A Project entitled

Interactions of heavy metals in soil to the occurrence of tree fungal diseases in urban areas

Submitted by

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Declaration

I, Lee Chi Fung , declare that this research report represents my own work under the supervision of Associate Professor; Associate Dean (Quality Assurance and Enhancement) of FLASS, Dr Li Wai Chin, and that it has not been submitted previously for examination to any tertiary institution.

Signed ___________________

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Abstract

Heavy metals are one of the potentially harmful pollutants in most contaminated soils. Some findings pointed out that there were various sources of the existence of heavy metals in terms of natural sources and artificial sources. Also, some fungi can bring up the positive or negative effects to the tree health, thus there was no concrete answers about this issue. The aims of this present study are to analyze the heavy metals concentration and major physical and chemical parameters in the soil samples. The samples collected from the randomly selected study sites and examined the relationship among 1) physical and chemical parameters, 2) heavy metals concentration in the soil samples, and 3) presence of wood decay fungi grown in the infected trees. Results of the study showed that heavy metals were one of the contaminants in soil may weaken the tree growth. The high load of heavy metals was determined in soil samples that possible to cause the fungal infection. For the level of heavy metals, Arsenic (As), Mercury (Hg) and Cadmium (Cd) were examined and counted as the "contaminated' land in the related areas. They were exceeded 5, 1.7 and 6 times of the Dutch Target Values respectively and were also correlated with fungi occurrence. The principal component analysis (PCA) results demonstrated that pH, Electrical Conductivity (EC), Total Organic Carbon (TOC) and Sulphur (S) were the parameters that correlated with fungal species. The significant difference of the results was found in most of the parameters. To conclude, there was no direct relationship between the fungal infection and heavy metals' concentration. The fungal infection worked based on the weakening effects to tree by the heavy metals stress.

1. Introduction

1.1 Background

The natural geological processes and artificial activities were the sources of heavy metals in the environment. The natural sources included the minerals from excessive weathering and the metal ions from rocks, displacement of various contaminants from subsurface layers of soil or groundwater, atmospheric deposition from volcanic activity and continental dusts transportation (Ernst, 1998). For the human routes, it is determined that the disposal of industrial effluents and solid waste, sewage sludge application, mining and smelting of metalliferous ores, electroplating, exhausted gas, production of energy and fuel, municipal waste generation (Lasat, 2000).

Heavy metal(loid)s were the current contaminants in the soil environment that prompted the awareness of the general publics and the governments to initiate the environmental laws, programs and policies for addressing the concerns of the excessive content of metals that potentially significant hazard to animals, human and ecosystem health (Blaylock & Hunag, 2000).

For instance, a literature discovered that the existence of heavy metals in soil that may pose the negative effects to the tree health. Fung et.al (2007) determined that the range of Pb (in mg/kg) in the soil samples was 4.39 – 9.85 in five sampling locations. As the heavy metals exhibit the toxic activity towards soil that may interact with the fungi in tree, however Baldrian (2003) and Jean-Phillippe (2011) pointed out that heavy metal can enhance and inhibit the tree fungal infection respectively and such issue is controversial in Hong Kong urban areas. Thus, it is not clear to illustrate the correlation between heavy metals and fungi, as a few research findings discussing about this issue.

1.2 Literature Review

Leung et.al (2010) determined that fungi could contribute to the phytoremediation of contaminated soil. Mycorrhizal fungi were the symbiotic microorganisms that connect with soil and roots by interacted with the host plants in the metal contaminated sites. It is determined that these fungi contain the high tolerance in the heavy metals stress conditions. It is vital to the toxicology to plants and heavy metal availability in environments (Remy et.al, 1994). About the role of mycorrhizal fungi, they can provide the nutrients element that scarcely available to the plant and enhance the uptake of heavy metal by the plant host. They were beneficial to the metal tolerance of the plants and binding the metals in the fungal hyphae polyphosphate that may relieve the metal toxicity in the plants (Barea et.al, 2005).

