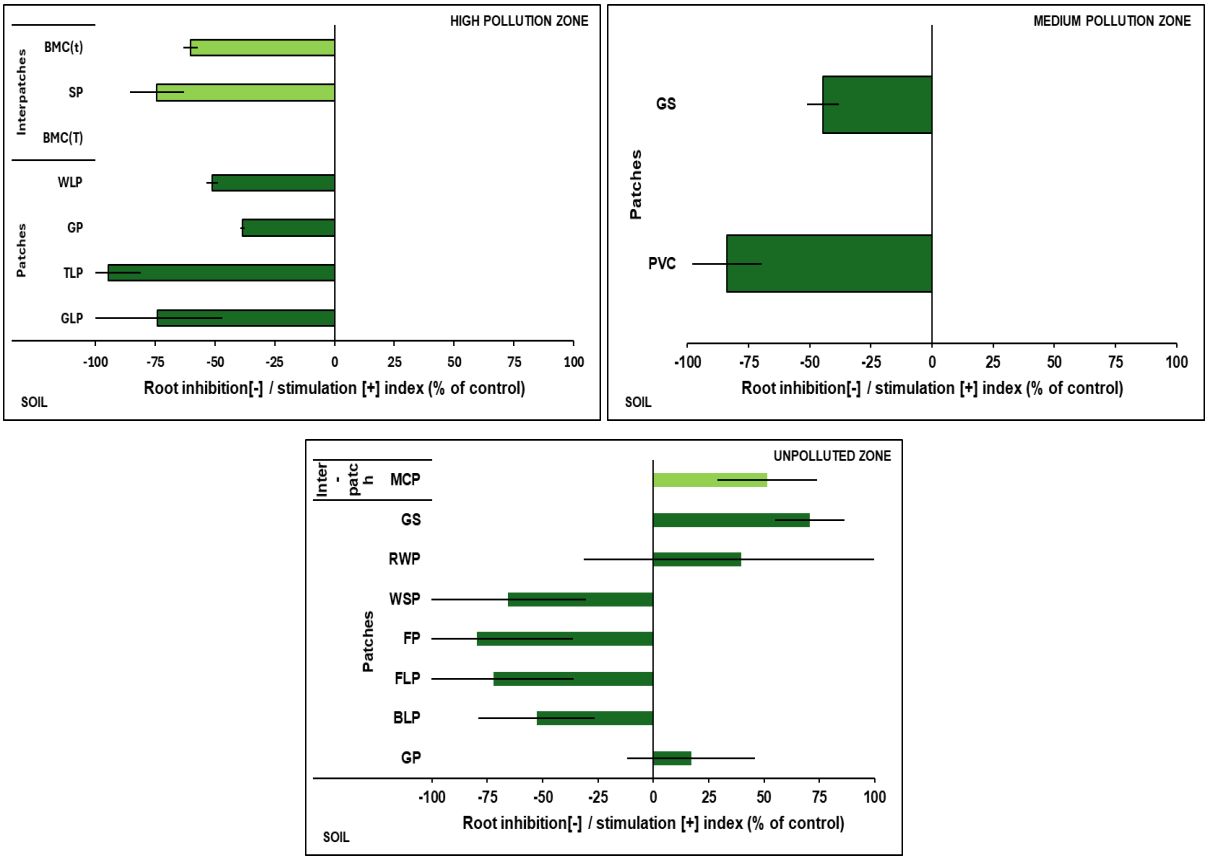


Figure 4.40: The effect of patch type on the inhibition/stimulation of root length of *Medicago sativa* grown in **SOILS of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *M. sativa* grown in soils (**Figure 4.41**), the high and medium pollution zones similarly had a stimulatory response from the bare and tree patch categories. The grass patch categories had an overall negative effect on root length, relative to the similar unpolluted patch categories.

Figure 4.41 is found on the next page.

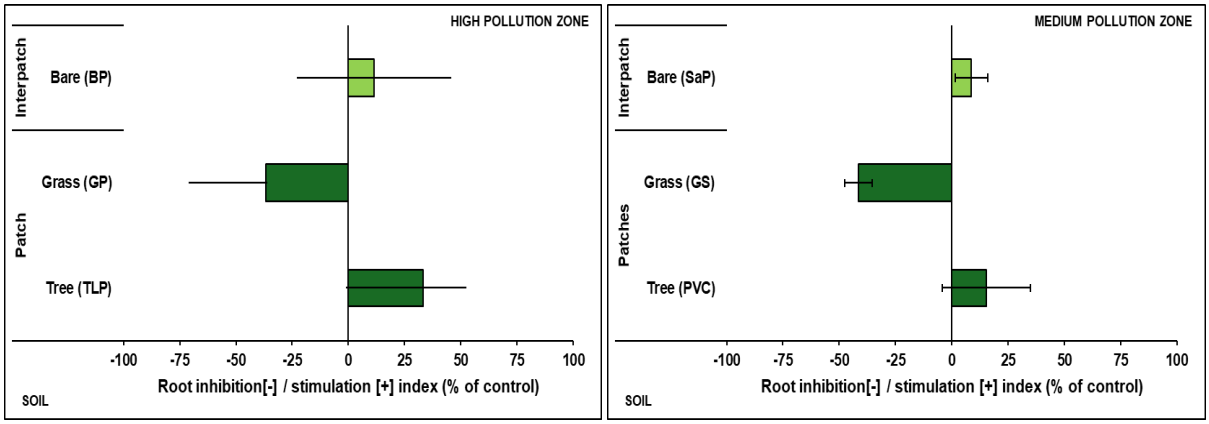


Figure 4.41: The effect of pollution level on the inhibition/stimulation of root length of *Medicago sativa* grown in polluted **SOILS** (treatment) relative to unpolluted **SOILS** (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.5. Germination and root elongation of *Brassica nigra*

For the FLP tests (**Figure 4.42**), germination of *B. nigra* started on day 1. The high pollution zone had a slight exponential growth after the start of germination, and peaked after day 6, within a range of 17 – 61%. The medium pollution zone peaked on day 6, within a range of 23 – 40%. In comparison, the unpolluted zone reached peak germination on day 2, within a germination range of 16 – 42%. No significant differences in peak germination were found along the zones or between patches and interpatches within each zone.

Figure 4.42 is found on the next page.

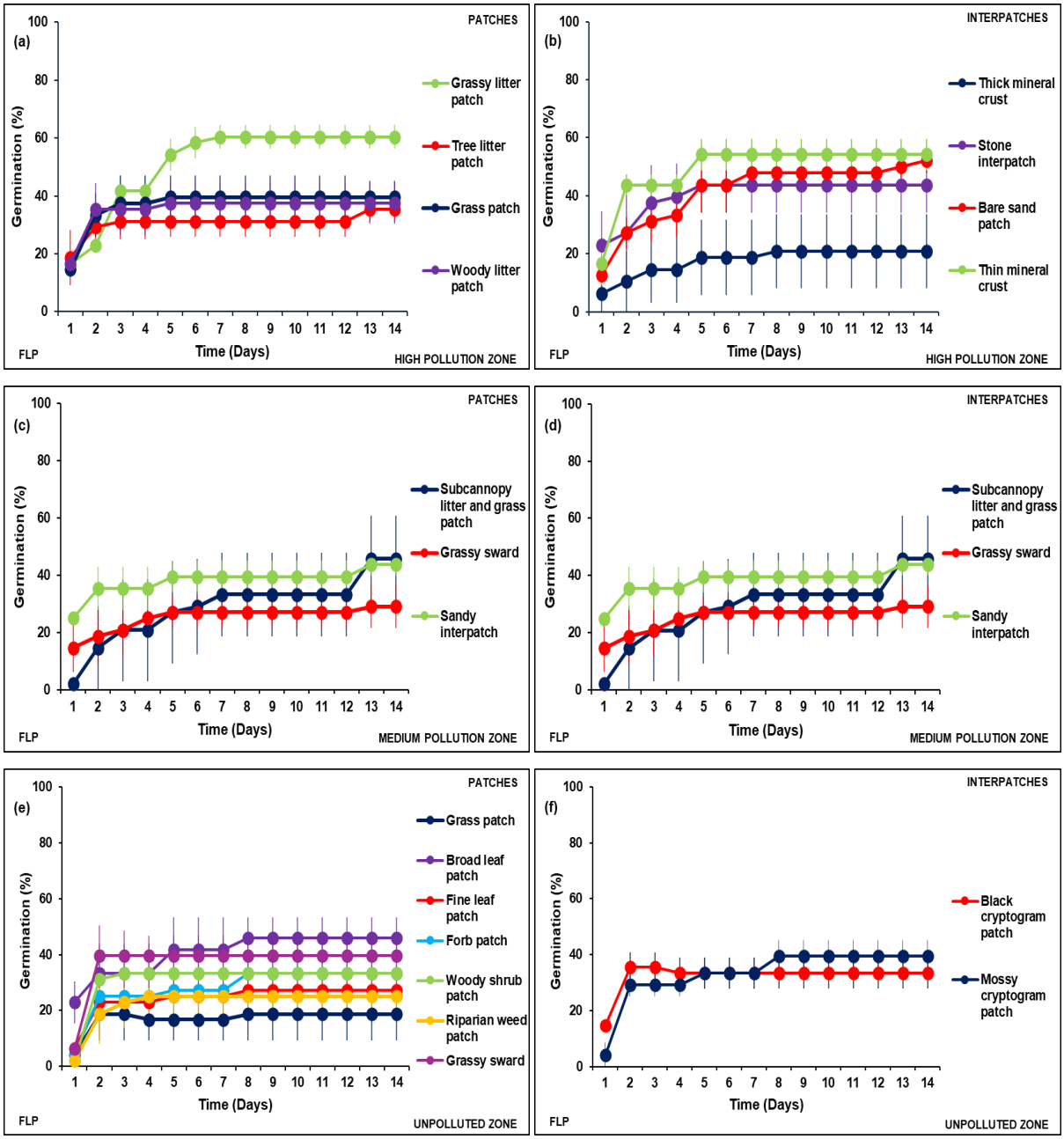


Figure 4.42: Time-germination curves for the mean (± standard error) germination of *Brassica nigra* after exposure to **FLP** solutions of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. There were no significant differences in the peak germination in patches/interpatches amongst pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *B. nigra* grown in FLP solutions (**Figure 4.43**), the high pollution zone had an overall inhibitory effect on root length, however, the thick bare crust interpatch seemed to have a slight inhibitory effect (almost no effect). In

contrast, the patches in the medium pollution zone had an overall stimulatory effect of root length relative to the plainest patch category control. The unpolluted zone had an overall inhibitory effect on root length, except the stimulatory effect of the forb patch (FP).

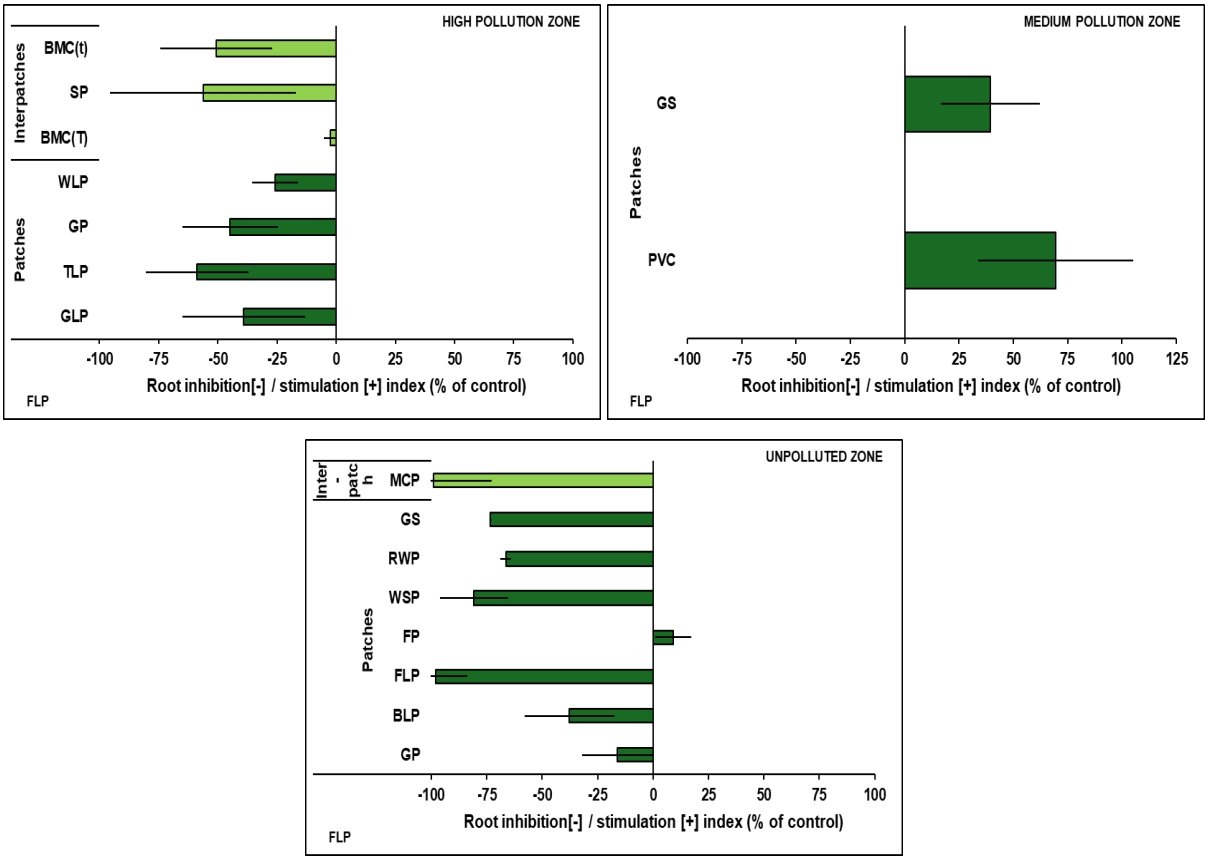


Figure 4.43: The effect of patch type on the inhibition/stimulation of root length of *Brassica nigra* grown in **FLP** solutions **of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

The effect of pollution level on the root length of *B. nigra* grown in FLP solutions (**Figure 4.44**): The high pollution zone showed an overall stimulation of root lengths relative to the unpolluted controls. The patches in the medium pollution zone also showed a stimulatory effect, whereas the bare interpatch had an inhibitory effect on root length.

Figure 4.44 is found on the next page.

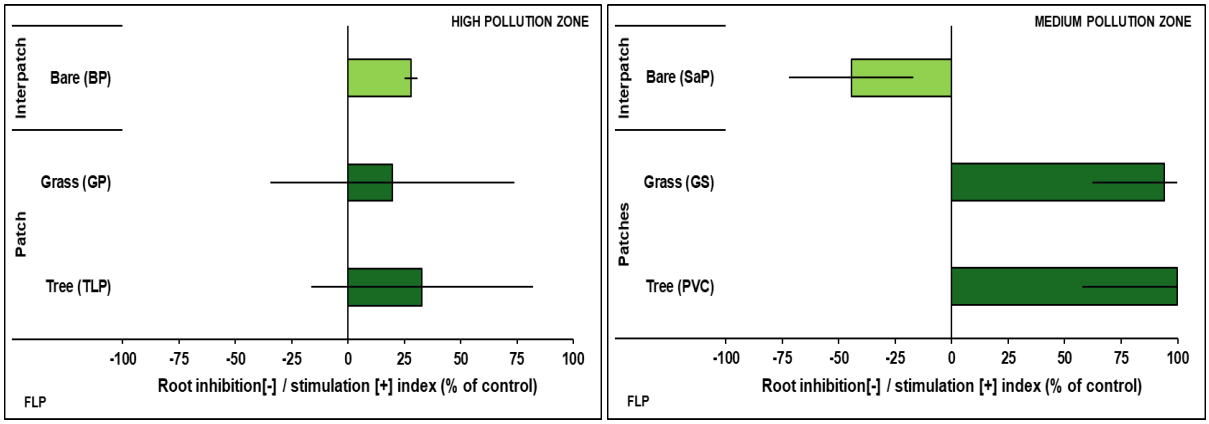


Figure 4.44: The effect of pollution level on the inhibition/stimulation of root length of *Brassica nigra* grown in polluted **FLP** solutions (treatment) relative to unpolluted **FLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:

For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.

For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP test (**Figure 4.45**), germination also started on day 1. The high pollution zone had a peak germination on day 9, within a range of 6 – 42%. The medium pollution zone also had peak germination on day 9, within a range of 26 – 43%. As with the FLP test above, the unpolluted zone had a peak on day 2, within a range of 18 – 57%. No significant differences in peak germination were found along the zones or between patches and interpatches within each zone.

Figure 4.45 is found on the next page.

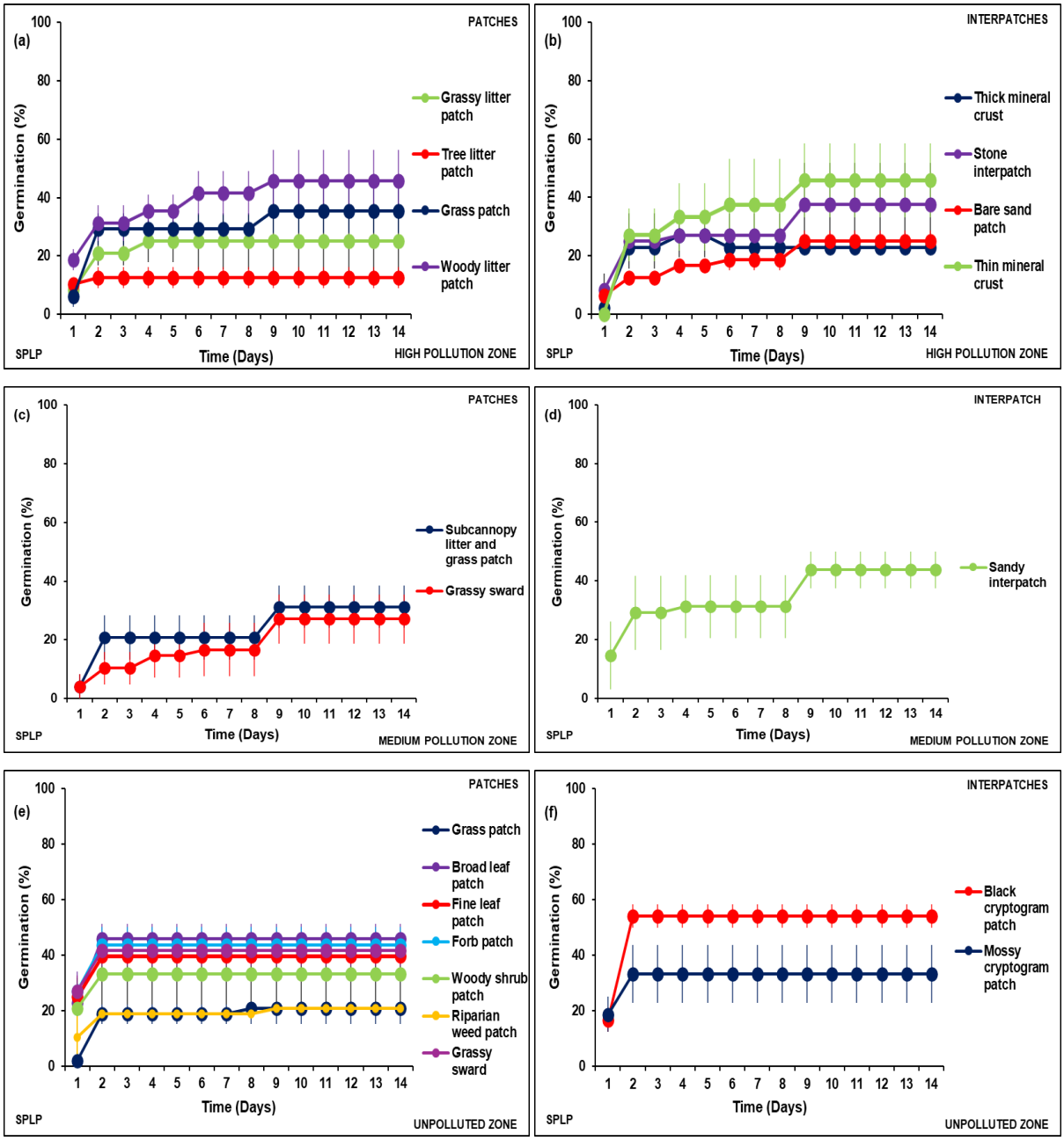


Figure 4.45: Time-germination curves for the mean (± standard error) germination of *Brassica nigra* after exposure to **SPLP** solutions of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. There were no significant differences in the peak germination in patches/interpatches amongst pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones

For the effect of patch type on the root length of *B. nigra* grown in SPLP solutions (**Figure 4.46**), all patch categories from the high pollution zone had an inhibitory effect, with the stone interpatch having a highly variable result. All patch categories from the medium pollution zone were stimulated in reference to the control. The unpolluted zone had a greater

extent of an inhibitory response, except for the stimulatory response from the broad leaf patch (BLP) and grass patch (GP).

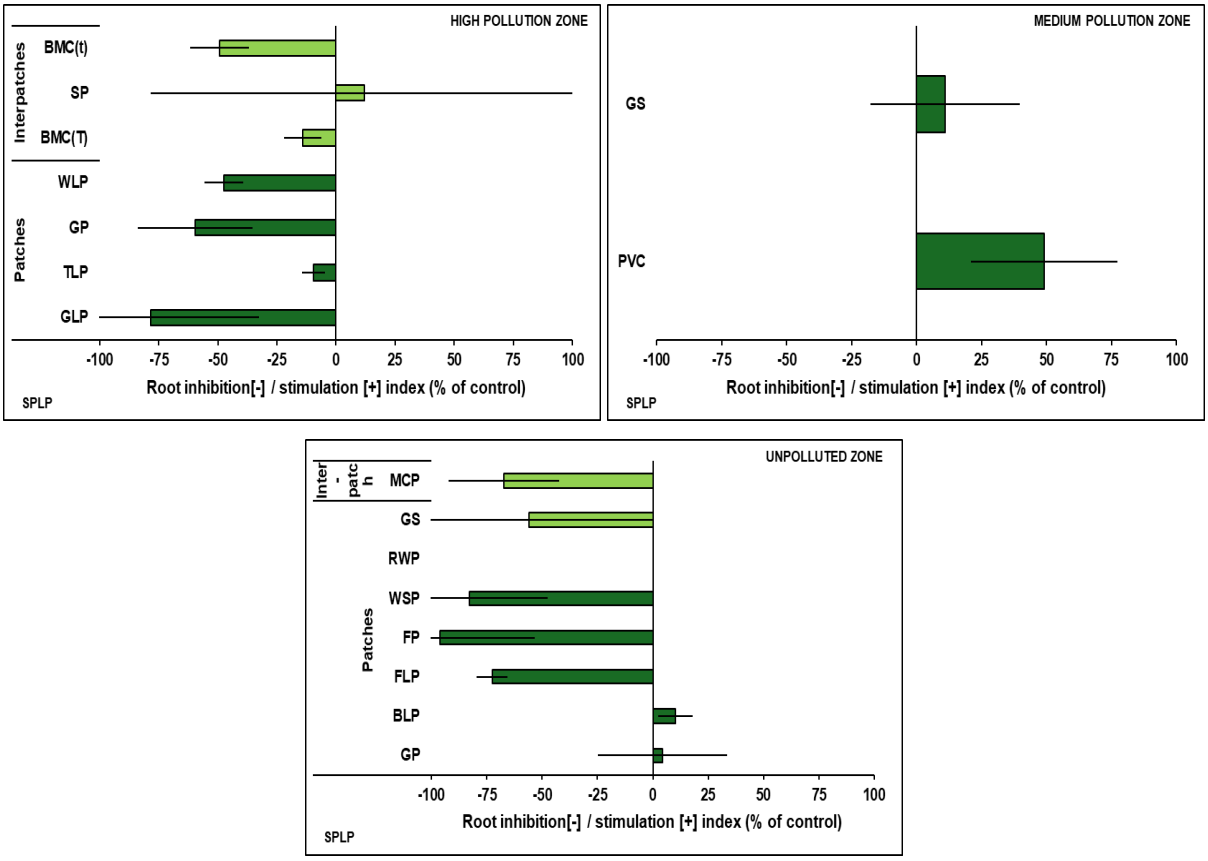


Figure 4.46: The effect of patch type on the inhibition/stimulation of root length of *Brassica nigra* grown in **SPLP** solutions of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *B. nigra* grown in SPLP solutions (**Figure 4.47**), both the high and medium pollution zone had an inhibitory effect on root length in relation to the plainest patch category within that pollution zone.

Figure 4.47 is found on the next page.

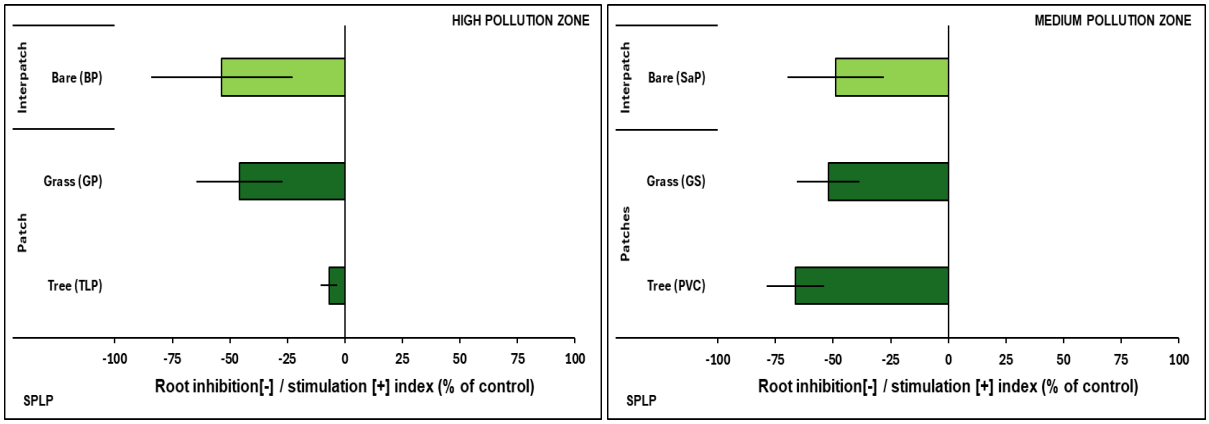


Figure 4.47: The effect of pollution level on the inhibition/stimulation of root length of *Brassica nigra* grown in polluted **SPLP** solutions (treatment) relative to unpolluted **SPLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.
For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the direct soil tests (**Figure 4.48**), the high and medium pollution zones had a peak germination on day 7. The unpolluted zone had a peak germination much quicker, on day 3.

Figure 4.48 is found on the next page.

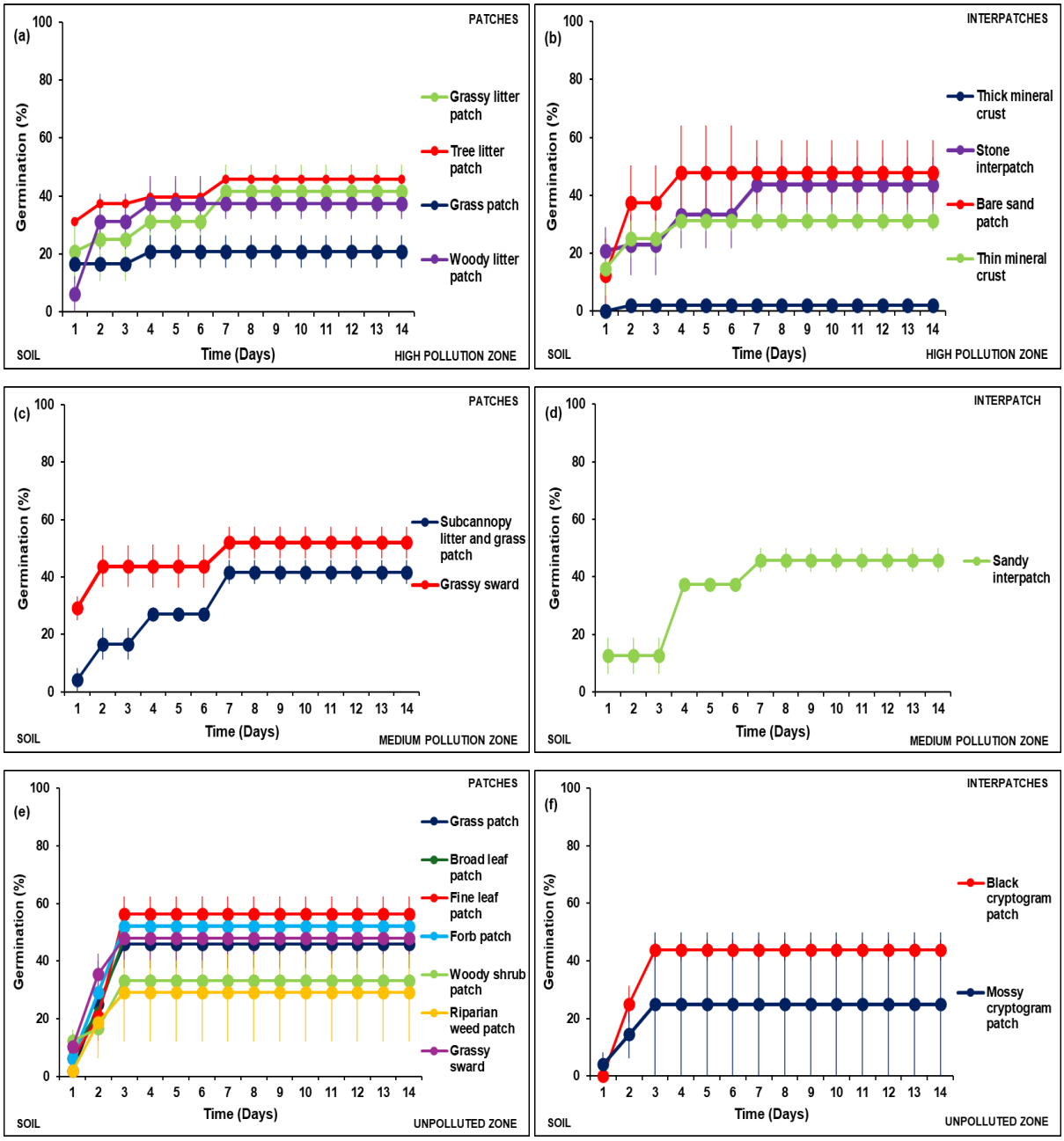


Figure 4.48: Time-germination curves for the mean (± standard error) germination of *Brassica nigra* after exposure to **SOILS** of patches and interpatches from the high pollution (**a** and **b**) medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. No significant differences existed in the peak germination in patches/interpatches amongst pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *B. nigra* grown in SPLP solutions (**Figure 4.49**), the high and medium pollution zone had an inhibitory effect on root length. The thick mineral crust interpatch (BMC[T]) and the sub-canopy litter and grass patch had no effect.

The unpolluted zone had an equal extent of an inhibitory and stimulatory effect, with the grass patch being variable in this regard.

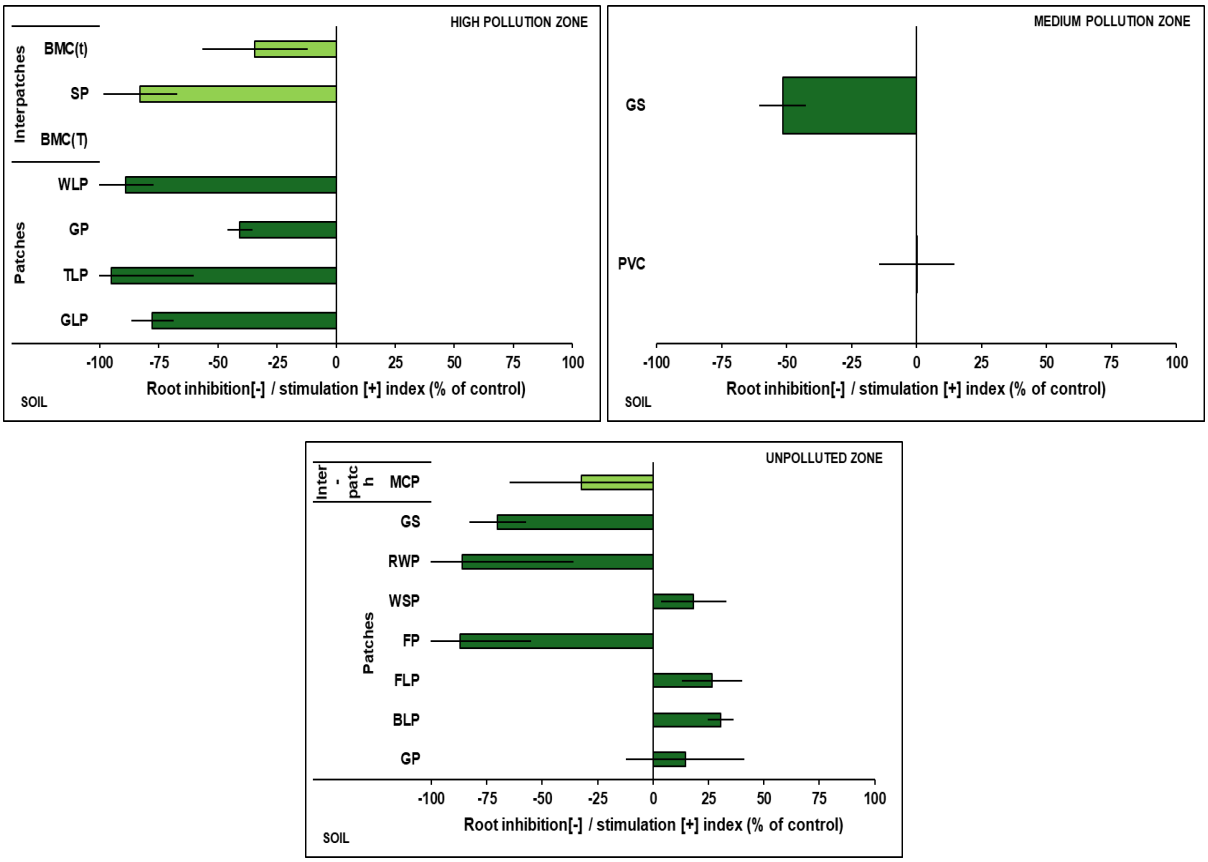


Figure 4.49: The effect of patch type on the inhibition/stimulation of root length of *Brassica nigra* grown in **SOILS** of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *B. nigra* grown in SPLP solutions (**Figure 4.50**), patch categories along the high and medium pollution zone had an inhibitory response on root length.

Figure 4.50 is found on the next page.

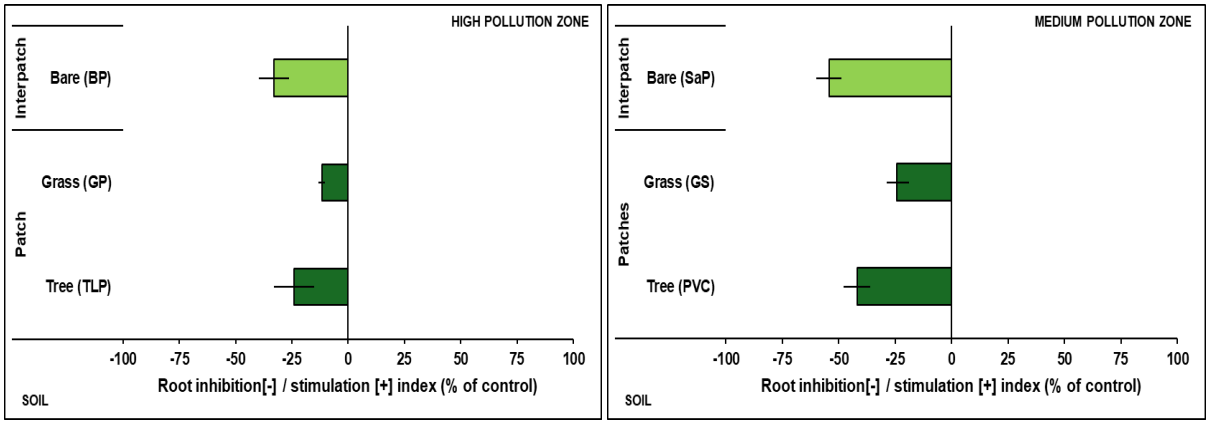


Figure 4.50: The effect of pollution level on the inhibition/stimulation of root length of *Brassica nigra* grown in polluted **SOILS** (treatment) relative to unpolluted **SOILS** (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone.
For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.6. Germination and root elongation of *Sorghum bicolor*

For the FLP tests (**Figure 4.51**), almost 50% of *S. bicolor* germinated on day 1 along the high and medium pollution zones. Peak germination was reached on day 5 for both the high and medium pollution zones, in a range of 80 – 95% and 85 – 95%, respectively. The FLP tests of the unpolluted zone reached 50% germination on day 1, and peak germination was reached on day 7, within a range of 61 – 90%. Patches along the high and medium pollution zones were found to have higher peak germination rates of *S. bicolor* compared to the unpolluted zone (high: $p = 0.005$; medium: $p = 0.008$). Similarly, interpatches along the high pollution zone stimulated a higher peak germination compared to the unpolluted zone ($p = 0.002$).

Figure 4.51 is found on the next page.

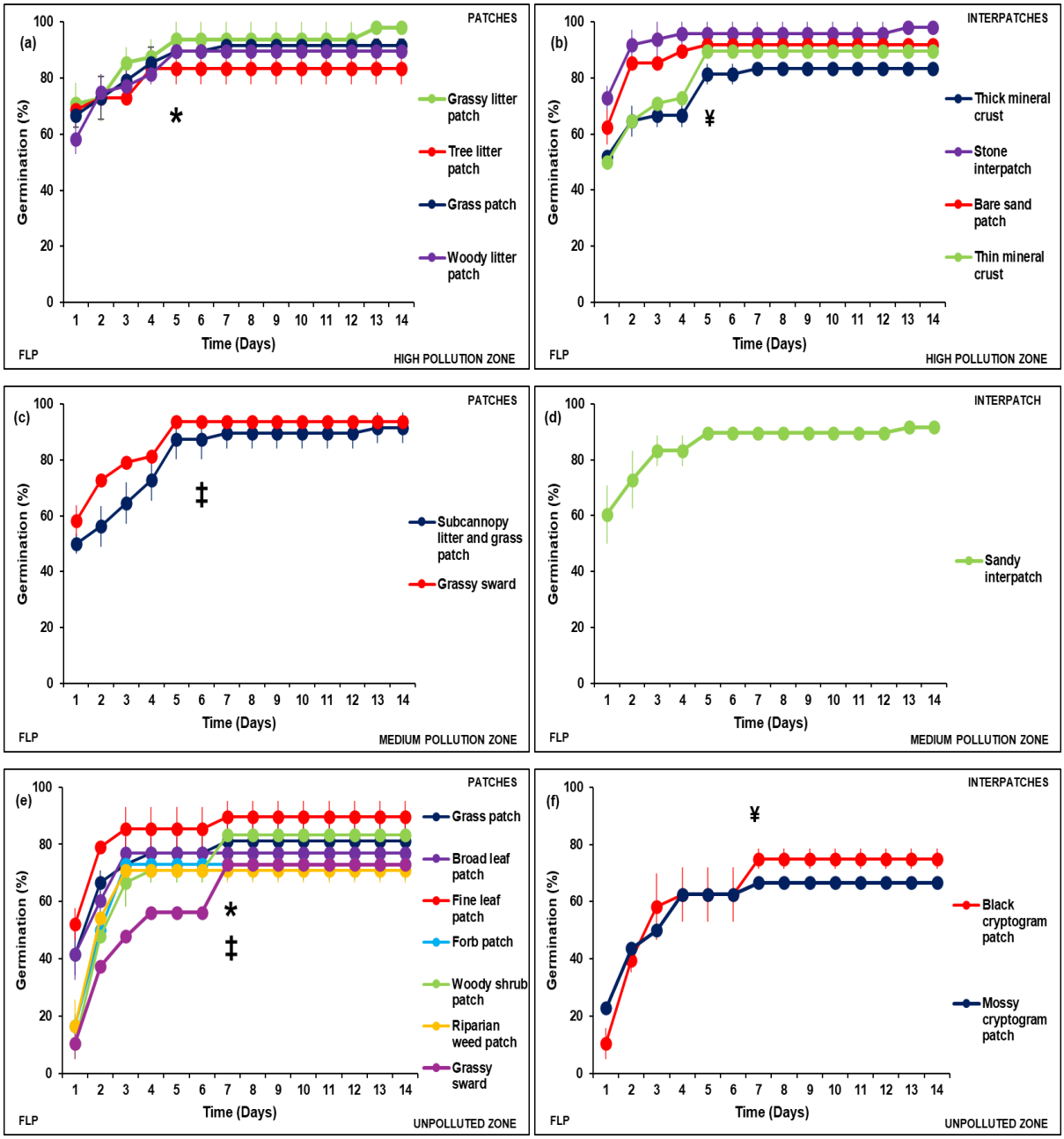


Figure 4.51: Time-germination curves for the mean (± standard error) germination of *Sorghum bicolor* after exposure to **FLP** solutions of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**), and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. Graphs with similar symbols indicate significant differences in peak germination between the patch/interpatch amongst the pollution zones. There were no significant differences in peak germination between patches and interpatches of each pollution zone.

For the effect of patch type on the root length of *S. bicolor* grown in FLP solutions (**Figure 4.52**), the tree and grass litter patch from the high pollution zone (TLP and GLP) were inhibited to ~83% of the control patches, indicating a 17% inhibition. The thick mineral crust

interpatch was also inhibited at 24% of the control – indicating a 74% inhibition. The thin mineral crust interpatch and stone interpatch were stimulated to 10-15% of the control – indicating an 80-85% stimulation. All patches from the medium pollution zone were stimulated in reference to the control. Most patch categories from the unpolluted zone were stimulated, except the grass patch (GP) and broad leaf patch (BLP) which had an inhibitory effect on the root length of *S. bicolor*.

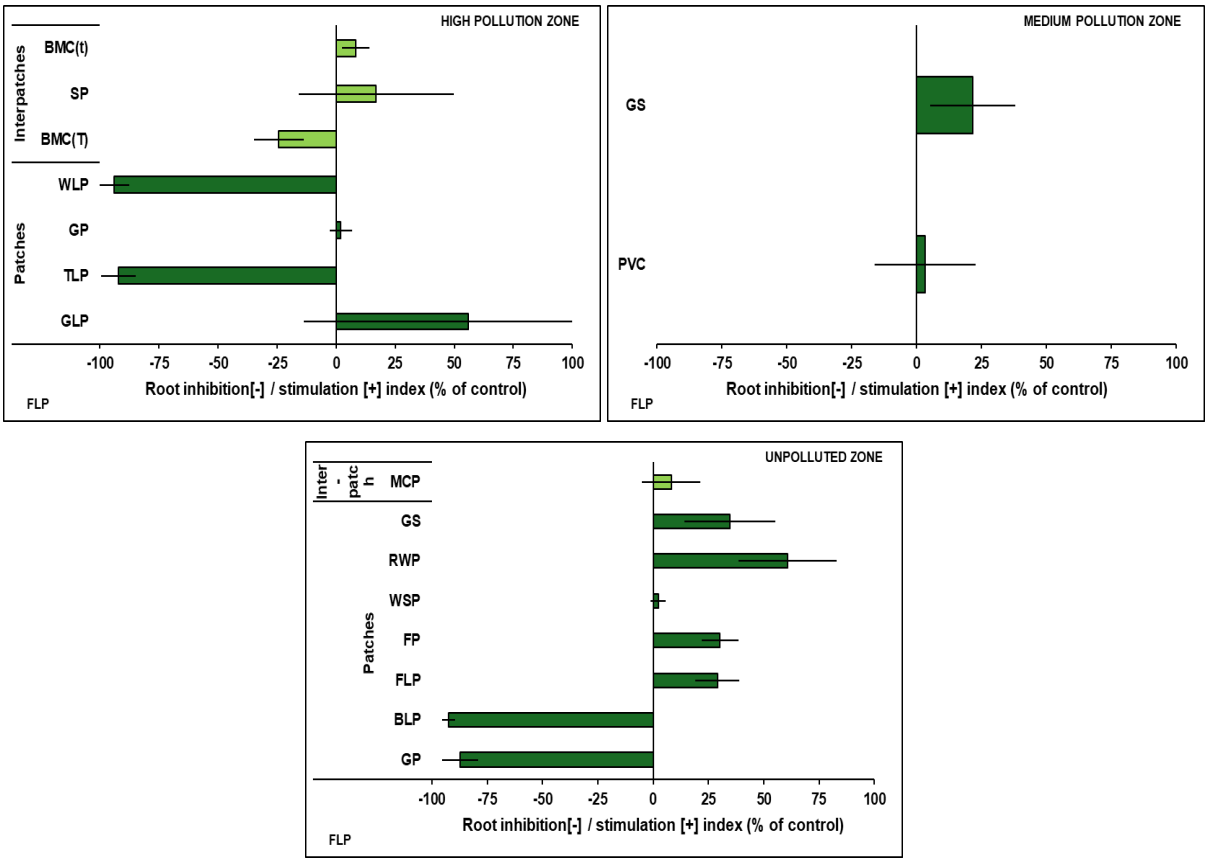


Figure 4.52: The effect of patch type on the inhibition/stimulation of root length of *Sorghum bicolor* grown in **FLP** solutions **of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *S. bicolor* grown in FLP solutions (**Figure 4.53**), the high and medium pollution zones resulted in a stimulatory effect in root length in reference to the control unpolluted patch categories.

Figure 4.53 is found on the next page.

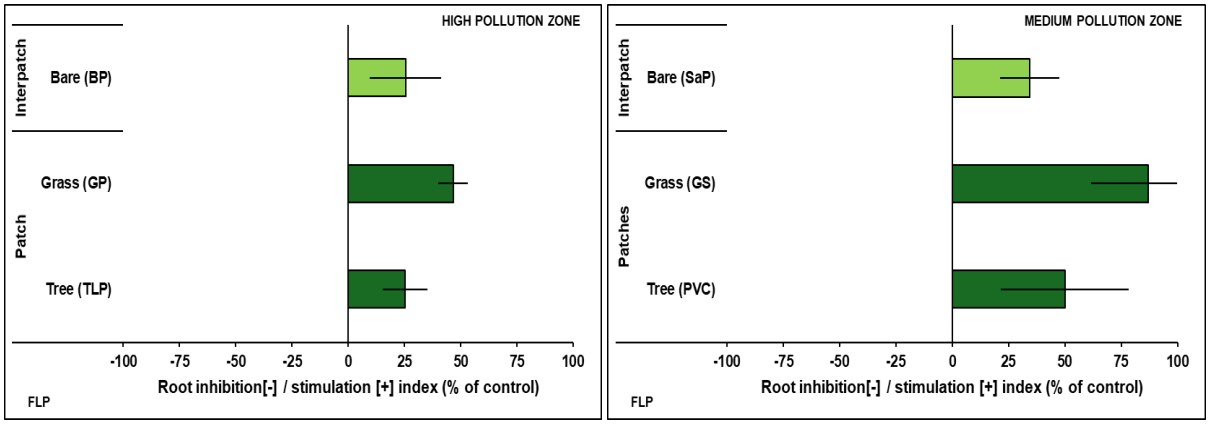


Figure 4.53: The effect of pollution level on the inhibition/stimulation of root length of *Sorghum bicolor* grown in polluted **FLP** solutions (treatment) relative to unpolluted **FLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the SPLP tests (**Figure 4.54**), germination started on day 1 and exponentially increased on day 2 for each zone. The high pollution zone reached peak germination day 3, within a range of 73 – 90%. Similarly, the medium pollution zone reached peak germination plateau after day 3, in a range of 70 – 90%. The unpolluted zone reached peak germination after day 4 within a range of 63 – 90%. No significant differences in peak germination were found along the zones or between patches and interpatches within each zone.

Figure 4.54 is found on the next page.