Nevertheless, the whit rot fungi, *Heterobasidion,* caused the wood decay in the Norway spruce forests. They were pathogenically damage the forest soil and agricultural soil with the high content of calcium (Ca) and pH value. In current study, it is hard to find the major variation of the fungal infection that correlated with the high pH or concentration of Ca. Since there were different variations of the infection of fungi, such as the absence of antagonistic soil fungi, the tree stump and wounds which relatively low protection of tree, and the reasons behind to pose the high pH value and high content of Ca (Hietala et.al, 2016). It was doubtful for discovering the dominant factors that causing negative effects of tree health, as aforementioned, there was not enough references to support the view.

1.3 Objectives of research

As the presence of heavy metals in the soil that may relate to the presence of the fungal disease in the urban tree, but the knowledge gap of their relationship especially tree grown in Hong Kong parks is unknown. Therefore, the research questions are shown below:

1 Is the heavy metals concentration being one of the possible contaminants in urban soil?

2 Is there any positive or negative effect to the tree grown in the urban areas when the tree decay fungi found in trees?

3 What is the relationship between physical and chemical properties, (e.g. heavy metals, nutrients in the soil nearby the plant root) and wood decay fungi grown in various types of trees located in Hong Kong public spaces?

To deal with the research questions, this study consisted of a couple of objectives:

1 To analyze the heavy metals concentration and major physical and chemical parameters in the soil samples that collected from the sampling sites

2 To examine the relationship between the physical and chemical parameters, the heavy metals concentration in the soil samples, and the presence of wood decay fungi grown in the infected trees

2. Materials and methods

2.1 Preliminary Investigation

The preliminary study in Kowloon Park was done. It is observed that the heavy metal contamination was determined in this site (i.e. Cd exceeded the Dutch guideline value) (ESDAT, 2000) in urban soil and found the fungal diseases in the urban parks.

2.2 Study sites

A total of around 200 pits were excavated at 20 to 30 locations in 18 randomly selected urban and rural areas for the investigation in this project, namely Central Kwai Chung Park (CWCP), Chai Wan Park (CWP), The Education University of Hong Kong (EDU), Hong Kong Park (HKP), Kowloon Park (KWP), Kowloon Walled City Park (KWCP), Lai Chi Kok Park (LCKP), Lion Rock Park (LRP), Ma On Shan Park (MOSP), Mui Shue Hang Playground (MSHP), North District Park (NDP), Po Hong Park (PHP), Hong Kong Science Park (SP), Sha Tin Park (STP), Tai Po Waterfront Park (TPWP), Tin Shui Wai Park (TSWP), Tuen Mun Park (TMP) and Yuen Long Park (YLP) (Fig. 1). The abbreviation, names of parks, district, area (ha) and opening year (since) of the sampling locations was listed in Table 1.