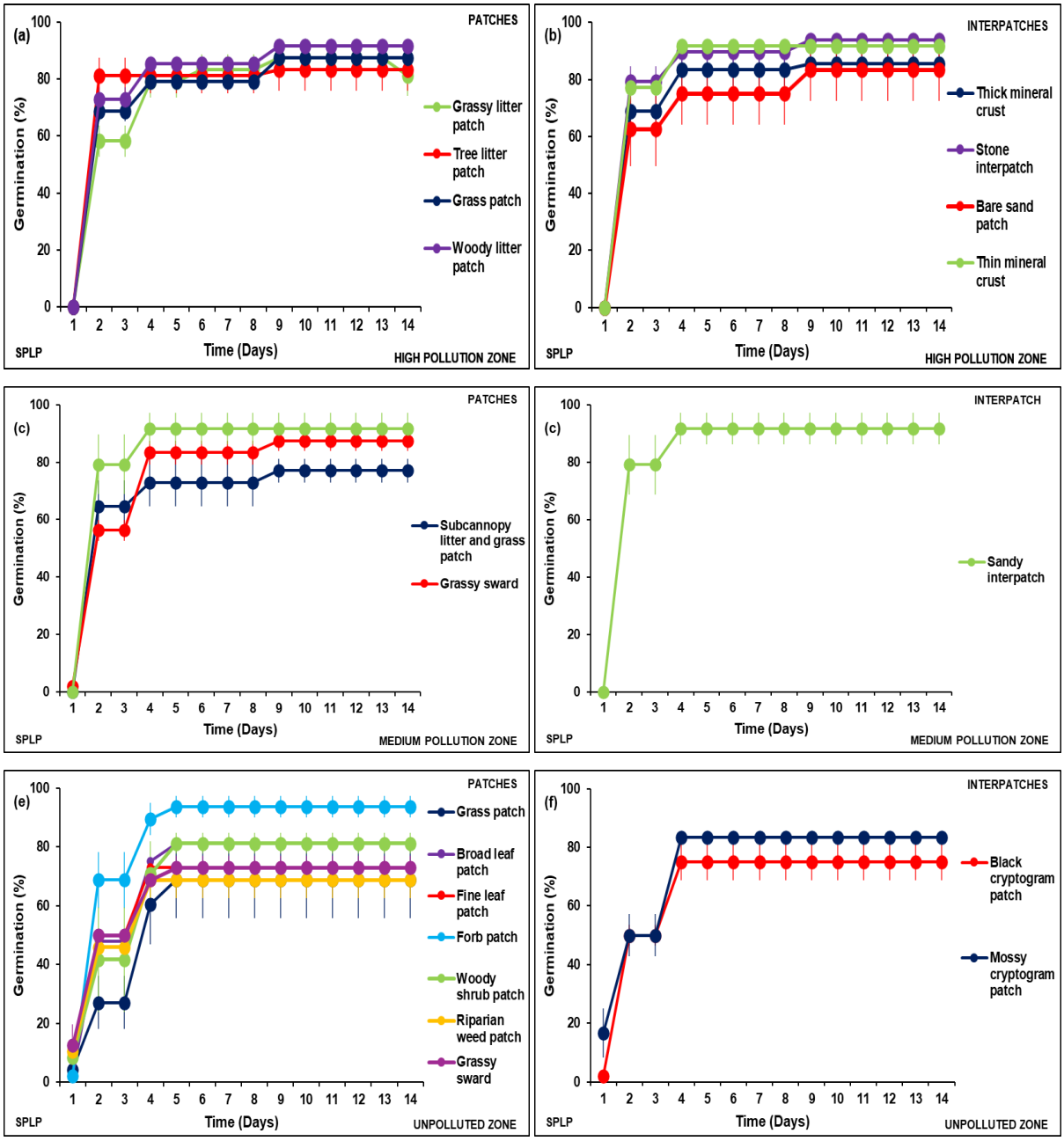


Figure 4.54: Time-germination curves for the mean (± standard error) germination of *Sorghum bicolor* after exposure to **SPLP** solutions of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. No significant differences existed in the peak germination in patches/interpatches amongst pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *S. bicolor* grown in SPLP solutions (**Figure 4.55**), most patch categories were inhibited from the high pollution zone, except the stone (SP) and the grass litter patch (GLP) which were stimulated compared to the plainest patch

category in that zone. All patches from the medium pollution zone were inhibited. There was a greater stimulatory response in the unpolluted zone, with just the fine leaf patch (FLP), riparian weed patch (RWP), and grassy sward (GS) having an inhibitory response.

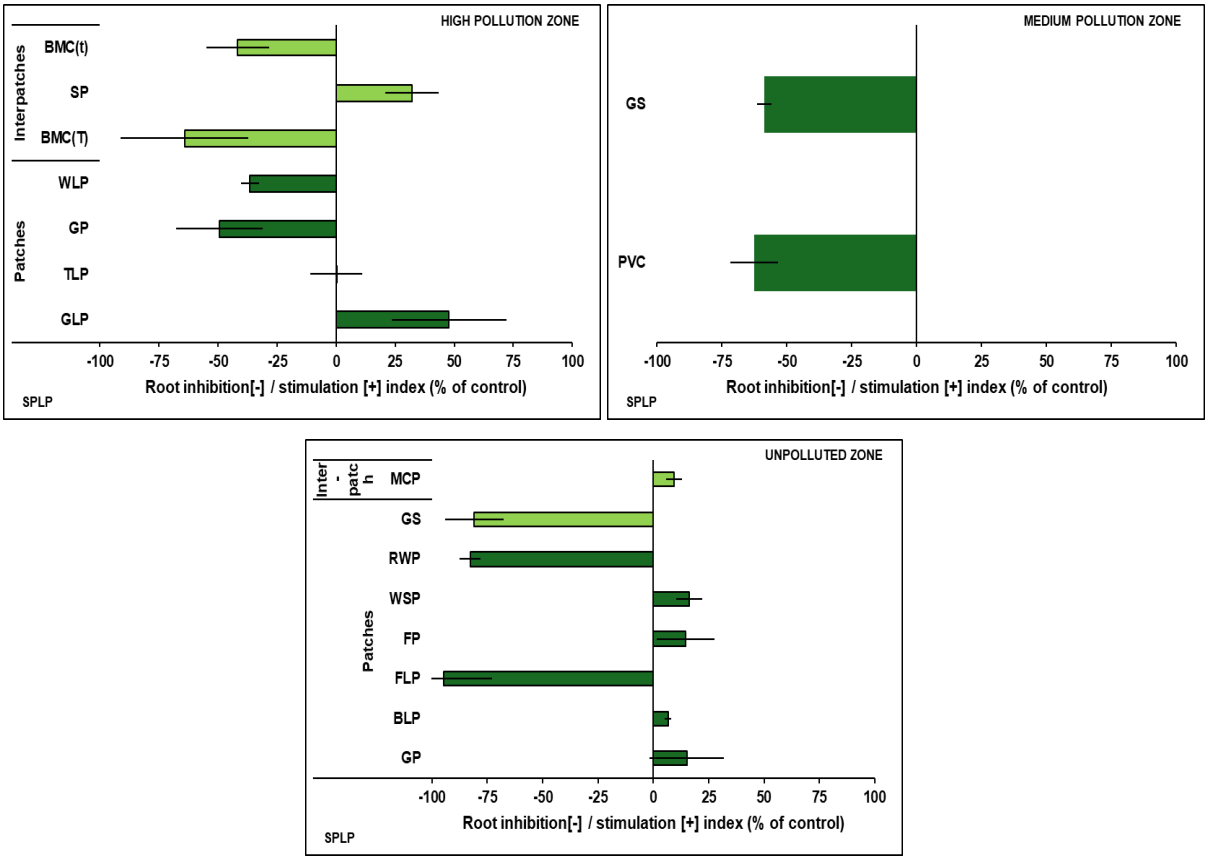


Figure 4.55: The effect of patch type on the inhibition/stimulation of root length of *Sorghum bicolor* grown in **SPLP** solutions **of** different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *S. bicolor* grown in SPLP solutions (**Figure 4.56**), the bare and tree patch categories from the high pollution zone had a stimulatory response, while the grass patch had an inhibitory effect. The bare patch category from the medium pollution zone had a stimulatory effect, compared to the grass and tree patches which had an inhibitory response of root length.

Figure 4.56 is found on the next page.

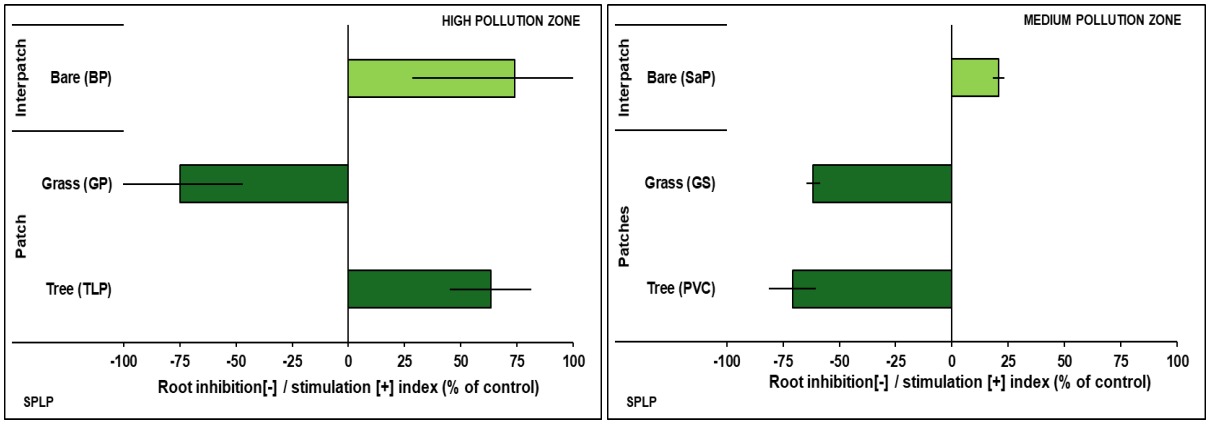


Figure 4.56: The effect of pollution level on the inhibition/stimulation of root length of *Sorghum bicolor* grown in polluted **SPLP** solutions (treatment) relative to unpolluted **SPLP** solutions (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.
Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

For the soil test (**Figure 4.57**), germination started on day 1 for each zone. The thick mineral crust of the high pollution zone did not stimulate any germination of *S. bicolor*. Peak germination along this zone was reached after day 2, and within a range of 63 – 82%. For the medium pollution zone, a peak was reached after day 6, within a range of 80 – 86%. For the unpolluted zone, germination started on day 1 and continued into day 3, resulting in a peak on day 6, within a range of 56 – 89%. No significant differences in peak germination were found along the zones or between patches and interpatches within each zone.

Figure 4.57 is found on the next page.

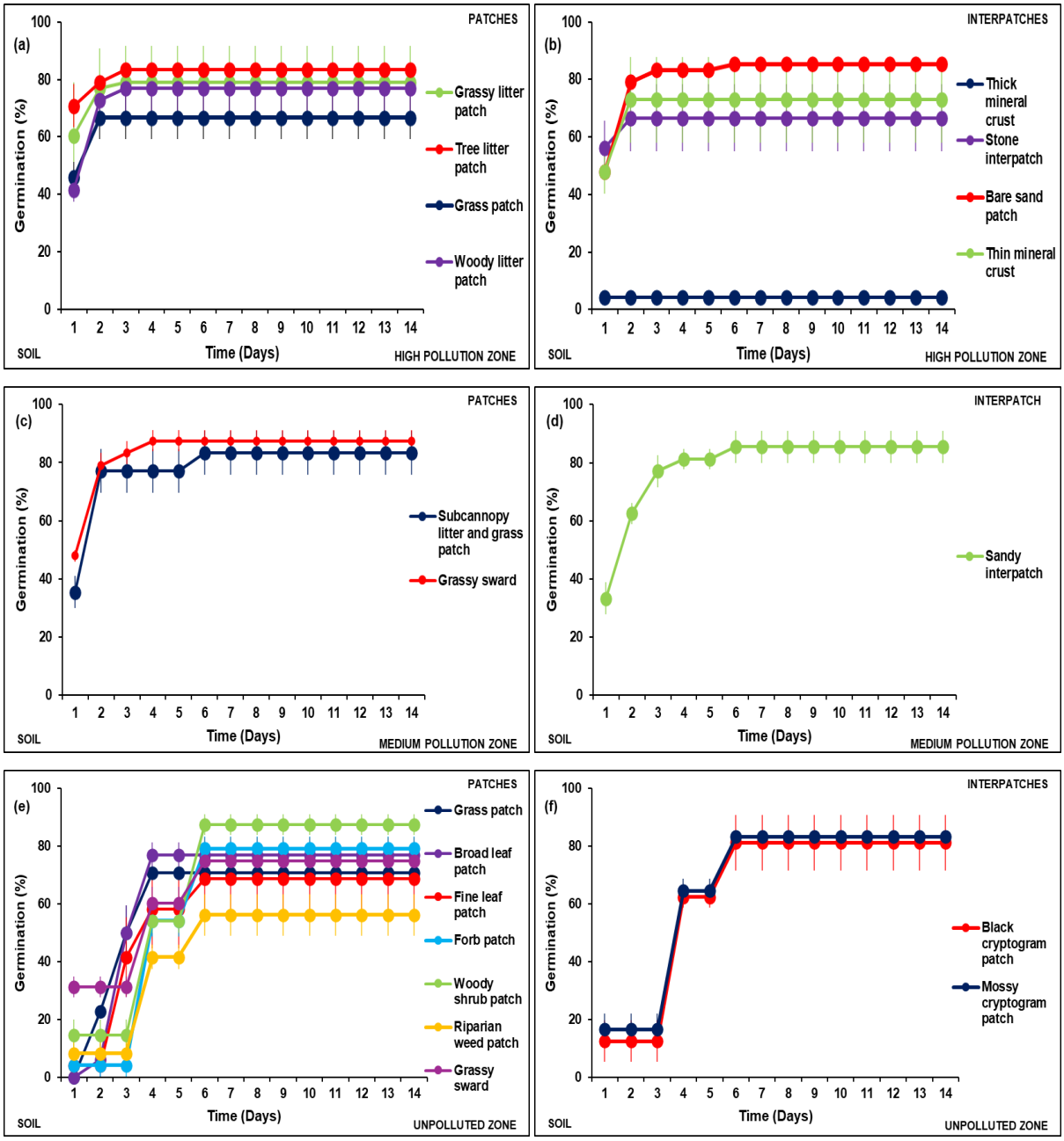


Figure 4.57: Time-germination curves for the mean (± standard error) germination of *Sorghum bicolor* after exposure to **SOILS** of patches and interpatches from the high pollution (**a** and **b**), medium pollution (**c** and **d**) and unpolluted (**e** and **f**) zones. Each curve shows when peak germination occurred within 14 days. Mean values are derived from a triplicate response of each patch/interpatch. There were no significant differences in the peak germination in patches/interpatches amongst pollution zones. There were no significant differences in peak germination between patches and interpatches of the pollution zones.

For the effect of patch type on the root length of *S. bicolor* grown directly in soils (**Figure 4.58**), the high pollution zone had an inhibitory response, except for the stone interpatch (SP).

Similarly, for the medium pollution zone, all patch categories had an inhibitory effect. The unpolluted zone had a total stimulatory response of root length.

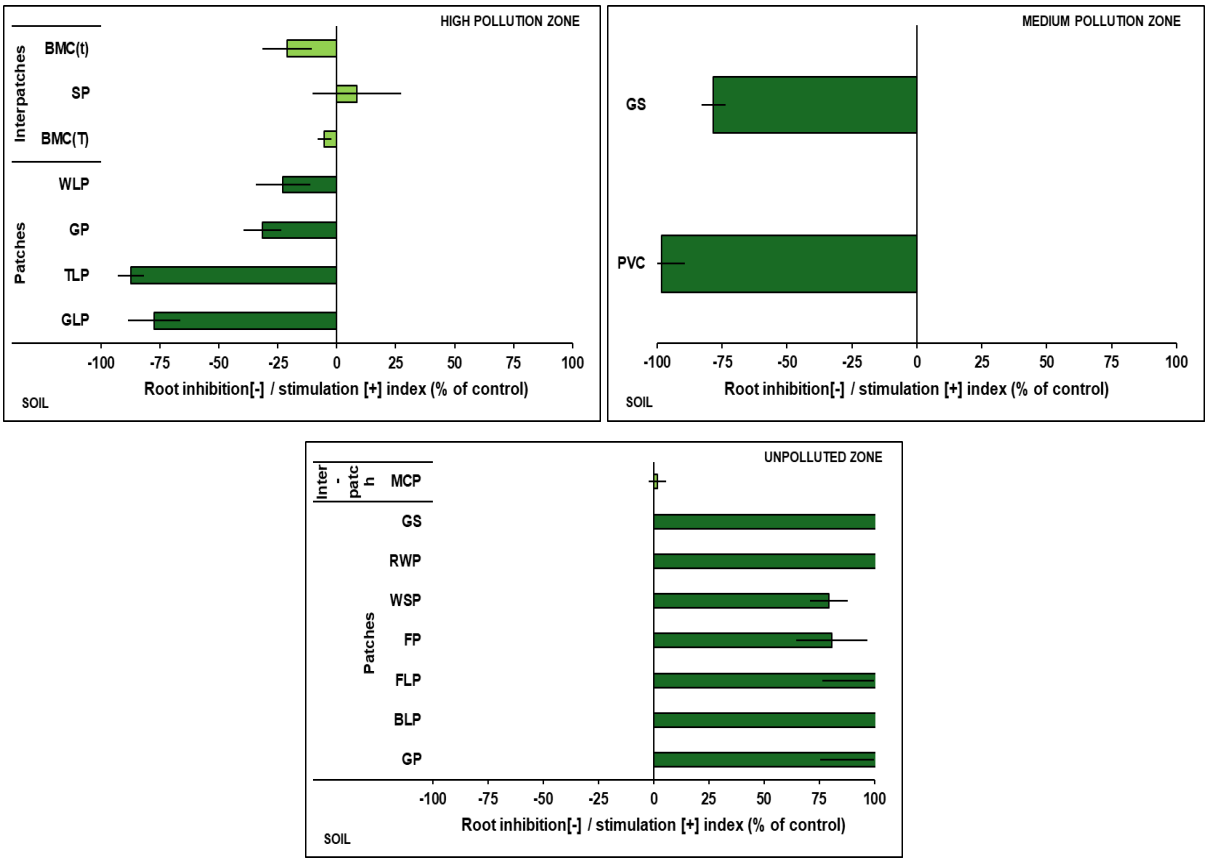


Figure 4.58: The effect of patch type on the inhibition/stimulation of root length of *Sorghum bicolor* grown in **SOILS** of different patch categories (treatment) relative to the plainest patch category found in that pollution zone (control). Each zone was divided into its respective patch/interpatch type (the medium polluted zone contained a single interpatch used as a control). Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note: Controls for each pollution zone are: (a) bare patch (BP) from the high pollution zone, (b) sandy patch (SaP) from the medium pollution zone, and (c) bare cryptogram patch (BCP) from the unpolluted zone.

For the effect of pollution level on the root length of *S. bicolor* grown in SPLP solutions (**Figure 4.59**), the grass and tree patches from the high and medium polluted zones had an inhibitory effect. Whereas the bare patch category had differing responses, with an inhibition along the high pollution zone and a stimulation along the medium pollution zone.

Figure 4.59 is found on the next page.

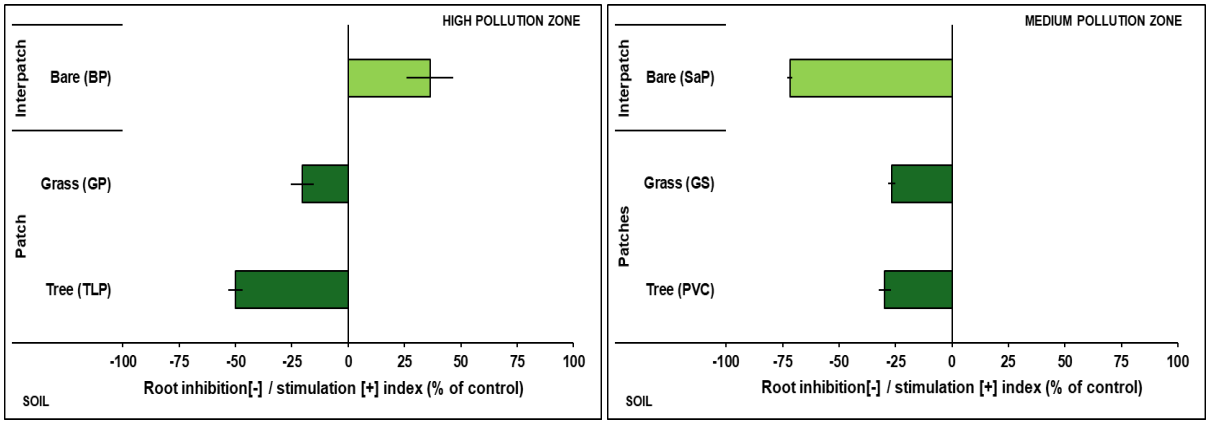


Figure 4.59: The effect of pollution level on the inhibition/stimulation of root length of *Sorghum bicolor* grown in polluted **SOILS** (treatment) relative to unpolluted **SOILS** (control). Patch categories that were similar between each pollution zone are included in this analysis: **tree**, **grass**, and **bare** patches. Patch categories from the unpolluted zone were used as controls. Each bar represents the mean (± standard error) of a triplicate response from each patch category.

Note:
For the high pollution zone: Tree – TLP (tree litter patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GP (grass patch) was compared to GS (grassy sward) from the unpolluted zone. Bare – BP (bare patch) was compared to BCP (black cryptogram patch) from the unpolluted zone. For the medium pollution zone: Tree – PVC (perennial vegetation patch) was compared to BLP (broad leaf patch) from the unpolluted zone. Grass – GS (grassy sward) was compared to GS (grassy sard) from the unpolluted zone. Bare: SaP (sandy patch) was compared to BCP from the unpolluted zone.

4.3.7. Correlation between soil properties and seed germination and root elongation

For each pollution zone, correlation coefficients were determined between peak seed germination and properties of the FLP, SPLP and soil test substances (**Figure 4.60**).

For the germination of *A. hybridus*, there was a negative relationship with pH from the high pollution zone (**Figure 4.60a**), and a negative relationship with electrical conductivity, and concentrations of Cu and Pb. For the medium pollution zone (**Figure 4.60b**), only correlations within patches are included in this explanation, since the single interpatch identified had only three replicates, which is an unreliable estimate. FLP germination in patches along this zone was positively influenced by soil organic matter, whereas pH had a negative relationship. For the unpolluted zone (**Figure 4.60c**), pH was negatively related to peak germination, and soil organic matter had a positive relationship.

For the peak germination of *D. australe*, pH, and soil organic matter of tests from the high pollution zone (**Figure 4.60a**) had a positive relationship, whereas electrical conductivity had a negative relationship. Only patches were considered in the medium pollution zone (**Figure 4.60b**), and no strong relationships were present. Soil organic matter and pH had a mixed

relationship in the unpolluted zone (**Figure 4.60c**), while electrical conductivity was negatively related to peak germination.

For *L. sativum*, peak germination was negatively related to electrical conductivity in the high-pollution zone (**Figure 4.60a**), while pH and soil organic matter had a positive relationship. For patches in the medium pollution zone (**Figure 4.60.b**), there was a negative relationship with electrical conductivity and a mixed response with soil organic matter. Only pH had a positive relationship in the unpolluted zone (**Figure 4.60c**).

For *M. sativa,* the high pollution zone (**Figure 4.60a**) had a mixed relationship with pH and peak germination, and only the soil interpatch tests displayed a positive and negative relationship with soil organic matter and electrical conductivity, respectively. Patches of the medium pollution zone (**Figure 4.60b**) showed a negative relationship with pH and a single positive relationship with Pb concentrations.

For *B. nigra,* there was a positive relationship between pH, soil organic matter and Pb concentrations and peak germination from the high-pollution zone (**Figure 4.60a**). For the medium pollution zone patches (**Figure 4.60b**), there was just a single negative correlation between soil organic matter and peak germination. For the unpolluted zone (**Figure 4.60c**), electrical conductivity showed a mixed relationship with peak germination, whereas pH and soil organic matter had a negative relationship.

For *S. bicolor*, pH had a single positive relationship with peak germination in the high-pollution zone (**Figure 4.60a**), whereas electrical conductivity had a negative relationship. For the patches of the medium pollution zone (**Figure 4.60b**), soil organic matter was negatively related to peak germination. The unpolluted zone (**Figure 4.60c**) had a mixed response to pH and a single negative relationship with electrical conductivity.

Figure 4.60 is found on the next page.

(a) High pollution zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 12 Interpatch: N = 12		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	**Patch**	0,09	0,58	0,13	-0,27	0,21	0,01	-0,44	-0,07	-0,16	-0,54	0,07	-0,26	0,12	-0,31	0,63	-0,31	-0,21	0,23
	Interpatch	0,40	0,76	0,87	0,53	0,56	0,76	0,51	0,35	0,92	-0,32	-0,22	0,70	0,64	0,40	0,86	0,33	0,28	0,95
Electrical conductivity	**Patch**	-0,16	0,43	-0,34	-0,20	0,23	0,34	-0,05	-0,53	0,19	0,06	-0,20	-0,30	-0,14	0,49	-0,15	0,00	0,34	-0,19
	Interpatch	-0,13	-0,62	-0,43	-0,44	-0,61	-0,35	-0,31	-0,25	-0,47	0,15	0,14	-0,61	-0,46	-0,09	-0,29	-0,92	-0,01	-0,28
Soil organic matter	**Patch**	0,19	-0,09	-0,54	-0,13	0,16	0,20	0,04	-0,40	-0,47	-0,30	0,07	-0,10	-0,24	0,22	-0,23	-0,04	0,44	-0,32
	Interpatch	0,42	0,51	0,43	0,50	0,12	0,55	0,38	-0,15	0,60	0,00	-0,14	0,67	0,35	-0,13	0,50	0,22	-0,10	-0,32
Cu	**Patch**	-	-0,59	-	-	0,47	-	-	0,07	-	-	-0,41	-	-	0,54	-	-	0,03	-
	Interpatch	-	-0,61	-	-	-0,54	-	-	-0,17	-	-	0,29	-	-	-0,13	-	-	-0,16	-
Pb	**Patch**	-	-0,46	-	-	0,39	-	-	-0,17	-	-	-0,44	-	-	0,67	-	-	0,30	-
	Interpatch	-	-0,59	-	-	-0,49	-	-	-0,16	-	-	0,37	-	-	-0,13	-		-0,19	-

(b) Medium pollution zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 6 Interpatch: N = 3		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	**Patch**	-0,01	-0,32	0,16	0,81	-0,70	0,48	0,02	-0,09	0,41	0,35	-0,74	-0,61	-0,02	0,32	-0,14	0,42	-0,30	-0,03
	Interpatch	0,98	-0,78	1,00	-0,79	-0,70	-0,79	-0,14	-0,16	0,99	0,92	-0,99	0,32	0,79	-0,99	0,37	-0,99	0,88	-0,89
Electrical conductivity	**Patch**	0,42	0,49	-0,28	-0,78	0,22	0,16	0,25	-0,71	-0,77	-0,28	-0,06	-0,13	-0,43	0,25	0,07	0,29	-0,53	0,21
	Interpatch	0,90	0,88	0,97	-0,92	0,82	-0,92	0,11	-0,02	0,93	0,99	1,00	-0,06	0,92	1,00	0,61	-0,99	-0,95	-0,98
Soil organic matter	**Patch**	0,54	-0,10	-0,23	-0,76	0,23	-0,29	0,52	0,26	-0,64	-0,03	0,37	0,26	-0,87	0,02	0,20	0,74	0,19	0,52
	Interpatch	-0,98	-1,00	-0,92	0,50	-0,99	0,50	0,50	0,50	-0,96	-0,70	-0,87	0,66	-0,50	-0,86	0,00	0,86	0,98	0,65
Cu	**Patch**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb	**Patch**	-	-0,60	-	-	0,53	-	-	0,37	-	-	0,80	-	-	0,26	-	-	0,10	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

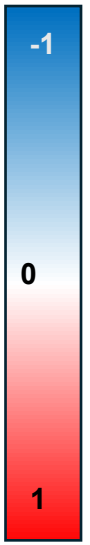


Figure 4.60 is continued to the next page.

(c) Unpolluted zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 21 Interpatch: N = 6		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	**Patch**	0,02	0,45	-	0,00	-0,01	-	0,06	0,47	-	0,16	-0,33	-	0,19	0,34	-	-0,21	-0,10	-
	Interpatch	-0,82	-0,51	-	-0,37	-0,52	-	0,58	-0,24	-	0,27	-0,66	-	-0,54	-0,55	-	-0,52	0,72	-
Electrical conductivity	**Patch**	0,10	0,28	-	-0,05	-0,13	-	0,00	0,21	-	-0,22	0,09	-	0,14	0,09	-	0,01	0,06	-
	Interpatch	0,27	-0,14	-	-0,49	-0,11	-	0,43	0,05	-	0,51	-0,84	-	0,60	-0,47	-	-0,23	0,82	-
Soil organic matter	**Patch**	-0,23	0,07	-0,22	-0,07	-0,19	-0,04	0,19	0,18	0,45	0,29	-0,11	0,10	0,34	-0,10	0,10	0,12	-0,10	0,36
	Interpatch	0,20	0,54	0,37	0,45	0,35	0,18	-0,49	-0,05	-0,40	0,13	0,27	0,26	-0,17	-0,59	-0,71	-0,18	-0,08	-0,37
Cu	**Patch**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb	**Patch**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

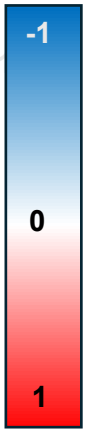


Figure 4.60: Heat map of Pearson correlation coefficients between peak germination and soil properties (pH, electrical conductivity soil organic matter, and concentrations of Cu and Pb under the **FLP, SPLP,** and **soil** test conditions in the from the (**a**) high pollution zone, (**b**) medium pollution zone, and (**c**) unpolluted zone. Correlation results are presented for: *Amaranthus hybridus*, *Dactyloctenium australe*. *Lepidum sativum*, *Medicago sativa*, *Brassica nigra*, and *Sorghum bicolor*. Positive correlations are highlighted in red, and negative correlations are highlighted in blue. A dash (-) indicates that it was not applicable to test for a correlation. Interpatches of the medium pollution zone were included tentatively and only for completion since a sample size of three is not a reliable estimate of any relationship.

Figure 4.61 presents correlations between root length and the properties of FLP, SPLP, and soil tests**.**

Electrical conductivity, soil organic matter, and concentrations of Cu and Pb were all negatively related to the root length of *A. hybridus* in the high pollution zone (**Figure 4.61a**). In contrast, pH displayed a positive correlation. For patches of the medium pollution zone (**Figure 4.61b**), electrical conductivity had a positive relationship with root length. Only pH showed a negative relationship with root length in the unpolluted zone (**Figure 4.61c**).

For *D. australe*, there was a positive relationship between pH and root length, and a negative relationship between soil organic matter and Pb concentrations with root length from the high pollution zone (**Figure 4.61a**). For the patches of the medium pollution zone (**Figure 4.61b**), single correlations were found for pH (positive) and soil organic matter and Pb concentrations (both negative). For the unpolluted zone (**Figure 4.6c**), there was a single negative correlation between electrical conductivity and root length.

For *L. sativum*, there was a mixed response with pH, soil organic matter and electrical conductivity from the high pollution zone (**Figure 4.61a**), whereas the concentrations of Pb and Cu had a negative relationship with root length. There was another mixed response between pH from the patches of the medium pollution zone (**Figure 4.61b**), while electrical conductivity and soil organic matter both had a single negative response. pH was the only relationship from the unpolluted zone (**Figure 4.6c**), and this was positive.

The root length of M. sativa from the high pollution zone (**Figure 4.61a**) had a mixed relationship with pH, electrical conductivity, and soil organic matter. Concentrations of Cu and Pb were negatively related to root length at this zone. For patches from the medium pollution zone (**Figure 4.61b**), only the soil tests showed a positive relationship with pH and electrical conductivity. For the unpolluted zone, there was a negative relationship between root length, electrical conductivity, and soil organic matter.

For *B. nigra*, pH negatively influenced root length from the high pollution zone (**Figure 4.61a**), and the concentration of Pb followed suit, whereas soil organic matter was positively related to root length. The concentration of Pb in the medium polluted zone (**Figure 4.61b**) was the only property that related to root length. There was a mixed response for pH from the unpolluted zone (**Figure 4.61c**).

For *S, bicolor*, pH was positively related to root length from the high pollution zone (**Figure 4.61a**), whereas electrical conductivity and the concentrations of Cu and Pb had a negative relationship. Soil organic matter had a mixed relationship with root length along this zone. For the patches from the medium pollution zone (**Figure 4.61b**), soil organic matter had a positive relationship with root length, while Pb concentrations had a negative relationship. The unpolluted zone (**Figure 4.61b**) showed a positive relationship towards pH and electrical conductivity, whereas soil organic matter had a mixed relationship.

Figure 4.61 is found on the next page.

(a) High pollution zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 12 Interpatch: N = 12		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	Patch	-0,03	0,20	-0,09	-0,25	0,50	0,32	0,06	0,22	-0,08	-0,46	0,17	0,43	-0,04	0,19	0,30	0,12	0,37	0,39
	Interpatch	0,59	0,74	0,67	0,52	0,38	0,54	0,71	0,53	0,56	0,24	0,55	0,51	0,74	0,54	0,59	0,73	0,42	0,70
Electrical conductivity	Patch	-0,52	-0,42	-0,18	-0,23	-0,46	0,45	0,07	-0,36	0,16	-0,30	-0,43	-0,27	-0,40	0,00	-0,05	-0,21	-0,21	-0,48
	Interpatch	-0,26	-0,68	-0,07	-0,17	-0,37	0,07	-0,39	-0,60	-0,05	0,24	-0,72	-0,10	0,22	-0,45	0,20	-0,54	-0,39	-0,52
Soil organic matter	Patch	-0,27	-0,57	-0,42	-0,36	-0,54	0,17	-0,39	-0,51	-0,46	-0,42	-0,57	-0,42	-0,22	0,12	0,01	0,06	-0,31	-0,52
	Interpatch	0,46	0,34	0,32	0,29	0,42	0,45	0,44	-0,08	0,27	0,36	0,65	0,57	0,51	-0,11	-0,14	0,43	0,05	0,28
Cu	Patch	-	-0,55	-	-	-0,32	-	-	-0,24	-	-	-0,54	-	-	-0,24	-	-	-0,48	-
	Interpatch	-	-0,74	-	-	-0,03	-	-	-0,59	-	-	-0,59	-	-	-0,41	-	-	-0,51	-
Pb	Patch	-	-0,78	-	-	-0,62	-	-	-0,43	-	-	-0,65	-	-	-0,26	-	-	-0,72	-
	Interpatch	-	-0,85	-	-	0,02	-	-	-0,63	-	-	-0,56	-	-	-0,41	-	-	-0,67	-

(b) Medium pollution zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 6 Interpatch: N = 3		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	Patch	-0,13	0,22	0,72	-0,12	0,58	0,43	-0,62	0,05	0,58	0,36	-0,11	0,56	0,43	0,71	0,21	0,45	-0,01	0,14
	Interpatch	-0,48	0,58	-0,99	-0,27	0,24	-0,96	-0,81	-	-0,98	-0,03	-	-0,97	-	-0,81	-0,75	-0,31	-	0,30
Electrical conductivity	Patch	-0,25	0,85	0,16	0,04	0,51	0,33	0,80	0,30	0,32	-0,42	-0,22	0,53	-0,13	0,30	0,39	-0,12	0,24	0,45
	Interpatch	-0,68	-0,71	-0,93	0,10	-0,41	-0,86	-0,64	-	-1,00	-	-	-1,00	-	0,69	-0,55	-	0,98	0,03
Soil organic matter	Patch	-0,24	0,39	-0,17	0,33	-0,46	0,37	0,78	0,36	-0,10	-0,23	-0,08	0,27	-0,31	0,26	0,14	0,51	-0,16	0,31
	Interpatch	0,11	0,96	0,96	0,51	0,80	0,99	0,97	-	0,83	-	-	0,81	-	-0,26	0,95	-0,08	-0,77	-0,63
Cu	Patch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb	Patch	-	-0,71	-	-	-0,91	-	-	-0,39	-	-	-0,10	-	-	-0,69	-	-	-0,80	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

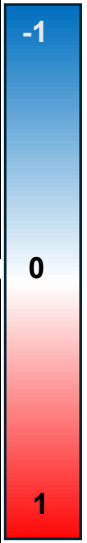


Figure 4.61 continues to the next page.

(c) Unpolluted zone		*Amaranthus hybridus*			*Dactyloctenium australe*			*Lepidum sativum*			*Medicago sativa*			*Brassica nigra*			*Sorghum bicolor*		
Patches: N = 21 Interpatch: N = 6		FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL	FLP	SPLP	SOIL
pH	**Patch**	0,06	0,42	-	-0,27	0,16	-	0,37	0,54	-	-0,03	-0,13	-	-0,16	0,57	-	0,31	0,02	-
	Interpatch	-0,81	-0,18	-	-0,37	-0,23	-	-0,17	0,32	-	-0,32	-0,21	-	-0,53	0,24	-	-0,04	0,63	-
Electrical conductivity	**Patch**	-0,01	0,03	-	0,03	0,11	-	0,15	0,09	-	-0,52	-0,05	-	-0,28	0,03	-	-0,07	0,07	-
	Interpatch	-0,33	0,00	-	-0,79	-0,17	-	-0,38	0,46	-	0,30	0,02	-	0,18	0,36	-	-0,17	0,82	-
Soil organic matter	**Patch**	-0,30	0,17	-0,18	-0,17	0,47	0,05	-0,27	0,19	0,17	0,29	0,29	-0,66	-0,17	-0,06	0,05	-0,49	-0,09	0,02
	Interpatch	0,36	-0,10	-0,14	0,00	-0,42	0,42	0,37	0,47	0,07	0,24	0,24	0,23	-0,25	0,17	0,60	0,71	-0,57	-0,15
Cu	**Patch**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb	**Patch**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Interpatch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

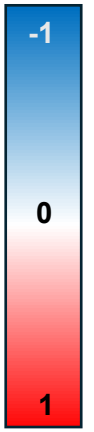


Figure 4.61: Heat map of Pearson correlation coefficients between root lengths and soil properties (pH, electrical conductivity soil organic matter, and concentrations of Cu and Pb under the **FLP, SPLP,** and **soil** test conditions in the from the (**a**) high pollution zone, (**b**) medium pollution zone, and (**c**) unpolluted zone. Correlation results are presented for: *Amaranthus hybridus*, *Dactyloctenium australe*. *Lepidium sativum*, *Medicago sativa*, *Brassica nigra*, and *Sorghum bicolor*. Positive correlations are highlighted in red, and negative correlations are highlighted in blue. A dash (-) indicates that it was not applicable to test for a correlation. Interpatches of the medium pollution zone were included tentatively and only for completion since a sample size of three is not a reliable estimate of any relationship.

4.4 Discussion

Overall, seed germination and root elongation were most affected in the highly polluted zones, with sensitivity varying among species. Patch type had little influence under polluted conditions but showed clearer effects in the unpolluted zone, indicating that contamination overrides natural patch–interpatch dynamics in determining plant responses.

4.4.1. *Amaranthus hybridus*

Regarding seed germination, *A. hybridus* exhibited a higher peak germination in the high pollution zone than in the unpolluted zone for the patch and interpatch FLP, SPLP, and soil tests. This outcome did not vary between patches and interpatches, except for the SPLP tests from the medium polluted zone (although this zone only compared two patches to a single interpatch). For root elongation, *A. hybridus* grew longer in patches compared to interpatches for each test, due to the lower pH and electrical conductivity compared to interpatches. The higher concentration of Cu and Pb was also correlated with the smaller root lengths along the interpatches, mostly for the SPLP solutions, which can mobilise and increase the leachable concentration of salts and Cu and Pb (Wuana and Okieimen, 2011). Additionally, the root length from seeds grown in the medium pollution zone was longer than in the high pollution zone, in reference to the root length from the unpolluted zone.

The strong correlation between pH and peak germination may imply that the lower pH could reduce seed dormancy and influence premature radicle formation due to increased enzyme activity (Duke and Egley, 2018). Even though germination was quicker in the polluted tests, root length was shorter, indicating that factors such as low pH and high electrical conductivity led to faster germination and may have increased damage to root cells as germination proceeded, resulting in shorter roots (Shahid *et al*., 2014).

4.4.2. *Dactyloctenium australe*

Dactyloctenium australe started to germinate at a faster rate and had a higher peak germination along the unpolluted zone compared to the high and medium-polluted zones. This result was consistent with the FLP, SPLP, and soil tests. Due to the low pH and high Cu along the high and medium pollution zones. The high organic matter may also result in increased solubility of Cu at these zones, inhibiting germination rates (Fesuk, 2006). Differences between the patch and interpatch germination rates were only present for soil tests from the unpolluted zone.

There was no difference between the root length of *D. australe* in patches and interpatches for the FLP and SPLP tests, however, root length due to the direct soil tests did result in shorter roots in patches and interpatches from the high and medium pollution zones compared to the unpolluted zone. It should be noted that the shorter root length in soil tests may be due to soil compacting root growth compared to the aqueous solutions (Bengough, 2003). Therefore, *D. australe* was sensitive to pH and the high concentration of Cu.

4.4.3. *Lepidium sativum*

Germination of *L. sativum* commenced at a faster rate in the high and medium pollution zones compared to the unpolluted zone for the FLP and SPLP tests, but not for the direct soil tests. Additionally, peak germination was much higher during the FLP tests from the high and medium-pollution zones compared to the unpolluted zone, but this was not observed for the SPLP and soil tests. According to the correlation results, these results may be influenced by the acidic pH from the high and medium pollution zones and soil organic matter. Differences between patch and interpatch peak germination were only present in soil tests conducted in the unpolluted zone.

Root lengths of *L. sativum* appeared to respond similarly to the effect of patch type between the high and unpolluted zones for each test. However, for the effect of pollution in the FLP tests, the root length of *L. sativum* was significantly longer in the high and medium pollution zones compared to the unpolluted one, and similarly for the grass and tree patches during the SPLP tests. This pattern was the opposite during the soil tests; root length was longer in the unpolluted soils compared to the polluted soils. Correlation results point to pH, EC, and concentrations of Cu and Pb affecting the root length results.

These results were not expected, since the high concentrations of Cu and Pb and low pH should inhibit root length and germination (Pavel *et al.,* 2013). However, low concentrations of heavy metals and an acidic pH are known to stimulate root length and germination for the initial period of growth (Nouri *et al*., 2020). Therefore, *L. sativum* displayed a high degree of tolerance to pH, salinity, and concentrations of Cu and Pb. The greater germination rate and root length may also point to a hormetic effect, but this can only be concluded with tests of a longer duration.

4.4.4. *Medicago sativa*

Germination of *M. sativa* started earlier in the high pollution zone compared to the medium and unpolluted zones for each test. Additionally, a higher peak germination was recorded for the high and medium pollution zones compared to the unpolluted zone. Differences between patch and interpatch peak germination were only present in FLP tests of the unpolluted zone. Correlation results show that the acidic pH, high salinity, and soil organic matter influenced the results from the high pollution zone, while pH and the concentration of Pb influenced the results from the medium pollution zone.

The root length of *M. sativa* was longer in interpatches of the high and medium pollution zones compared to the unpolluted zone, specifically for the FLP tests and to a smaller extent for the soil tests, whereas the opposite occurred during the SPLP test. When considering the effect of pollution levels, the polluted zones appeared to respond in an inconsistent pattern in relation to the unpolluted zone. Correlation results point to soil organic matter, pH, and concentrations of Cu and Pb leading to these effects along the high pollution zone.

The stimulation of root length and germination along the high and medium pollution zones was unexpected, considering the sensitivity of *M. sativa* to acidity and heavy metal concentrations (Aydinalp and Marinova, 2009). Therefore, *M. sativa* showed tolerance to pH, salinity, and concentrations of Cu and Pb.

4.4.5. *Brassica nigra*

The initial and peak germination of *B. nigra* did not differ between tests or pollution zones. No difference was also evident between patches and interpatches of each zone. The root length of *B. nigra* in patches and interpatches did not differ between pollution zones, however, the root length was longer in the unpolluted zone compared to the polluted zones. According to the correlation results, pH was the common property that led to the results in the high and medium-polluted zones. Therefore, *B. nigra* had a slight sensitivity in root length due to the low pH.

4.4.6. *Sorghum bicolor*

Germination of *S. bicolor* reached peak germination much quicker in the high and medium pollution zones compared to the unpolluted zone, which was a consistent pattern for the FLP, SPLP and soil tests. However, the root length of *S. bicolor* was greater in the unpolluted zones compared to the high and medium-polluted zones, except in the FLP solutions. This

pattern was especially evident when considering the effect patch type; for which the length in the unpolluted zone was greater than the polluted zones. Correlation results indicate that pH, EC, Pb and Cu contributed to the root length results, while pH and electrical conductivity promoted the germination rate results. The germination of *S. bicolor* is known to be sensitive towards pH and concentrations of Cu and Pb (Ertekin *et al.,* 2020).

Potential limitations to this study design include:

- With *L. sativum,* a possible hormetic effect could be understood if the test were conducted for a longer time.
- Simply correlating the soil properties with the total percentage germination and root lengths does not imply the causation of these results. More tests may be necessary to confirm these effects.
- The current study shows the short-term effect of ecotoxicity on germination rate and root elongation, though long-term outcomes may differ.
- The sample size of patches and the interpatch in the medium pollution zone was too small to have any meaningful interpretation.

4.5 Conclusion

The aim of this chapter was to assess the phytotoxicity of soils and leachate solutions to the germination and root elongation of six species of vascular plants, considering the effect of patch type and pollution level. Seeds were grown in three different substrata. The results show that patch–interpatch distinctions have limited influence under polluted conditions, as contamination levels largely determine biological responses. In unpolluted zones, patch type still contributes to differences in plant performance, reflecting functional heterogeneity. However, in polluted soils, this spatial structure breaks down, confirming that contamination overrides the ecological role of patch type and weakens its predictive value within the LFA framework. *Amaranthus hybridus* and *D. australe* showed sensitivity in terms of germination rate and root length. *Lepidium sativum* showed a great tolerance to the high and medium pollution zones. The germination rate and root length of M. sativa showed tolerance to low pH, high salinity, Cu and Pb. *Brassica nigra* exhibited no significant response. The root length of *S. bicolor* was sensitive to low pH and high salinity, whereas the germination rate implies a degree of tolerance. Therefore, root length is the most sensitive seedling test of ecotoxicity. However, the germination rate results can allow a deeper insight to the sensitivity and/or tolerance results.

Chapter five – Synthesis and conclusion

5.1 Introduction

The Landscape Function Analysis (LFA) model has been used widely as a monitoring procedure to rapidly assess landscape restoration or the extent of rehabilitation along a degraded site (Tongway and Hindley, 2004). Initially developed for use along Australian rangelands, it has now been adopted as a model for examining the extent of landscape dysfunction due to degradation stemming from mining and smelting processes. Since its adoption by the mining industry, the underlying conceptual model has not changed to accommodate the effects that heavy metal contaminants can have on the landscape. While the LFA model does indirectly consider the soil physicochemical and biological properties in its design, it does not consider how heavy metal contamination and salinity can alter these properties. This poses the need to directly assess these properties to acquire a more holistic view of the landscape in question, thereby potentially complementing the existing framework.

This study aimed to investigate whether integrating soil physicochemical and ecotoxicological properties from patches and interpatches along a pollution gradient can enhance the ability of the LFA model to evaluate landscape function. The informing science behind this aim is based on the Trigger-Transfer-Reserve-Pulse (TTRP) framework, which views a landscape as a self-sustaining system with a balanced capture and cycle of water, nutrients, and organic matter (Ludwig and Tongway, 1997). When heavy metal contaminants are introduced into the system, the feedback loops maintaining the TTRP model could be disrupted through altered soil structure and a decoupling of the biotic-abiotic feedback mechanism in the soil (Rajkumar *et al*., 2012). This can result in a mismatch between the results of the LFA model and the true state of the landscape.

This chapter aims to synthesise the results from the three data chapters of this dissertation to determine how physicochemical and ecotoxicological factors can complement the traditional LFA model, and to evaluate how these combined findings can address the central aim. I will first integrate the findings on landscape function and soil physicochemical properties (Chapter 2), followed by soil leachate toxicity to aquatic organisms (Chapter 3), and then phytotoxicity to vascular plants (Chapter 4). I will then conclude by reflecting on the

implications of these findings for the LFA framework by proposing an integrated conceptual model and end by identifying recommendations for future work.

5.2 Interpretation of key findings

5.2.1. Landscape function and soil properties

The research question that guided chapter two was: "What is the relationship between LFA indices (stability, infiltration, and nutrient cycling) and soil properties of patches and interpatches across a gradient of soil pollution?". To answer this question, I used the LFA model on site along three zones of varying pollution levels (high, medium, and unpolluted). After analysing the results of the final three indices of the LFA model (stability, infiltration, and nutrient cycling) from each pollution zone, only the unpolluted zone showed significant differences between patches and interpatches. In contrast, the high- and medium-pollution zones did not show significant patch/interpatch differences. This may suggest that the LFA model may not adequately capture landscape function in the presence of contaminants. It may also suggest a deviation in spatial heterogeneity due to the presence of contaminants. In addition, correlations between the LFA indices and soil physicochemical properties in terms of pH, electrical conductivity, soil organic matter, and metal concentrations (Cu, Pb, Ni, and SO_4) were mostly weak.

Overall, these results indicate that heavy metal contamination interferes with the relationships that underpin the TTRP framework. In a healthy landscape, patches serve as resource reservoirs and are then cycled through the landscape system (Tongway and Hindley, 2004). Heavy metal contamination can result in a change in soil structure, reduced biological activity, and a reduction in infiltration and nutrient cycling (Ginocchio *et al*., 2004). Contaminated landscapes may therefore have a reduction in the patch-interpatch relationships, and in the process, reduce overall resource retention within the landscape. While the LFA model is a useful tool in physically degraded environments, it may fail to detect the dysfunction due to heavy metal contaminants.

5.2.2. Soil leachate toxicity to *Daphnia magna* and *Aliivibrio fischeri*

Chapter three aimed to answer the research question: "How does the ecotoxicity of soil leachates to *D. magna* and *A. fischeri* change between patches and interpatches across a gradient of soil pollution, and how is this related to the properties of each solution?". To answer this question, I assessed the sensitivity of *D. magna* immobilisation and *A. fischeri*

inhibition of bioluminescence after exposure to soil leachates. *Daphnia magna* revealed high immobilisation rates in samples from the high and medium pollution zones, corresponding to the elevated concentrations of soluble metals and high salt levels. Whereas *A. fischeri* revealed both inhibition and occasional stimulation in response to samples from the high and medium pollution zones. This is likely to indicate a stress-hormesis effect (Agathokleous *et al*., 2020).

These results cover the landscape-scale interpretation by linking terrestrial contamination to aquatic risk. The leachate assays simulate the "transfer" component of the TTRP framework, but functioning at contaminated conditions, where rainfall mobilises soluble metals into the groundwater. Instead of transferring nutrients through the system, contaminants are now transferred. When integrating these results with the results from Chapter 2, the patches and interpatches that scored high stability indices mask a deeper functional impairment, as toxicity is only revealed once the contaminants are mobilised. Therefore, the aquatic assays reveal a plausible extension to the scope of the LFA model, since these results indicate that a functional assessment of the landscape should consider connections between soil processes and downstream ecological receptors.

5.2.3. Phytotoxicity to vascular plants

Chapter four addressed the following research question: "How does patch/interpatch type influence the phytotoxicity of soils and leachate solutions to plants, and how is this related to the properties of each test substrate?". The results from the plant bioassays revealed species-specific and concentration-dependent responses across each pollution zone. In general, *Amaranthus hybridus* and *Dactyloctenium australe* revealed a greater sensitivity to contaminants by the strong inhibition of germination and root elongation. *Lepidium sativum*, *Sorghum bicolor*, and *Medicago sativa* displayed moderate tolerance, whereas *Brassica nigra* showed little to no sensitivity to any substrate. Root elongation proved to be more sensitive to contamination as compared to germination alone, making it a reliable indicator of toxicity.

Patch type did not influence the phytotoxicity results for the high and medium pollution zones, referencing a deviation in spatial heterogeneity due to the presence of contaminants, as uncovered in **5.2.1**. Whereas in the unpolluted zone, patches generally revealed greater germination and root elongation than the interpatches. This is consistent with the LFA model in that patches are areas of resource accumulation, whereas interpatches are areas of resource

loss. Since the high and medium pollution zones do not show much difference in terms of a response to patch type, it can be attributed to heavy metal contaminants overriding the effect of spatial heterogeneity (Zeng *et al*., 2023). Integrating these results with the results of chapters two and three reveals a pattern: as contamination increases, physicochemical and ecotoxicological results communicate a dysfunctional landscape, whereas the LFA model appears insensitive.in comparison. Therefore, assessing phytotoxicity provides a critical biological insight into landscape degradation that the three indices (stability, infiltration, and nutrient cycling) of the LFA model fail to achieve.

5.3 Addressing the research problem and aim

The overarching problem statement identified in Chapter 1 stated that while the LFA model is widely used by the mining industry, it may not serve as an accurate portrayal of the landscape function under heavy metal stress. This is due to the heavy metal contaminants altering the fundamental dynamics that the LFA model aims to quantify and was shown throughout all three data chapters.

Chapter two showed that the final LFA indices lose predictive power under high concentrations of heavy metal contaminants, indicating that the visual and physical surface indicators assessed above-ground may not correspond to the functional processes occurring below-ground level. Chapter three revealed that leachates derived from these soils result in measurable toxicity to aquatic organisms, indicating that contamination has functional consequences beyond the terrestrial area. Chapter four extended this finding to terrestrial plants by indicating root inhibition and varied germination responses. These findings (**Figure 5.1**) demonstrate that integrating physicochemical and ecotoxicological metrics with the LFA model provides a more comprehensive understanding of landscape function along heavy metal contaminated sites.

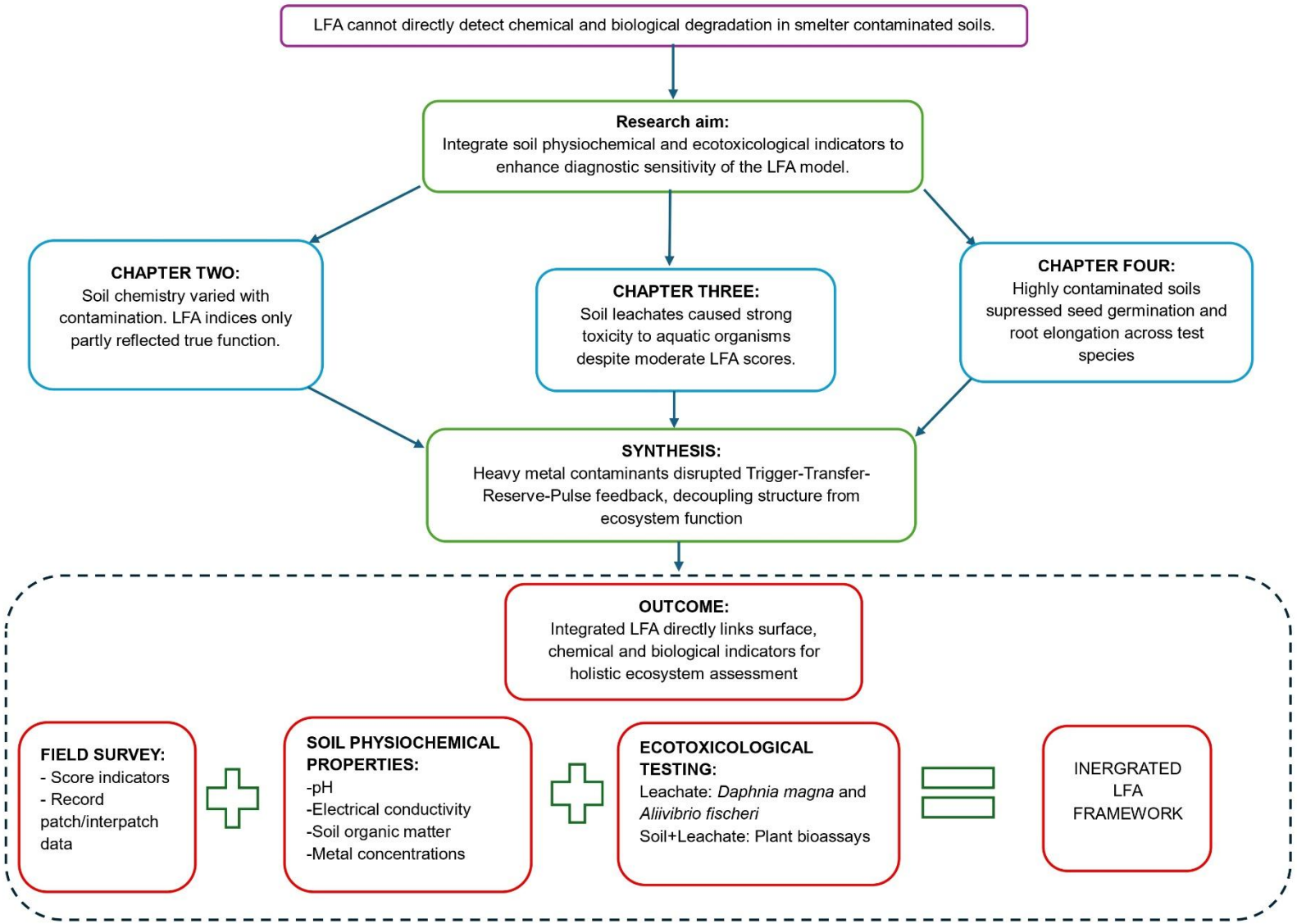


Figure 5.1: Synthesis of results and conceptual development of the integrated Landscape Function Analysis (LFA) framework

5.4 Future directions

Future research should aim to use this integrated LFA framework across diverse landscapes and climatic regions that display smelter-related contamination. Longitudinal monitoring may also be able to determine how this integrated framework responds to rehabilitation interventions over time, providing baseline monitoring indices for functional recovery (McKenna *et al*., 2020). In addition, the LFA protocol could have more defined rules for selecting the various patch categories when on-site. These rules can be specific to the landscape in question. For example, patch types could be combined based on key plant traits, which relate to their carbon versus nitrogen ratio (Erdenebileg *et al*., 2023). For example, grassy sward patches should be compared to woody patches; fine leaf tree patches should be compared to broad leaf tree patches. This is because different functional traits contribute differently to carbon and nitrogen dynamics for nutrient cycling, litter breakdown, or stability (Carrillo *et al*., 2012).

From a theoretical perspective of using the LFA model along heavy-metal contaminated sites, capturing how contamination alters the TTRP framework aligns with the broader ecological theory on functional integrity and resilience (Lucas *et al*., 2014). Expanding the framework to cover these gaps may be a critical step in understanding ecosystem degradation and recovery. This can also contribute to global restoration efforts such as the UN Decade on Ecosystem Restoration, which calls for diagnostic methods capable of tracking progress toward ecological functionality (Young *et al*., 2022).

5.5 Conclusion

This study can advance our understanding of landscape function in heavy-metal-stressed environments. While the LFA model is effective at diagnosing structural degradation along rangelands, it understates dysfunction in chemically contaminated systems since it relies on the visible and physical surface indicators only. The approach used by this study provides an expanded diagnostic framework that can capture both the visible and invisible indicators of landscape function. This can redefine the LFA model as a monitoring procedure that offers a more holistic assessment of overall landscape function. The results of this project could also serve as baseline monitoring indices for rehabilitative procedures at the Loole study site in Palabora Copper Mine.

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Appendix A

Table A1: Soil Surface Assessment criteria for each indicator scored along identified query zones. (After Tongway and Hindley, 2004)

1. Vegetation canopy /rock cover (rain splash protection)		
Projected cover	**Class**	**Interpretation of rain splash protection**
1% or less	1	No protection
1-15%	2	Low protection
15-30%	3	Moderate protection
30-50%	4	High protection
More than 50%	5	Very high protection
2. Perennial vegetation cover		
Basal/canopy cover	**Class**	**Interpretation of subsurface contribution**
1% or less	1	No contribution
1-10%	2	Low contribution
10-20%	3	Moderate contribution
More than 20%	4	High contribution
3. Litter		
Plant litter cover	**Class**	
<10%	1	
10-25%	2	
25-50%	3	
50-75%	4	
75-100%	5	
100%; >20mm thick	6	
100%; 21-70mm thick	7	
100%; 70-120%	8	
100%; 120-170mm thick	9	
100%; >170mm thick	10	
Litter origin	**Symbol**	
Locally derived	L	

Transported	T
Litter Decomposition criteria	
Nil	N
Slight	S
Moderate	M
Extensive	E

4. Cryptogam cover		
Biological Soil Crust cover	**Class**	**Interpretation of cover**
Not applicable	0	No stable crust present
1% or less	1	No cover
1-10%	2	Slight cover
10-50%	3	Moderate cover
More than 50%	4	Extensive cover

5. Physical crust brokeness	
Crust Brokeness	**Class**
No crust present	0
Crust present; extensively broken	1
Crust present; moderately broken	2
Crust present; slightly broken	3
Crust present; intact, smooth	4

6. Soil erosion type and severity				
Erosion severity	**Insignificant**	**Slight**	**Moderate**	**Severe**
Erosion type	**Class**	**Class**	**Class**	**Class**
Sheeting(E)	4	3	n/a	n/a
Pedestal(P)	n/a	n/a	2	1
Terracette(T)	n/a	n/a	2	1
Rill(R)	n/a	n/a	2	1
Scalding(S)	n/a	n/a	n/a	1

7. Deposited materials (Aluvium)		
Amount Deposited	**Class**	**Interpretation**
>50%	1	Extensive
20-50%	2	Moderate
5-20%	3	Slight
0-5%	4	None/small amount

8. Soil surface roughness		
Surface Roughness	**Class**	**Interpretation**
<3mm relief	1	Smooth retention
3-8mm relief	2	Low retention
8-25mm relief	3	Moderate retention
Deep depressions with visible base	4	Large retention

Very deep depressions/cracks; >100m	5	Extensive retention
9. Surface resistance to disturbance		
Soil surface nature	**Class**	**Interpretation**
Loose sandy surface	1	Surface not crusted
Easily broken	2	Surface easily penetrated
Moderately hard	3	Moderately hard physical crust present
Hard, brittle crust	4	Needs metal instrument to break hard surface. Subcrust is also very hard, coherent, and brittle
Non-brittle	5	Soil surface shows "springiness"; self-mulching clay OR Surface has no physical crust and is under dense perennial vegetation of considerable cover: trees, grass swards (i.e. not just an isolated plant).
10. Slake test		
Behaviour observed	**Class**	**Interpretation**
Not applicable	0	No coherent fragments
Very Unstable	1	Fragment collapses in less than 5 sec
Unstable	2	Fragment collapses in 5-10 sec; >50% of subcrust material slumps
Moderately stable	3	Surface crust remains intact; <50% sub-crust slumping
Very Stable	4	Fragment remains intact with no swelling
11. Soil texture		
Soil texture	**Class**	**Interpretation**
Silty clay to heavy clay	1	Very slow infiltration rate
Sandy clay loam to sandy clay	2	Slow infiltration rate
Sandy loam to silt loam	3	Moderate infiltration rate
Sandy to clay sand	4	High infiltration rate

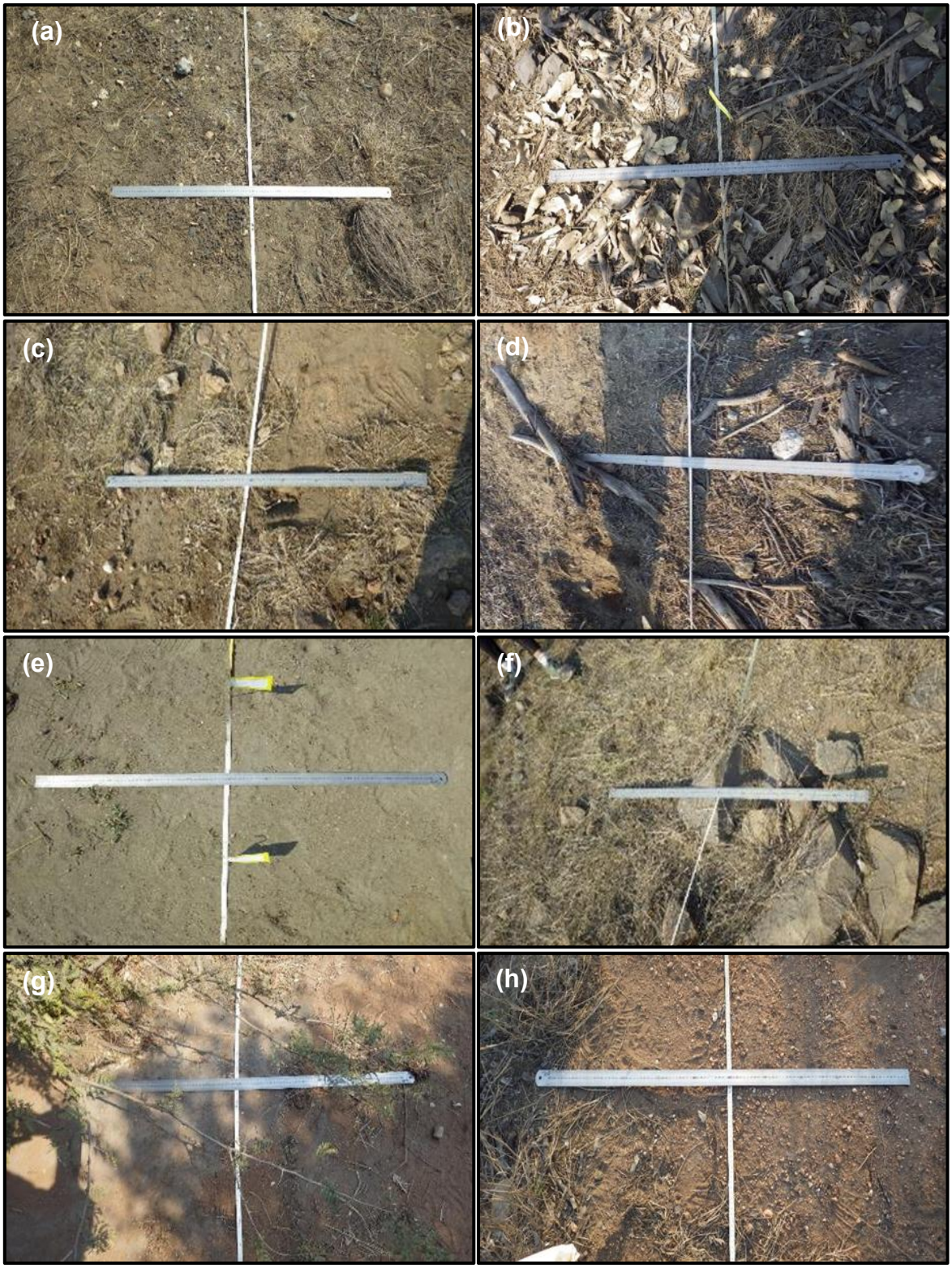


Figure A1: Soil Surface Assessment query zones along HIGH POLLUTION ZONE. (a) Grass litter patch, (b) Tree litter patch, (c) Grass patch, (d) Woody litter patch, (e) Thick bare interpatch, (f) Stone patch, (g) Thin bare interpatch, and (h) Bare patch

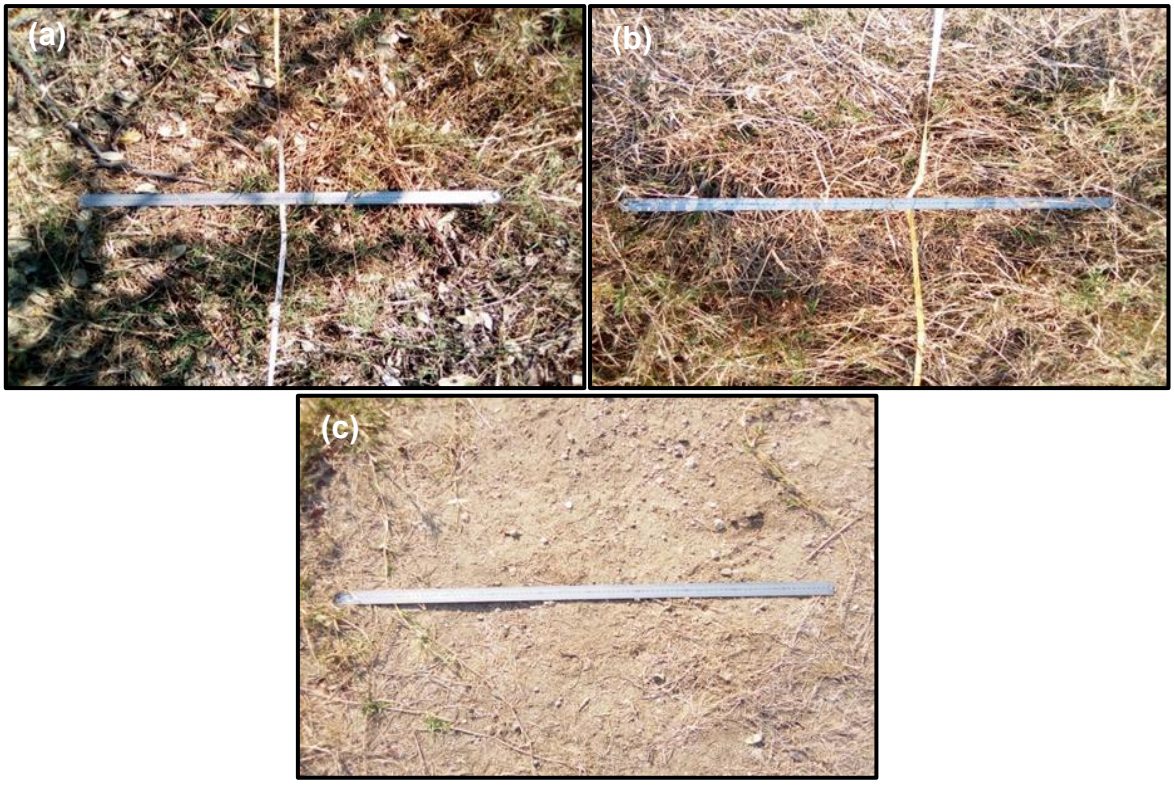


Figure A2 Soil Surface Assessment query zones along MEDIUM POLLUTION ZONE (a) Subcanopy litter and grass patch, (b) Grass sward, (c) Sandy interpatch





Figure A3: Soil Surface Assessment query zones along UNPOLLUTED ZONE: (a) Grass patch; (b) Broad leaf tree patch; (c) Fine leaf tree patch; (d) Forb patch; (e) Woody shrub patch; (f) Riparian weed patch; (g) Grass sward; (h) Black cryptogram patch; (i) Mossy cryptogram patch.

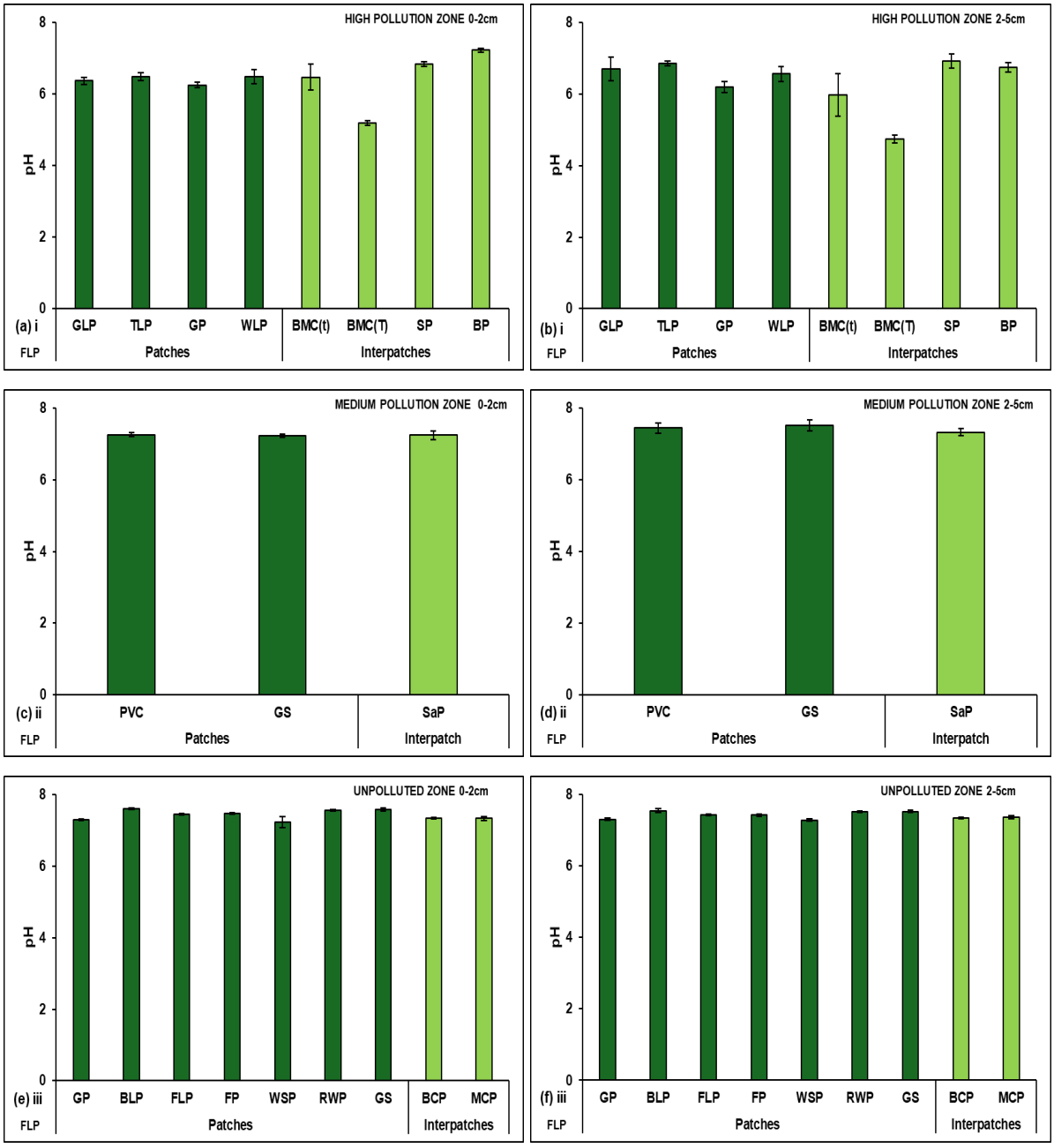


Figure A4: pH values of FLP leachates for patch categories making up the patches and interpatches of the high (**a** and **b**), medium (**c** and **d**) and unpolluted zones (**e** and **f**) at two soil depths: 0-2 cm (a, c, e) and 2-5 cm (b, d, f).

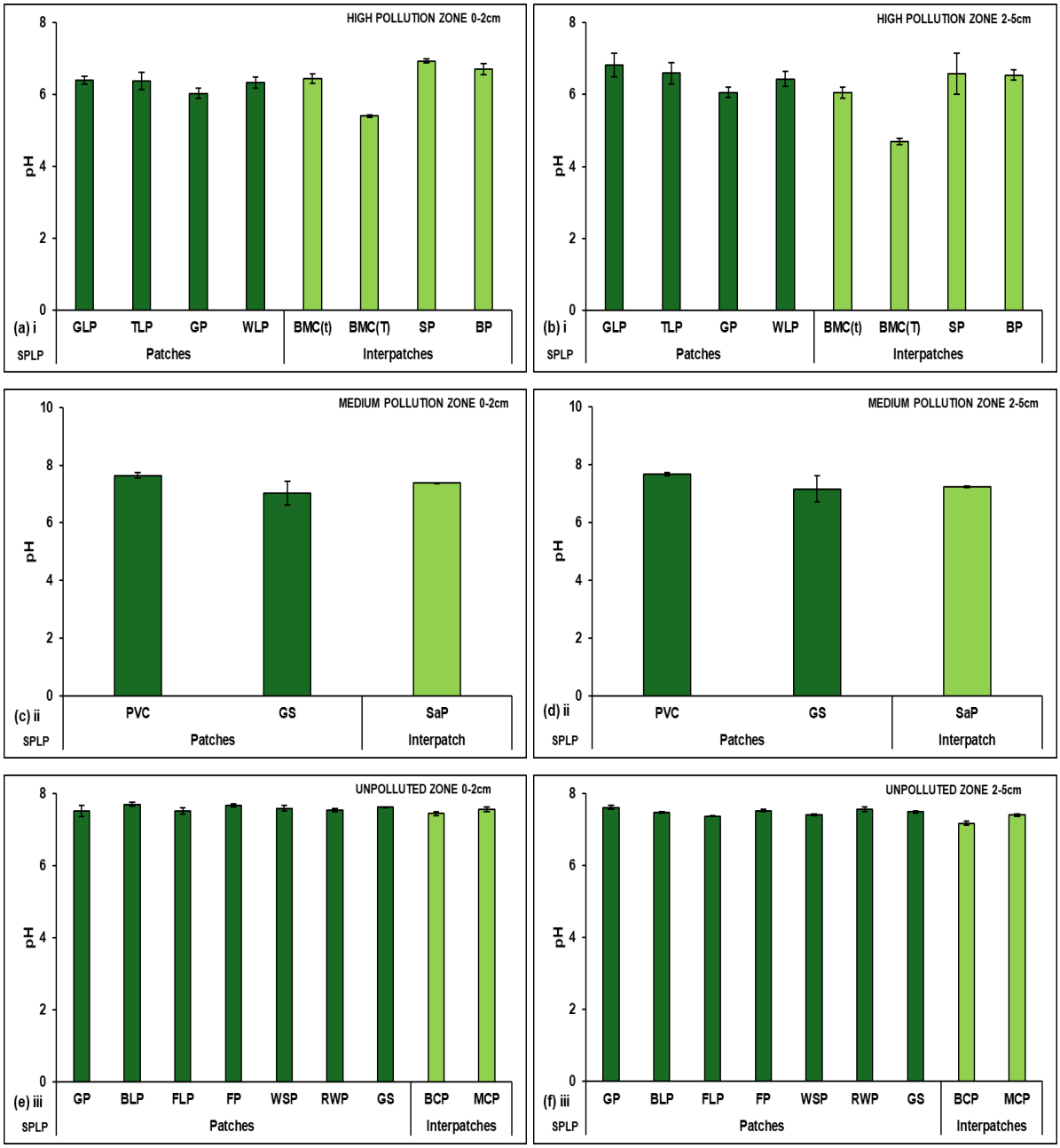


Figure A5: pH values of SPLP leachates for patch categories making up the patches and interpatches of the high (**a** and **b**), medium (**c** and **d**) and unpolluted zones (**e** and **f**), at two soil depths: 0-2 cm (a, c, e) and 2-5 cm (b, d, f)

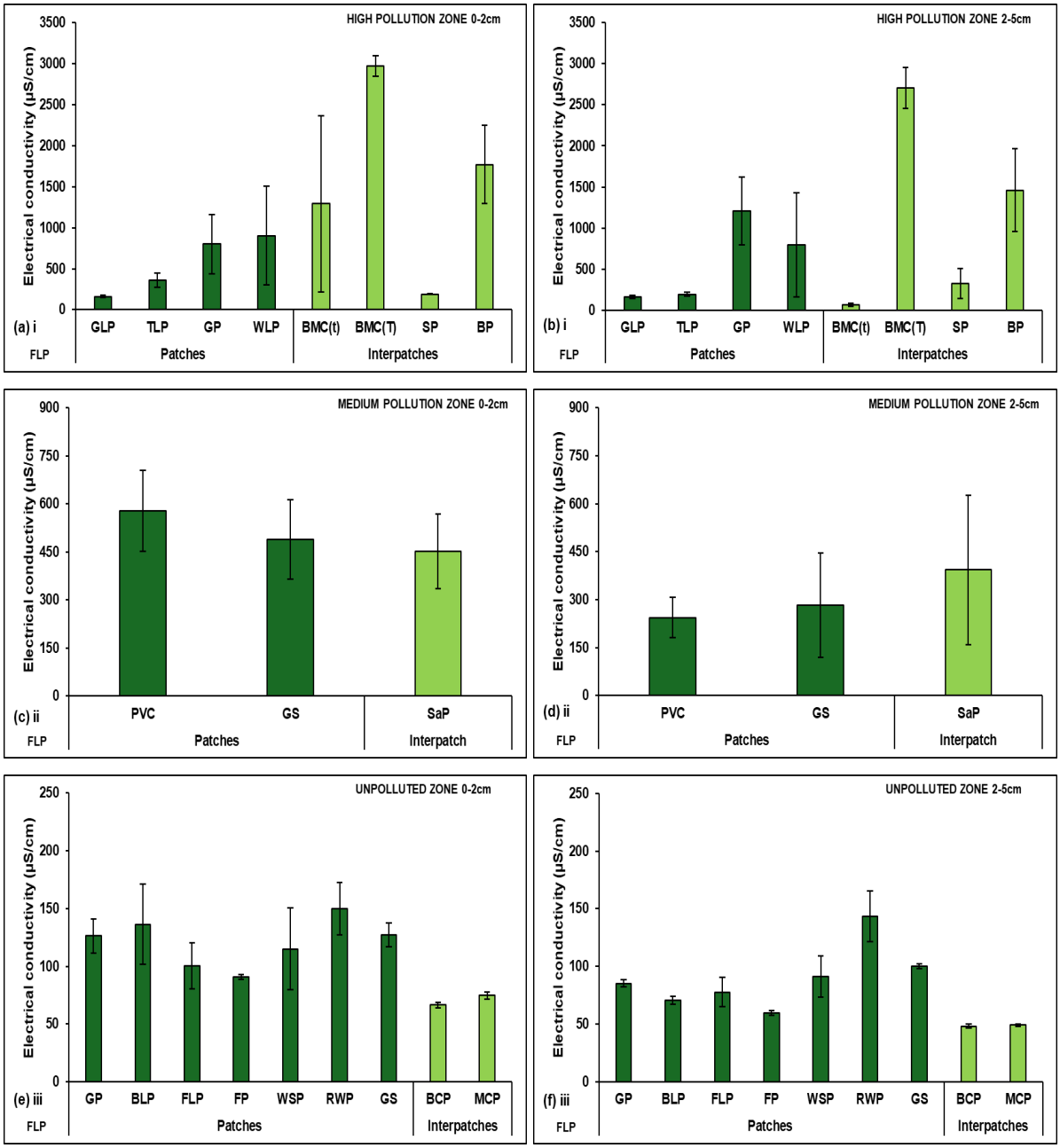


Figure A6: Electrical conductivity values of FLP leachates for patch categories making up the patches and interpatches of the high (**a** and **b**), medium (**c** and **d**) and unpolluted zones (**e** and **f**), at two soil depths: 0-2 cm (a, c, e) and 2-5 cm (b, d, f)

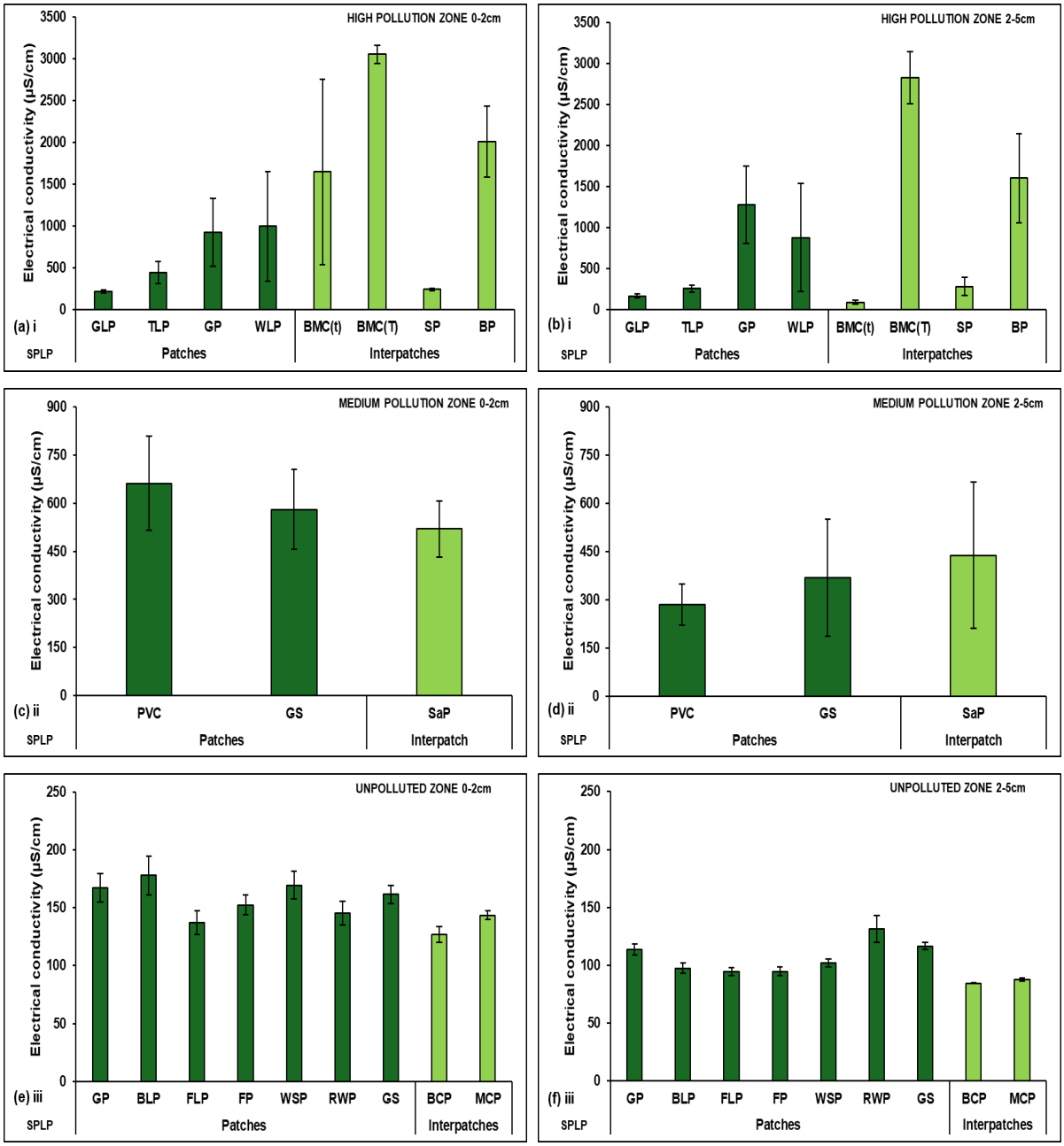


Figure A7: Electrical conductivity values of SPLP leachates for patch categories making up the patches and interpatches of the high (a and b), medium (c and d) and unpolluted zones (e and f), at two soil depths: 0-2 cm (a, c, e) and 2-5 cm (b, d, f).

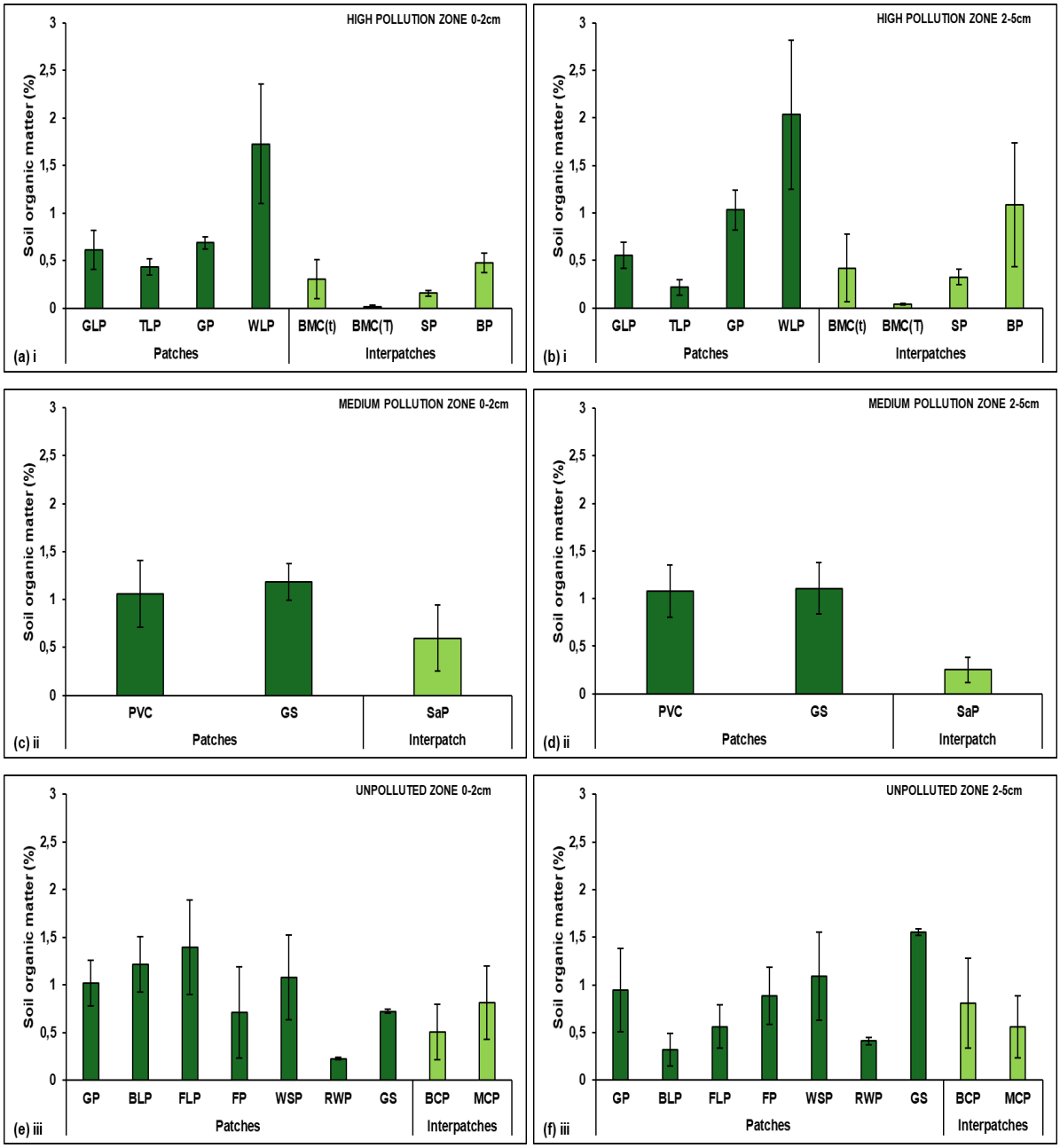


Figure A8: Soil organic matter at 0-2cm and 2-5cm for patch categories making up the patches and interpatches of the high (**a** and **b**), medium (**c** and **d**) and unpolluted zones (**e** and **f**), at two soil depths: 0-2 cm (a, c, e) and 2-5 cm (b, d, f)

Table A2: Mean (± standard error) semi-quantitative concentrations of soluble Cu, Pb, Br, Ni, and SO_4 in FLP soil extractions from patches and interpatches at depths of 0-2cm and 2-5cm. Dashes (-) indicate samples that were below the detection limit.

FLP	Patch/ Interpatch	Patch category	Depth:	Cu (mg/ℓ)	Pb (mg/ℓ)	Br (mg/ℓ)	Ni (mg/ℓ)	SO_4 (mg/ℓ)
HIGH POLLUTION ZONE	Patches	Grassy litter patch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
		Tree litter patch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
		Grass patch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
		Woody litter patch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
	Interpatches	Thick mineral crust	0 – 2cm	30.00 (0)	50.00 (0)	6.67 (1.67)	10.00 (0)	400 (0)
			2 – 5cm	200.00 (0)	100.00 (0)	15.00 (0)	10.00 (0)	400.00 (0)
		Thin mineral crust	0 – 2cm	3.33 (3.33)	11.67 (11.67)	1.67 (1.67)	-	200.00 (0)
			2 – 5cm	10.00 (0)	16.67 (16.67)	2.50 (2.50)	10.00 (0)	333.33 (66.67)
		Stone interpatch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
		Bare sand interpatch	0 – 2cm	11.67 (11.67)	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
MEDIUM POLLUTION ZONE	Patches	Subcanopy grass and litter patch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
		Grassy sward	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)
	Inter-patch	Sandy interpatch	0 – 2cm	-	-	-	-	200.00 (0)
			2 – 5cm	-	-	-	-	200.00 (0)

Table A3: Mean (± standard error) semi-quantitative concentrations of soluble Cu, Pb, Br, Ni, and SO_4 in SPLP soil extractions from patches and interpatches at depths of 0-2 cm and 2-5 cm. Dashes (-) indicate samples that were below the detection limit.

SPLP	Patch/ Interpatch	Patch type:	Depth:	Cu (mg/ℓ)	Pb (mg/ℓ)	Br (mg/ℓ)	Ni (mg/ℓ)	SO_4 (mg/ℓ)
HIGH POLLUTION ZONE	Patches	Grassy litter patch	0 – 2cm	1.00 (0)	20.00 (0)	1.00 (0)	-	400.00 (200)
			2 – 5cm	0.67 (0.33)	13.33 (6.67)	1.00 (0)	-	200.00 (0)
		Tree litter patch	0 – 2cm	6.67 (3.33)	20.00 (0)	3.67 (1.33)	-	200.00 (0)
			2 – 5cm	1.00 (0)	33.33 (16.67)	3.67 (1.33)	-	200.00 (0)
		Grass patch	0 – 2cm	16.67 (6.67)	50.00 (0)	30.00 (22.55)	-	200.00 (0)
			2 – 5cm	16.67 (6.67)	66.67 (16.67)	6.67 (1.67)	-	200.00 (0)
		Woody litter patch	0 – 2cm	40.00 (30.0)	66.67 (1.67)	6.67 (1.67)	-	400.00 (200)
			2 – 5cm	16.67 (6.67)	40.00 (10)	5.00 (0)	-	333.33 (66.67)
	Interpatches	Thick mineral crust	0 – 2cm	100.00 (0)	100.00 (0)	13.33 (3.33)	-	333.33 (66.67)
			2 – 5cm	100.00 (0)	100.00 (0)	10.00 (0)	-	533.33 (133.33)
		Thin mineral crust	0 – 2cm	46.67 (27.28)	66.67 (16.67)	8.33 (1.67)	-	400.00 (200.00)
			2 – 5cm	10.00 (10.00)	33.33 (33.33)	4.00 (3.00)	-	400 (200.00)
		Stone interpatch	0 – 2cm	7.00 (3.00)	20.00 (0)	1.00 (0)	-	200.00 (0)
			2 – 5cm	4.00 (3.00)	30.00 (10.00)	3.67 (1.33)	-	200.00 (0)
		Bare sand interpatch	0 – 2cm	10.33 (9.84)	23.33 (14.53)	1.67 (1.67)	-	400.00 (200.00)
			2 – 5cm	6.67 (3.33)	33.33 (16.67)	3.33 (1.67)	-	400.00 (200.00)
MEDIUM POLLUTION ZONE	Patches	Subcannopy grass and litter patch	0 – 2cm	1.00 (0)	0.33 (0.33)	-	-	200.00 (0)
			2 – 5cm	1.00 (0)	-	-	-	200.00 (0)
		Grassy sward	0 – 2cm	1.00 (0)	0.33 (0.33)	-	-	200.00 (0)
			2 – 5cm	1.00 (0)	-	-	-	200.00 (0)
	Inter-patch	Sandy interpatch	0 – 2cm	1.00 (0)	1.00 (0)	-	-	200.00 (0)
			2 – 5cm	1.00 (0)	0.33 (0.33)	0.67 (0.33)	-	200.00 (0)

Table A4: Summary of results from Mann Whitney U test used to test the difference between patch and interpatch responses for each physicochemical propert, at two depths, measured within each of the three pollution zones: high, medium, and unpolluted. Significant differences are indicated in yellow highlight ($p < 0.05$).

Site	Comparison between:	Depth	U	p-value
FLP pH				
High	Patches and interpatches	0 – 2cm	51.50	0.2481
Medium	Patches and interpatches	0 – 2cm	12.00	0.5476
Unpolluted	Patches and interpatches	0 – 2cm	102.50	0.0228
High	Patches and interpatches	2 – 5cm	88.00	0.8955
Medium	Patches and interpatches	2 – 5cm	12.00	0.6130
Unpolluted	Patches and interpatches	2 – 5cm	94.50	0.0703
SPLP pH				
High	Patches and interpatches	0 – 2cm	52.50	0.2726
Medium	Patches and interpatches	0 – 2cm	12.00	0.6013
Unpolluted	Patches and interpatches	0 – 2cm	85.00	0.2097
High	Patches and interpatches	2 – 5cm	95.50	0.1841
Medium	Patches and interpatches	2 – 5cm	15.00	0.1539
Unpolluted	Patches and interpatches	2 – 5cm	112.50	0.0042
FLP Electrical conductivity				
High	Patches and interpatches	0 – 2cm	50.00	0.2189
Medium	Patches and interpatches	0 – 2cm	11.00	0.7143
Unpolluted	Patches and interpatches	0 – 2cm	118.00	0.0015
High	Patches and interpatches	2 – 5cm	63.50	0.6441
Medium	Patches and interpatches	2 – 5cm	6.50	0.6041
Unpolluted	Patches and interpatches	2 – 5cm	126.00	0.0003
SPLP Electrical conductivity				
High	Patches and interpatches	0 – 2cm	41.00	0.0780
Medium	Patches and interpatches	0 – 2cm	11.00	0.7143
Unpolluted	Patches and interpatches	0 – 2cm	104.00	0.0181
High	Patches and interpatches	2 – 5cm	61.00	0.5443

Medium	Patches and interpatches	2 – 5cm	8.00	0.9048
Unpolluted	Patches and interpatches	2 – 5cm	124.00	0.0004
Soil Organic Matter (%)				
High	Patches and interpatches	0 – 2cm	127.00	0.0016
Medium	Patches and interpatches	0 – 2cm	15.00	0.1667
Unpolluted	Patches and interpatches	0 – 2cm	80.00	0.3358
High	Patches and interpatches	2 – 5cm	109.50	0.0326
Medium	Patches and interpatches	2 – 5cm	18.00	0.0238
Unpolluted	Patches and interpatches	2 – 5cm	71.00	0.6618

Table A5: Summary of statistical results from Kruskal-Wallis tests used to assess the effect of pollution level in patches and interpatches from each pollution zone. When a significant result was found ($p < 0.05$), a post hoc Dunn's test using a Bonferroni correction was used to understand which group of sites significantly differed between the three pollution zones, only if the Kruskal-Wallis test had significant results. Significant results are shown in yellow.

Patch type	Comparison between pollution zones (Mean rank score)	H-statistic	Degrees of freedom	p-value	Post-Hoc Dunn's test – Bonferroni correction (mean rank difference; p-value)
FLP pH					
Patches and interpatches at 0 – 2cm	High (13.52), Medium (31.28), Unpolluted (45.33).	42.19	2	p<0.0001	High – Medium (-17.76; p = 0.0093) High – Unpolluted (-31.82; p < 0.0001)
Patches at 0 – 2cm	High (6.5), Medium (17.33), Unpolluted (28.48).	28.77	2	p<0.0001	High – Unpolluted (-21.98; p < 0.0001)
Interpatches at 0 – 2cm	High (7.17), Medium (14.33), Unpolluted (17).	11.06	2	0.0040	High – Unpolluted (-9.83; p = 0.0020)
Patches and interpatches at 2 – 5cm	High (13.17), Medium (42.00), Unpolluted (42.07).	39.42	2	p<0.0001	High – Medium (-28.83; p < 0.0001) High – Unpolluted (-28.91; p < 0.0001)
Patches at 2 – 5cm	High (6.92), Medium (28.25), Unpolluted (25.12).	23.19	2	p<0.0001	High – Medium (-21.33; p = 0.0002). High – Unpolluted (-18.20; p < 0.0001)
Interpatches at 2 – 5cm	High (6.75), Medium (15.33), Unpolluted (17.33).	13.36	2	0.0001	High – Unpolluted (-1.58; p = 0.0006)
SPLP pH					
Patches and interpatches at 0 – 2cm	High (13.13), Medium (35.33), Unpolluted (44.33).	41.40	2	p<0.0001	High – Medium (-22.21; p = 0.0011) High – Unpolluted (-31.21; p < 0.0001)

Patches at 0 – 2cm	High (7.08), Medium (21.67), Unpolluted (26.90).	23.25	2	p<0.0001	High – Medium (-14.58; p = 0.0105). High – Unpolluted (-19.82; p < 0.0001)
Interpatches at 0 – 2cm	High (6.50), Medium (14.83), Unpolluted (18.08).	15.29	2	0.0005	High – Unpolluted (-11.58; p = 0.0002)
Patches and interpatches at 2 – 5cm	High (), Medium (), Unpolluted ().				
Patches at 2 – 5cm	High (7.04), Medium (27.67), Unpolluted (25.21).	22.63	2	p<0.0001	High – Medium (-20.63; p = 0.0003). High – Unpolluted (-18.17; p < 0.0001)
Interpatches at 2 – 5cm	High (6.96), Medium (14.83), Unpolluted (17.17).	12.17	2	0.0023	High – Unpolluted (-10.21; p = 0.0010))
FLP Electrical conductivity (µS/cm)					
Patches and interpatches at 0 – 2cm	High (42.71), Medium (45.11), Unpolluted (14.78).	39.91	2	p<0.0001	High – Unpolluted (27.93; p < 0.0001). Medium – Unpolluted (30.33; p < 0.0001)
Patches at 0 – 2cm	High (28.50), Medium (32.83), Unpolluted (11.48).	26.01	2	p<0.0001	High – Unpolluted (17.02; p < 0.0001). Medium – Unpolluted (21.36; p < 0.0001)
Interpatches at 0 – 2cm	High (14.25), Medium (13.00), Unpolluted (3.50).	12.38	2	0.0021	High – Unpolluted (10.75; p = 0.0005)
Patches and interpatches at 2 – 5cm	High (41.67), Medium (39.56), Unpolluted (17.56).	27.07	2	p < 0.0001	High – Unpolluted (24.11; p < 0.0001). Medium – Unpolluted (22.00; p = 0.0011)
Patches at 2 – 5cm	High (29.96), Medium (27.00),	20.97	2	p < 0.0001	High – Unpolluted (17.65; p < 0.0001).

	Unpolluted (12.31).				Medium – Unpolluted (14.69; p = 0.0054)
Interpatches at 2 – 5cm	High (13.75), Medium (13.00), Unpolluted (4.50).	9.26	2	0.0098	High – Unpolluted (9.25; p = 0.0029)
SPLP Electrical conductivity (µS/cm)					
Patches and interpatches at 0 – 2cm	High (43.65), Medium (44.33), Unpolluted (14.20).	42.76	2	p < 0.0001	High – Unpolluted (29.44; p < 0.0001). Medium – Unpolluted (30.13; p < 0.0001)
Patches at 0 – 2cm	High (28.92), Medium (32.83), Unpolluted (11.24).	27.35	2	p < 0.0001	High – Unpolluted (17.38; p < 0.0001). Medium – Unpolluted (21.60; p < 0.0001)
Interpatches at 0 – 2cm	High (14.25), Medium (12.00), Unpolluted (3.50).	12.66	2	0.0018	High – Unpolluted (8.92; p = 0.0013)
Patches and interpatches at 2 – 5cm	High (41.69), Medium (41.89), Unpolluted (16.76).	30.40	2	p < 0.0001	High – Unpolluted (24.93; p < 0.0001). Medium – Unpolluted (25.13; p = 0.0002)
Patches at 2 – 5cm	High (30.29), Medium (29.25), Unpolluted (11.48).	25.47	2	p < 0.0001	High – Unpolluted (18.82; p < 0.0001). Medium – Unpolluted (17.77; p = 0.0008)
Interpatches at 2 – 5cm	High (13.25), Medium (13.00), Unpolluted (5.50).	6.61	2	0.0367	High – Unpolluted (7.75; p = 0.0124)
Soil Organic Matter (%)					
Patches and interpatches at 0 – 2cm	High (23.60), Medium (37.06), Unpolluted (34.44).	6.39	2	0.8590	N/A
Patches at 0 – 2cm	High (17.92), Medium (24.5), Unpolluted (19.90).	1.34	2	0.5120	N/A

Interpatches at 0 – 2cm	High (9.21), Medium (12.50), Unpolluted (13.83).	2.44	2	0.2948	N/A
Patches and interpatches at 2 – 5cm	High (26.90), Medium (34.39), Unpolluted (32.41).	1.79	2	0.4084	N/A
Patches at 2 – 5cm	High (19.25), Medium (26.33), Unpolluted (18.62).	2.21	2	0.3310	N/A
Interpatches at 2 – 5cm	High (10.17), Medium (9.17), Unpolluted (13.58).	1.52	2	0.4676	N/A

Table A6: Summary of Mann-Whitney U test results used to understand the effect of each soil surface index between patches and interpatches. Significant results are shown in yellow highlight ($p < 0.05$).

Site	**Soil Surface Index**	**Comparison between:**	**U**	**p-value**
High pollution zone	Stability	Patches and interpatches	359	0.0051408
	Infiltration	Patches and interpatches	144	<0.0001
	Nutrient cycling	Patches and interpatches	140	<0.0001
Medium pollution zone	Stability	Patches and interpatches	18	0.0256
	Infiltration	Patches and interpatches	18	0.02624
	Nutrient cycling	Patches and interpatches	18	0.02497
Unpolluted zone	Stability	Patches and interpatches	68	0.7899
	Infiltration	Patches and interpatches	116	0.002026
	Nutrient cycling	Patches and interpatches	101.5	0.02491

Table A7: Results of the multiple regression test for the effect of pollution level between soil surface indices (stability, infiltration, and nutrient cycling), for the high, medium, and unpolluted zones. Values highlighted in yellow indicate significant regression models ($p < 0.05$).

Soil Surface Index	Soil physicochemical property	Coefficients	Standard error	t-statistic	p-value	Confidence interval
HIGH POLLUTION ZONE						
Stability						
	(a) 0-2cm	57,10	23,40	2,44	0,03	(7,94; 106,26)
	FLP pH	-0,32	5,49	-0,06	0,95	(-11,85; 11,21)
	SPLP pH	-0,57	6,95	-0,08	0,94	(-15,17; 14,03)
	FLP EC	0,02	0,01	1,30	0,21	(-0,01; 0,04)
	SPLP EC	-0,02	0,01	-1,28	0,22	(-0,04; 0,01)
	SOM	5,00	2,75	1,82	0,09	(-0,77; 10,77)
	(b) 2-5cm	22,74	22,01	1,03	0,32	(-23,50; 68,98)
	FLP pH	1,91	4,04	0,47	0,64	(-6,59; 10,40)
	SPLP pH	2,35	5,19	0,45	0,66	(-8,56; 13,27)
	FLP EC	0,01	0,02	0,32	0,75	(-0,04; 0,06)
	SPLP EC	0,00	0,02	-0,21	0,84	(-0,05; 0,04)
	SOM	1,63	2,32	0,70	0,49	(-3,24; 6,50)
Infiltration						
	(a) 0-2cm	86,73	30,32	2,86	0,01	(23,02; 150,44)
	FLP pH	2,05	7,11	0,29	0,01	(-12,89; 16,99)
	SPLP pH	-11,29	9,01	-1,25	0,02	(-30,21; 7,63)
	FLP EC	0,01	0,02	0,38	0,01	(-0,03; 0,04)
	SPLP EC	-0,01	0,02	-0,57	0,04	(-0,04; 0,03)
	SOM	12,79	3,56	3,60	0,00	(5,31; 20,26)
	(b) 2-5cm	74,51	34,73	2,15	0,05	(1,55; 147,47)
	FLP pH	2,82	6,38	0,44	0,66	(-10,59; 16,22)
	SPLP pH	-10,05	8,20	-1,23	0,24	(-27,27; 7,17)
	FLP EC	-0,05	0,04	-1,43	0,17	(-0,14; 0,03)
	SPLP EC	0,05	0,04	1,29	0,21	(-0,03; 0,12)
	SOM	9,63	3,66	2,63	0,02	(1,95; 17,32)
Nutrient cycling						
	(a) 0-2cm	48,50	30,08	1,61	0,01	(-14,69; 111,69)

	FLP pH	-1,30	7,05	-0,18	0,03	(-16,12; 13,52)
	SPLP pH	-3,54	8,93	-0,40	0,04	(-22,31; 15,23)
	FLP EC	0,01	0,02	0,65	005	(-0,02; 0,05)
	SPLP EC	-0,01	0,02	-0,80	0,03	(-0,05; 0,02)
	SOM	14,01	3,53	3,97	0,00	(6,59; 21,42)
	(b) 2-5cm	45,79	34,40	1,33	0,20	(-26,48; 118,07)
	FLP pH	2,55	6,32	0,40	0,69	(-10,72; 15,83)
	SPLP pH	-6,83	8,12	-0,84	0,41	(-23,89; 10,22)
	FLP EC	-0,05	0,04	-1,22	0,24	(-0,13; 0,03)
	SPLP EC	0,04	0,03	1,11	0,28	(-0,03; 0,11)
	SOM	9,30	3,62	2,57	0,02	(1,69; 16,91)
MEDIUM POLLUTION ZONE						
Stability						
	(a) 0-2cm	-705,14	890,49	-0,79	0,49	(-3539,09; 2128,82)
	FLP pH	110,92	125,22	0,89	0,44	(-287,57; 509,42)
	SPLP pH	-10,28	17,38	-0,59	0,60	(-65,59; 45,04)
	FLP EC	-0,24	0,57	-0,42	0,70	(-2,05; 1,57)
	SPLP EC	0,22	0,57	0,39	0,72	(-1,60; 2,05)
	SOM	27,51	24,09	1,14	0,34	(-49,15; 104,18)
	(b) 2-5cm	36,23	34,56	1,05	0,03	(-73,76; 146,21)
	FLP pH	35,05	4,92	7,12	0,01	(19,40; 50,71)
	SPLP pH	-32,23	3,88	-8,30	0,00	(-44,59; -19,87)
	FLP EC	0,35	0,06	6,18	0,01	(0,17; 0,52)
	SPLP EC	-0,39	0,06	-6,83	0,01	(-0,57; -0,21)
	SOM	44,04	2,63	16,72	0,00	(35,66; 52,42)
Infiltration						
	(a) 0-2cm	-1024,97	834,95	-1,23	0,31	(-3682,16; 1632,22)
	FLP pH	150,33	117,41	1,28	0,29	(-223,31; 523,97)
	SPLP pH	-8,83	16,30	-0,54	0,63	(-60,70; 43,03)
	FLP EC	-0,40	0,53	-0,76	0,50	(-2,10; 1,29)
	SPLP EC	0,41	0,54	0,77	0,50	(-1,30; 2,12)
	SOM	12,99	22,59	0,58	0,61	(-58,89; 84,87)

	(b) 2-5cm	-59,27	98,84	-0,60	0,59	(-373,83; 255,29)
	FLP pH	28,16	14,07	2,00	0,14	(-16,63; 72,95)
	SPLP pH	-15,25	11,11	-1,37	0,26	(-50,61; 20,11)
	FLP EC	0,22	0,16	1,37	0,26	(-0,29; 0,73)
	SPLP EC	-0,24	0,16	-1,45	0,24	(-0,76; 0,29)
	SOM	37,06	7,53	4,92	0,02	(13,08; 61,03)
Nutrient cycling						
	(a) 0-2cm	-1136,43	909,74	-1,25	0,30	(-4031,62; 1758,75)
	FLP pH	162,84	127,92	1,27	0,29	(-244,26; 569,95)
	SPLP pH	-9,13	17,76	-0,51	0,64	(-65,64; 47,38)
	FLP EC	-0,44	0,58	-0,75	0,51	(-2,29; 1,41)
	SPLP EC	0,44	0,59	0,75	0,51	(-1,42; 2,30)
	SOM	24,15	24,61	0,98	0,40	(-54,17; 102,47)
	(b) 2-5cm	3,82	40,71	0,09	0,03	(-125,72; 133,37)
	FLP pH	30,78	5,80	5,31	0,01	(12,33; 49,22)
	SPLP pH	-27,31	4,58	-5,97	0,01	(-41,87; -12,75)
	FLP EC	0,41	0,07	6,26	0,01	(0,20; 0,62)
	SPLP EC	-0,45	0,07	-6,65	0,01	(-0,67; -0,23)
	SOM	51,27	3,10	16,52	0,00	(41,39; 61,14)
UNPOLLUTED ZONE						
Stability						
	(a) 0-2cm	-1,07	63,64	-0,02	0,99	(-133,42; 131,29)
	FLP pH	13,76	7,25	1,90	0,07	(-1,31; 28,84)
	SPLP pH	-5,65	7,82	-0,72	0,48	(-21,90; 10,60)
	FLP EC	0,04	0,03	1,29	0,21	(-0,03; 0,11)
	SPLP EC	-0,03	0,06	-0,44	0,67	(-0,15; 0,10)
	SOM	-1,70	1,80	-0,94	0,36	(-5,45; 2,05)
	(b) 2-5cm	-24,54	62,35	-0,39	0,02	(-154,21; 105,13)
	FLP pH	18,41	7,65	2,41	0,03	(2,50; 34,32)
	SPLP pH	-8,46	6,55	-1,29	0,03	(-22,09; 5,16)
	FLP EC	0,10	0,04	2,58	0,02	(0,02; 0,17)
	SPLP EC	-0,01	0,06	-0,11	0,91	(-0,12; 0,11)
	SOM	1,44	1,44	1,01	0,01	(-1,54; 4,43)

Infiltration						
	(a) 0-2cm	-95,66	84,81	-1,13	0,02	(-272,04; 80,71)
	FLP pH	28,11	9,66	2,91	0,01	(8,02; 48,20)
	SPLP pH	-13,23	10,41	-1,27	0,03	(-34,89; 8,43)
	FLP EC	0,02	0,04	0,46	0,04	(-0,07; 0,11)
	SPLP EC	0,12	0,08	1,54	0,03	(-0,04; 0,29)
	SOM	0,77	2,40	0,32	0,02	(-4,23; 5,76)
	(b) 2-5cm	-261,83	86,41	-3,03	0,01	(-441,52; -82,14)
	FLP pH	26,34	10,60	2,48	0,02	(4,29; 48,38)
	SPLP pH	13,43	9,08	1,48	0,01	(-5,45; 32,30)
	FLP EC	0,11	0,05	2,18	0,04	(0,01; 0,22)
	SPLP EC	-0,07	0,08	-0,87	0,03	(-0,23; 0,10)
	SOM	-1,65	1,99	-0,83	0,04	(-5,79; 2,49)
Nutrient cycling						
	(a) 0-2cm	-122,27	72,35	-1,69	0,01	(-272,73; 28,19)
	FLP pH	21,55	8,24	2,61	0,02	(4,41; 38,68)
	SPLP pH	-2,32	8,88	-0,26	0,03	(-20,80; 16,16)
	FLP EC	0,01	0,04	0,16	0,02	(-0,07; 0,08)
	SPLP EC	0,05	0,07	0,70	0,01	(-0,09; 0,19)
	SOM	-2,37	2,05	-1,16	0,05	(-6,64; 1,89)
	(b) 2-5cm	-203,28	70,02	-2,90	0,01	(-348,89; -57,68)
	FLP pH	28,80	8,59	3,35	0,00	(10,94; 46,67)
	SPLP pH	1,34	7,35	0,18	0,03	(-13,95; 16,63)
	FLP EC	0,08	0,04	1,81	0,08	(-0,01; 0,16)
	SPLP EC	0,00	0,06	-0,02	0,00	(-0,13; 0,13)
	SOM	-0,88	1,61	-0,55	0,00	(-4,24; 2,47)

Appendix B

Table B1: Summary of statistical results from Kruskal-Wallis tests used to identify the effect of pollution level on *Daphnia magna*. When a significant result was found ($p < 0.05$), a *post hoc* Dunn's test using a Bonferroni correction was used to understand which group of sites significantly differed between the three sites, only if the Kruskal-Wallis test had significant results. Significant results are shown in yellow.

Patch type	Leachate type	Food	Comparison between pollution zones (mean rank score)	Chi-square	Degrees of freedom	p-value	Post Hoc Dunn's test (mean rank difference; p-value)
$t_{1\text{-}2\%}$ immobilisation							
Patch	FLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Interpatch	FLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	FLP	Fed	High (4.60), medium (4.33), unpolluted (18.00).	16.67	2	0.0002	High – Unpolluted (-13.40; p<0.0001)
Interpatch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Unfed	High (3.21), medium (3.60), unpolluted (11.52).	16.57	2	0.0003	High – Unpolluted (-14.18; p<0.0001)
Interpatch	SPLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Interpatch	SPLP	Fed	High (5.70), medium (2.50), unpolluted (10.50).	9.29	2	0.0096	Medium – Unpolluted (-8; p=0.0030)
$t_{25\%}$ immobilisation							
Patch	FLP	Unfed	High (5.83), medium (5.5), unpolluted (18.05).	13.39	2	0.0012	High – Unpolluted (-12.22; p=0.0210); Medium – Unpolluted (-12.55; p=0.0020)
Interpatch	FLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Interpatch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Unfed	High (3.60), medium (43.00), unpolluted (19.63)	17.42	2	0.0002	High – Unpolluted (-13.83; p=0.0002); Medium – Unpolluted (-9.83; p=0.0070)
Interpatch	SPLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Fed	High (3.83), medium (8.5), unpolluted (18.38).	17.97	2	0.0001	High – Unpolluted (-14.54; p<0.0001)
Interpatch	SPLP	Fed	High (5.50), medium (4.00), unpolluted (12.50).	10.67	2	0.0048	High – Unpolluted (-7.00; p=0.0060); Medium – Unpolluted (-8.50; p=0.0060)

$t_{50\%}$ immobilisation							
Patch	FLP	Unfed	High (5.50), medium (7.30), unpolluted (20.31).	18.74	2	p<0.0001	High – Unpolluted (-14.81; p=0.0002); Medium – Unpolluted (-13.01; p=0.002)
Interpatch	FLP	Unfed	High (2.50), medium (6.00), unpolluted (10.50).	10.50	2	0.0052	High – Unpolluted (-8.00; p=0.0007)
Patch	FLP	Fed	High (4.75), medium (7.50), unpolluted (20.50).	20.65	2	p<0.0001	High – Unpolluted (-15.75; p<0.0001); Medium – Unpolluted (-13.00; p=0.0010)
Interpatch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Unfed	High (5.83), medium (10.50), unpolluted (22.00).	22.77	2	p<0.0001	High – Unpolluted (-16.17; p<0.0001); Medium – Unpolluted (-11.5; p=0.0080)
Interpatch	SPLP	Unfed	High (6.70), medium (8.00), unpolluted (16.00).	10.68	2	0.0048	High – Unpolluted (-9.30; p=0.0010)
Patch	SPLP	Fed	High (5.72), medium (11.75), unpolluted (24.39).	25.15	2	p<0.0001	High – Unpolluted (-18.67; p<0.0010); Medium – Unpolluted (-12.64; p=0.0050)
Interpatch	SPLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
$t_{75\%}$ immobilisation							
Patch	FLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Interpatch	FLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Interpatch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Unfed	High (6.65), medium (11.58), unpolluted (22.50).	21.48	2	p<0.0001	High – Unpolluted (-15.85; p<0.0001); Medium – Unpolluted (-10.92; p=0.0040)
Interpatch	SPLP	Unfed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Fed	High (5.80), medium (13.00), unpolluted (21.00).	20.37	2	p<0.0001	High – Unpolluted (-15.50; p<0.0001)
Interpatch	SPLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
$t_{100\%}$ immobilisation							
Patch	FLP	Unfed	High (8.08), medium (11.2), unpolluted (26.5).	26.50	2	p<0.0001	High – Unpolluted (-18.42; p<0.0001); Medium – Unpolluted (-15.30; p=0.0070)

Interpatch	FLP	Unfed	High (6.67), medium (13.33), unpolluted (18).	14.16	2	p=0.0008	High – Unpolluted (-11.33; p=0.0003)
Patch	FLP	Fed	High (7.64), medium (11.50), unpolluted (21.00).	15.58	2	p=0.0004	High – Unpolluted (-13.36; p<0.0001); Medium – Unpolluted (-9.50; p=0.0020)
Interpatch	FLP	Fed	*NC*	*NC*	*NC*	*NC*	*NC*
Patch	SPLP	Unfed	High (6.92), medium (14.83), unpolluted (26.44).	24.56	2	p<0.0001	High – Unpolluted (-19.52; p<0.0001); Medium – Unpolluted (-11.60; p=0.0137)
Interpatch	SPLP	Unfed	High (7.17), medium (12.00), unpolluted (18.17).	12.82	2	0.0002	High – Unpolluted (-11.00; p=0.0004)
Patch	SPLP	Fed	High (6.75), medium (14.40), unpolluted (22.50).	22.03	2	p<0.0001	High – Unpolluted (-15.75; p<0.0001)
Interpatch	SPLP	Fed	*NC*	*NC*	*C*	*NC*	*NC*

Table B2: Summary of results from Mann Whitney U test used to test the difference between patch and interpatch immobilisation responses for *Daphnia magna.* Significant differences are indicated in yellow highlight ($p < 0.05$).

Site	Comparison between:	Leachate type	Food	U	p-value
$T_{1\text{-}2\%}$ immobilisation					
High pollution zone	Patch and interpatch	FLP	Unfed	*NC*	*NC*
High pollution zone	Patch and interpatch	FLP	Fed	*C*	*NC*
High pollution zone	Patch and interpatch	SPLP	Unfed	0	0.004925
High pollution zone	Patch and interpatch	SPLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	FLP	Unfed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Unfed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Fed	16.5	0.05906
Unpolluted zone	Patch and interpatch	FLP	Unfed	37	0.4592
Unpolluted zone	Patch and interpatch	FLP	Fed	38	0.2107
Unpolluted zone	Patch and interpatch	SPLP	Unfed	64.5	0.08676
Unpolluted zone	Patch and interpatch	SPLP	Fed	68	0.0313
$T_{25\%}$ immobilisation					
High pollution zone	Patch and interpatch	FLP	Unfed	*NC*	*NC*
High pollution zone	Patch and interpatch	FLP	Fed	5	0.8544
High pollution zone	Patch and interpatch	SPLP	Unfed	5	0.1339
High pollution zone	Patch and interpatch	SPLP	Fed	10	0.2173
Medium pollution zone	Patch and interpatch	FLP	Unfed	1	0.05834
Medium pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Unfed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Fed	10.5	0.1147
Unpolluted zone	Patch and interpatch	FLP	Unfed	37	0.1625
Unpolluted zone	Patch and interpatch	FLP	Fed	59	0.8355
Unpolluted zone	Patch and interpatch	SPLP	Unfed	*NC*	*NC*
Unpolluted zone	Patch and interpatch	SPLP	Fed	82	0.01249
$T_{50\%}$ immobilisation					
High pollution zone	Patch and interpatch	FLP	Unfed	16	0.4399
High pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
High pollution zone	Patch and interpatch	SPLP	Unfed	35	0.4076

High pollution zone	Patch and interpatch	SPLP	Fed	17.5	0.1395
Medium pollution zone	Patch and interpatch	FLP	Unfed	0	0.03466
Medium pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Unfed	10.5	0.4506
Medium pollution zone	Patch and interpatch	SPLP	Fed	*NC*	*NC*
Unpolluted zone	Patch and interpatch	FLP	Unfed	40.5	0.3775
Unpolluted zone	Patch and interpatch	FLP	Fed	31.5	0.3078
Unpolluted zone	Patch and interpatch	SPLP	Unfed	73.5	0.001095
Unpolluted zone	Patch and interpatch	SPLP	Fed	73	0.03375
$T_{75\%}$ immobilisation					
High pollution zone	Patch and interpatch	FLP	Unfed	23.5	0.8949
High pollution zone	Patch and interpatch	FLP	Fed	29.5	1
High pollution zone	Patch and interpatch	SPLP	Unfed	42.5	0.5899
High pollution zone	Patch and interpatch	SPLP	Fed	63.5	0.5672
Medium pollution zone	Patch and interpatch	FLP	Unfed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Unfed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Fed	*NC*	*NC*
Unpolluted zone	Patch and interpatch	FLP	Unfed	22.5	0.06087
Unpolluted zone	Patch and interpatch	FLP	Fed	25.5	0.64
Unpolluted zone	Patch and interpatch	SPLP	Unfed	45	0.01112
Unpolluted zone	Patch and interpatch	SPLP	Fed	39	0.1691
$T_{100\%}$ immobilisation					
High pollution zone	Patch and interpatch	FLP	Unfed	80.5	0.6402
High pollution zone	Patch and interpatch	FLP	Fed	69.5	0.5727
High pollution zone	Patch and interpatch	SPLP	Unfed	74.5	0.9056
High pollution zone	Patch and interpatch	SPLP	Fed	67.5	0.8127
Medium pollution zone	Patch and interpatch	FLP	Unfed	0	0.03577
Medium pollution zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Medium pollution zone	Patch and interpatch	SPLP	Unfed	11	0.6924
Medium pollution zone	Patch and interpatch	SPLP	Fed	*NC*	*NC*
Unpolluted zone	Patch and interpatch	FLP	Unfed	31	0.2517
Unpolluted zone	Patch and interpatch	FLP	Fed	*NC*	*NC*
Unpolluted zone	Patch and interpatch	SPLP	Unfed	85	0.004995

Unpolluted zone	Patch and interpatch	SPLP	Fed	38	0.1045

Table B3: Summary of results from Mann Whitney U test used to test the effect of feeding for *Daphnia magna.* Significant differences are indicated in yellow highlight ($p < 0.05$).

Site	Comparison between:	Test solution:	Patch type:	U	p-value
$T_{1\text{-}2\%}$ immobilisation					
High pollution zone	Unfed and fed	FLP	Patch	8	0.7037
High pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
High pollution zone	Unfed and fed	SPLP	Patch	*NC*	*NC*
High pollution zone	Unfed and fed	SPLP	Interpatch	14	0.8174
Medium pollution zone	Unfed and fed	FLP	Patch	*NC*	*NC*
Medium pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
Medium pollution zone	Unfed and fed	SPLP	Patch	7.5	0.7937
Medium pollution zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
Unpolluted zone	Unfed and fed	FLP	Patch	112.5	0.0256
Unpolluted zone	Unfed and fed	FLP	Interpatch	13	0.7266
Unpolluted zone	Unfed and fed	SPLP	Patch	147.5	0.4968
Unpolluted zone	Unfed and fed	SPLP	Interpatch	13	0.5127
$T_{25\%}$ immobilisation					
High pollution zone	Unfed and fed	FLP	Patch	6	1
High pollution zone	Unfed and fed	FLP	Interpatch	4	1
High pollution zone	Unfed and fed	SPLP	Patch	14.5	1
High pollution zone	Unfed and fed	SPLP	Interpatch	10.5	0.4379
Medium pollution zone	Unfed and fed	FLP	Patch	*NC*	*NC*
Medium pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
Medium pollution zone	Unfed and fed	SPLP	Patch	3	0.1039
Medium pollution zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
Unpolluted zone	Unfed and fed	FLP	Patch	87	0.0013
Unpolluted zone	Unfed and fed	FLP	Interpatch	11	0.2867
Unpolluted zone	Unfed and fed	SPLP	Patch	73.5	0.1084
Unpolluted zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
$T_{50\%}$ immobilisation					
High pollution zone	Unfed and fed	FLP	Patch	20.5	0.7462
High pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*

High pollution zone	Unfed and fed	SPLP	Patch	37	0.7709
High pollution zone	Unfed and fed	SPLP	Interpatch	9	0.0108
Medium pollution zone	Unfed and fed	FLP	Patch	13.5	0.9153
Medium pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
Medium pollution zone	Unfed and fed	SPLP	Patch	9.5	0.3535
Medium pollution zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
Unpolluted zone	Unfed and fed	FLP	Patch	69.5	0.0030
Unpolluted zone	Unfed and fed	FLP	Interpatch	5.5	0.0949
Unpolluted zone	Unfed and fed	SPLP	Patch	123.5	0.6635
Unpolluted zone	Unfed and fed	SPLP	Interpatch	0	0.0107
$T_{75\%}$ immobilisation					
High pollution zone	Unfed and fed	FLP	Patch	52	0.8589
High pollution zone	Unfed and fed	FLP	Interpatch	10.5	0.8291
High pollution zone	Unfed and fed	SPLP	Patch	46	0.7809
High pollution zone	Unfed and fed	SPLP	Interpatch	59	0.8003
Medium pollution zone	Unfed and fed	FLP	Patch	*NC*	*NC*
Medium pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
Medium pollution zone	Unfed and fed	SPLP	Patch	7	0.0887
Medium pollution zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
Unpolluted zone	Unfed and fed	FLP	Patch	27	p<0.001
Unpolluted zone	Unfed and fed	FLP	Interpatch	2.5	0.0369
Unpolluted zone	Unfed and fed	SPLP	Patch	38	0.2629
Unpolluted zone	Unfed and fed	SPLP	Interpatch	1	0.0226
$T_{100\%}$ immobilisation					
High pollution zone	Unfed and fed	FLP	Patch	65	0.9753
High pollution zone	Unfed and fed	FLP	Interpatch	66	1
High pollution zone	Unfed and fed	SPLP	Patch	66.5	0.7507
High pollution zone	Unfed and fed	SPLP	Interpatch	59	0.4592
Medium pollution zone	Unfed and fed	FLP	Patch	10	0.4070
Medium pollution zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*
Medium pollution zone	Unfed and fed	SPLP	Patch	5	0.0766
Medium pollution zone	Unfed and fed	SPLP	Interpatch	*NC*	*NC*
Unpolluted zone	Unfed and fed	FLP	Patch	55.5	0.6393
Unpolluted zone	Unfed and fed	FLP	Interpatch	*NC*	*NC*

Unpolluted zone	Unfed and fed	SPLP	Patch	58	0.2163
Unpolluted zone	Unfed and fed	SPLP	Interpatch	1.5	0.0149

Table B4: Summary of results from Kruskal-Wallis tests used to evaluate the effect of pollution level on the inhibition of *A. fischeri* bioluminescence. Inhibition responses were compared across each site, first considering both patches and interpatches, then comparing the patch and interpatch responses to pollution levels along each site. Results were compared at 5, 15, 30 and 60 minutes of exposure to soil FLP and SPLP solutions. When a significant difference was found between the comparison of sites ($p<0.05$), a post hoc Dunn's test, using a Bonferroni correction, was used to understand where this difference was found between the group of sites. Significant results are indicated in yellow highlight.

Patch type	Sample size	Test solution	Comparison between sites (Mean rank score)	Chi-square	Degrees of freedom	p-value	Post-Hoc Dunn's test (mean rank difference; p-value)
Inhibition at t5mins							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (10.46), medium pollution zone (9.75), unpolluted zone (28.38)	24.63	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-17.92; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-18.63; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (9.75), medium pollution zone (7), unpolluted zone (15.5)	4.89	2	0.087	N/a
Patch	High pollution zone = 12	SPLP	High pollution zone (9.25), medium	28.38	2	P<0.001	HIGH POLLUTION ZONE –

	Medium pollution zone = 6 Unpolluted zone = 21		pollution zone (10), unpolluted zone (29)				UNPOLLUTED ZONE (-19.75; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-19; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (8.42), medium pollution zone (9), unpolluted zone (17.17)	8.32	2	0.016	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-8.75; p=0.005)
Inhibition at t15mins							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (8.88), medium pollution zone (17.17), unpolluted zone (28.6)	26.21	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-19.72; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-16.43; p=0.002)
Interpatch	High pollution zone = 12	FLP	High pollution zone (9.83), medium pollution zone	5.05	2	0.080	N/a

	Medium pollution zone = 3 Unpolluted zone = 6		(6.67), unpolluted zone (15.5)				
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (8.17), medium pollution zone (12.5), unpolluted zone (28.9)	28.35	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-20.74; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-16.40; p=0.002)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (8.5), medium pollution zone (8), unpolluted zone (17.5)	9.23	2	0.010	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-9; p=0.004)
Inhibition at t30mins							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (7.33), medium pollution zone (13.83), unpolluted zone (29)	29.73	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-21.67; p<0.001); MEDIUM POLLUTION

							ZONE – UNPOLLUTED ZONE (-15.17; p=0.004)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (9.17), medium pollution zone (9.33), unpolluted zone (15.5)	4.43	2	0.109	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (9.04), medium pollution zone (10.42), unpolluted zone (29)	28.46	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-19.96; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-18.58; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (9.46), medium pollution zone (8), unpolluted zone (15.58)	4.72	2	0.094	N/a
Inhibition at t60mins							
Patch	High pollution zone = 12	FLP	High pollution zone (8.08),	29.02	2	P<0.001	HIGH POLLUTION

	Medium pollution zone = 6 Unpolluted zone = 21		medium pollution zone (12.33), unpolluted zone (19)				ZONE – UNPOLLUTED ZONE (-20.92; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-16.67; p=0.002)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (9.38), medium pollution zone (8.83), unpolluted zone (15.33)	4.16	2	0.125	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (10.46), medium pollution zone (7.58), unpolluted zone (29)	28.67	2	P<0.001	HIGH POLLUTION ZONE – UNPOLLUTED ZONE (-18.54; p<0.001); MEDIUM POLLUTION ZONE – UNPOLLUTED ZONE (-21.42; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3	SPLP	High pollution zone (10), medium pollution zone	2.94	2	0.230	N/a

	Unpolluted zone = 6		(8), unpolluted zone (14.5)				

Table B5: Summary of results from Mann-Whitney U Tests to determine the effect of patch type on the inhibition of *A. fischeri* bioluminescence at 5, 15, 30, and 60 minutes after exposure to soil leachate samples from patches and interpatches along HIGH POLLUTION ZONE, MEDIUM POLLUTION ZONE and the UNPOLLUTED ZONE. No significant differences were found between patches and interpatches ($p<0.05$).

Site	Comparison between:	Test solution	U	p-value
Inhibition at t5mins				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	92.5	0.2480
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	18	0.0269
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	91	0.1086
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	98.9	0.1329
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	14.5	0.1948
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	92.5	0.0906
Inhibition at t15mins				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	84	0.5065
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	17	0.0476
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	77	0.4304
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	88	0.3707
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	16	0.0952
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	76.5	0.4479
Inhibition at t30mins				

High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	78	0.7506
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	16	0.0952
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	64	0.9766
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	72	0.9770
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	16	0.0919
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	61.5	0.9532
Inhibition at t60mins				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	63.5	0.6438
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	16	0.0919
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	30	0.0546
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	72	0.9769
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	9.5	1
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	37	0.1320

Appendix C

Table B1: Summary of Kruskal-Wallis test to understand the effect of pollution level on the peak germination of six plant species.

Patch type	Sample size	Test solution/substrate	Comparison between sites (Mean rank score)	Chi-square	Degrees of freedom	P-value	Post-Hoc Dunn's test (mean rank difference; p-value)
A. Hybidus							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (26.71), medium pollution zone (26.75), unpolluted zone (14.24).	11.88	2	0.002637	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (12.47; p=0.002); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (15.51; p=0.0165)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (16.67), medium pollution zone (11.17), unpolluted zone (7.58).	2.7461	2	0.2533	N/a
Patch	High pollution zone = 12	SPLP	High pollution zone (20.42), medium pollution zone	0.59	2	0.744	N/a

	Medium pollution zone = 6 Unpolluted zone = 21		(16.75), unpolluted zone (20.69).				
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (18.17), medium pollution zone (28.5), unpolluted zone (21.63).	4.39	2	0.111	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SOIL	High pollution zone (34.98), medium pollution zone (33.42), unpolluted zone (37.51).	0.24	2	0.887	N/a
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (17.37), medium pollution zone (17.4), unpolluted zone (20.75).	1.09	2	0.580	N/a
D. Australe							
Patch	High pollution zone = 12	FLP	High pollution zone (15.21), medium pollution zone	16.05	2	P<0.001	HIGH POLLUTION ZONE-UNPOLLUTED

	Medium pollution zone = 6 Unpolluted zone = 21		(7.5), unpolluted zone (26.31).				ZONE (-11.10; p=0.006); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (-18.81; p<.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (6.79), medium pollution zone (15.67), unpolluted zone (17.08).	13.24	2	0.001	HIGH POLLUTION ZONE- UNPOLLUTED ZONE (-10.29; p<0.001)
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (12.17), medium pollution zone (12.67), unpolluted zone (26.57).	15.31	2	P<0.001	HIGH POLLUTION ZONE- UNPOLLUTED ZONE (-14.40; p<0.001); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (p=0.008)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (8.25), medium pollution zone (10.5), unpolluted zone (16.75).	7.72	2	0.021	HIGH POLLUTION ZONE- UNPOLLUTED ZONE (-8.5; p=0.006)

Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SOIL	High pollution zone (19.71), medium pollution zone (29.75), unpolluted zone (17.38).	5.59	2	0.061	N/a
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (10.17), medium pollution zone (11.33), unpolluted zone (12.5).	0.59	2	0.746	N/a
L. Sativum							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (30.63), medium pollution zone (30.25), unpolluted zone (11).	29.12	2	P<0.001	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (19.63; p<0.001); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (19.25; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3	FLP	High pollution zone (13.08), medium pollution zone (15.67),	9.83	2	0.007	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (8.58; p=0.005);

	Unpolluted zone = 6		unpolluted zone (4.5).				MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (11.17; p=0.01)
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (18.79), medium pollution zone (8.33), unpolluted zone (24.02).	9.09	2	0.011	MEDIUM POLLUTION ZONE- UNPOLLUTED ZONE (-15.69; p=0.003)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (9.04), medium pollution zone (5), unpolluted zone (17.92).	11.58	2	0.003	HIGH POLLUTION ZONE- UNPOLLUTED ZONE (-8.88; p=0.004); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (-12.92; p=0.003)
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SOIL	High pollution zone (12.21), medium pollution zone (28.92), unpolluted zone (21.9).	9.98	2	0.007	HIGH POLLUTION ZONE – MEDIUM POLLUTION ZONE (-16.71; p=0.003)

Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (11.71), medium pollution zone (13.17), unpolluted zone (8.5).	1.52	2	0.467	N/a
M. Sativa							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (25.25), medium pollution zone (31), unpolluted zone (13.86).	15.33	2	P<0.001	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (11.39; p=0.004); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (17.14; p<0.001)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (13.04), medium pollution zone (9.33), unpolluted zone (7.75).	3.2	2	0.202	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6	SPLP	High pollution zone (21.58), medium pollution zone (30.33),	8.45	2	0.015	MEDIUM POLLUTION ZONE-UNPOLLUTED ZONE (14.19; p=0.004)

	Unpolluted zone = 21		unpolluted zone (16.14).				
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (13.04), medium pollution zone (9.33), unpolluted zone (7.75).	3.6	2	0.166	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SOIL	High pollution zone (29.83), medium pollution zone (26.42), unpolluted zone (12.55).	20.05	2	P<0.001	HIGH POLLUTION ZONE- UNPOLLUTED ZONE (17.29; p<0.001); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (13.87; p=0.008)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (11.42), medium pollution zone (13.67), unpolluted zone (8.83).	1.37	2	0.504	N/a
B. Nigra							
Patch	High pollution zone = 12	FLP	High pollution zone (40.25), medium	5.08	2	0.079	N/a

	Medium pollution zone = 6 Unpolluted zone = 21		pollution zone (31.67), unpolluted zone (27.55).				
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (12), medium pollution zone (12.33), unpolluted zone (8.33).	1.61	2	0.448	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SPLP	High pollution zone (17.79), medium pollution zone (17.58), unpolluted zone (21.95).	1.36	2	0.08	N/a
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (8.96), medium pollution zone (13.33), unpolluted zone (13.92).	3.09	2	0.214	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6	SOIL	High pollution zone (14.75), medium pollution zone (23.83),	3.87	2	0.145	N/a

	Unpolluted zone = 21		unpolluted zone (21.9).				
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (9.92), medium pollution zone (14.67), unpolluted zone (11.33).	1.46	2	0.481	N/a
S. Bicolor							
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	FLP	High pollution zone (25.71), medium pollution zone (28.25), unpolluted zone (14.38).	11.54	2	0.003	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (11.33; p=0.005); MEDIUM POLLUTION ZONE - UNPOLLUTED ZONE (13.87; p=0.008)
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	FLP	High pollution zone (13.63), medium pollution zone (14), unpolluted zone (4.25).	10.32	2	0.006	HIGH POLLUTION ZONE-UNPOLLUTED ZONE (9.34; p=0.002)
Patch	High pollution zone = 12	SPLP	High pollution zone (25.37), medium	4.68	2	0.096	N/a

	Medium pollution zone = 6 Unpolluted zone = 21		pollution zone (21), unpolluted zone (16.64).				
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SPLP	High pollution zone (12.54), medium pollution zone (14.17), unpolluted zone (6.33).	5.21	2	0.074	N/a
Patch	High pollution zone = 12 Medium pollution zone = 6 Unpolluted zone = 21	SOIL	High pollution zone (19.92), medium pollution zone (28.17), unpolluted zone (17.71).	4.02	2	0.134	N/a
Interpatch	High pollution zone = 12 Medium pollution zone = 3 Unpolluted zone = 6	SOIL	High pollution zone (9.29), medium pollution zone (14.67), unpolluted zone (12.58).	2.41	2	0.300	N/a

Table B1: Summary of Mann-Whitney U tests to understand the effect of patch type on the peak germination of six plant species.

Site	Comparison between:	Test solution/substrate	U	p-value
A. Hybridus				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	89	0.3361
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	13	0.3538
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	68	0.2659
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	83	0.5355
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	1	0.0488
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	64.5	0.0597
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	78	0.7479
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	8	0.8932
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	53	0.5759
D. Australe				

High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	111	0.0238
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	1.5	0.0545
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	86	0.1845
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	85.5	0.4473
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	10	0.8955
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	86	0.1837
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	83.5	0.5239
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	13.5	0.2956
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	38.5	0.1557
L. Sativum				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	89	0.3244
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	7.5	0.7835

Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	31	0.0581
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	101	0.0983
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	11.5	0.5913
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	54	0.6159
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	56.5	0.3850
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	12.5	0.4185
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	98	0.0413
M. Sativa				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	51	0.2352
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	11	0.6973
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	24.5	0.0165
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	57.5	0.4080

Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	14.5	0.1872
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	67	0.8130
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	99.5	0.1157
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	10	0.8951
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	37	0.1292
B. Nigra				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	66.5	0.7703
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	5	0.3601
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	45.5	0.3154
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	70	0.9305
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	4	0.2332
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	39	0.1664

High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	85	0.4672
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	9	0.8907
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	75	0.6757
S. Bicolor				
High pollution zone	Patch (n = 12) and interpatch (n = 12)	FLP	72.5	1
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	FLP	11	0.4602
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	FLP	82.5	0.2607
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SPLP	58	0.4089
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SPLP	4	0.2228
Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SPLP	58	0.7895
High pollution zone	Patch (n = 12) and interpatch (n = 12)	SOIL	88.5	0.3487
Medium pollution zone	Patch (n = 6) and interpatch (n = 3)	SOIL	9	0.8937

Unpolluted zone	Patch (n = 21) and interpatch (n = 6)	SOIL	39.5	0.1719