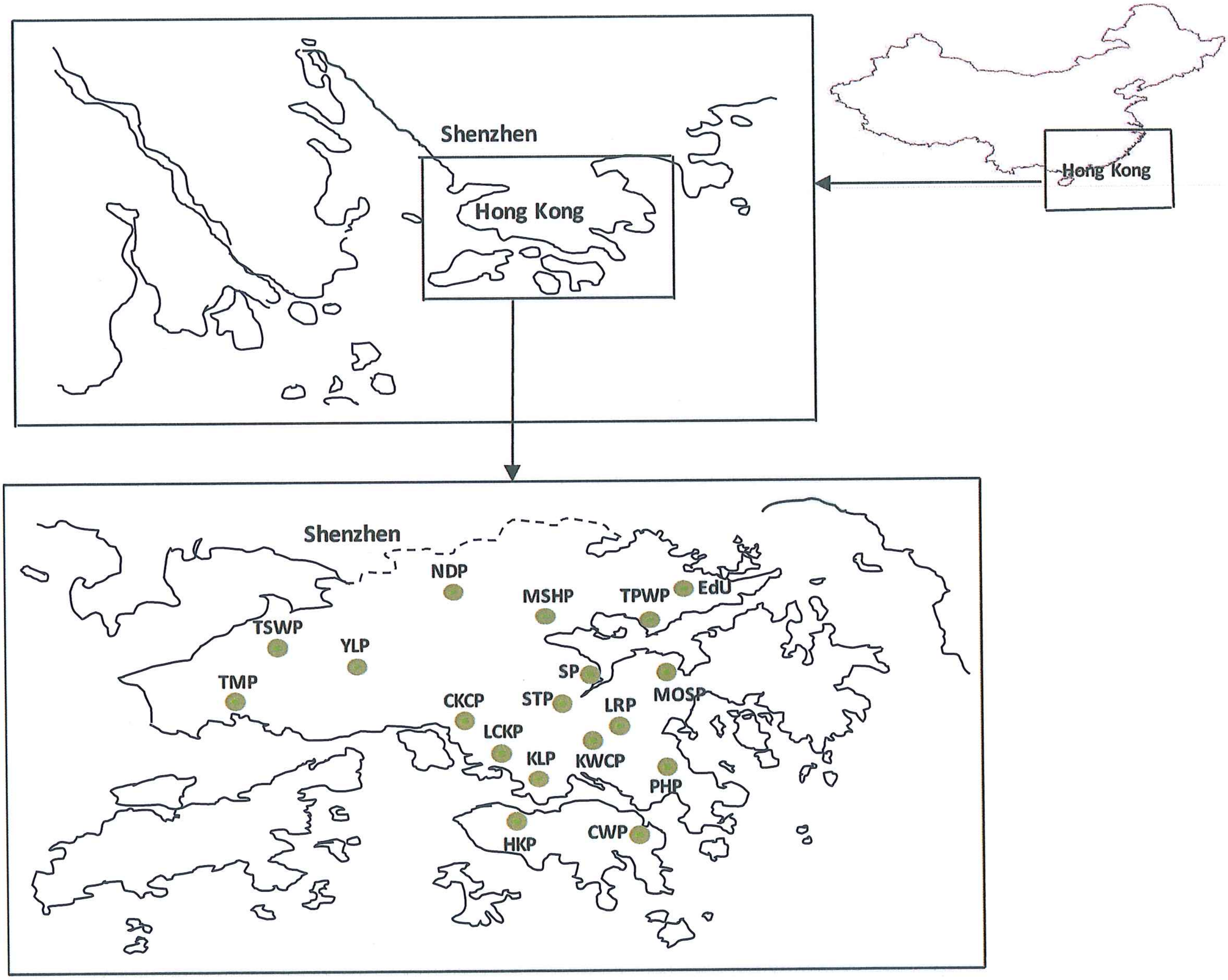

Fig. 1 Sampling locations

Table 1 The abbreviation, names of parks, district, area (ha) and opening year (since) of the sampling locations

No.	Abbreviation	English name of Parks	Chinese name of Parks	District	Area (ha)	Opening Year (Since)
1	CKCP/KCP	Central Kwai Chung Park	中葵涌公園	葵青	10.56	1986
2	CWP	Chai Wan Park	柴灣公園	東區	7.13	1993
3	EDU	Green spaces in the Education University of Hong Kong	香港教育大學	大埔	12.5 (Total area)	1994
4	HKP	Hong Kong Park	香港公園	中西區	8	1991
5	KLP/KWP	Kowloon Park	九龍公園	油尖旺	13.3	1970
6	KWCP	Kowloon Walled City Park	九龍寨城公園	九龍城	3.1	1994
7	LCKP	Lai Chi Kok Park	荔枝角公園	深水埗	17.65	1989
8	LRP	Lion Rock Park	獅子山公園	黃大仙	5.07	1966
9	MOSP	Ma On Shan Park	馬鞍山公園	沙田	5.5	1998
10	MSHP	Mui Shue Hang Playground	梅樹坑遊樂場	大埔	2.25	1991
11	NDP	North District Park	北區公園	北區	8.605	1990
12	PHP	Po Hong Park	寶康公園	西貢	4.13	1997
13	SP	Green spaces in the Hong Kong Science and Technology Park	香港科學園	沙田	22 (Total area)	2001
14	STP	Sha Tin Park	沙田公園	沙田	8.05	1988
15	TPWP	Tai Po Waterfront Park	大埔海濱公園	大埔	22	1994
16	TSWP	Tin Shui Wai Park	天水圍公園	元朗	14.86	1997
17	TMP	Tuen Mun Park	屯門公園	屯門	12.5	1985
18	YLP	Yuen Long Park	元朗公園	元朗	7.5	1991

2.3 Physical measurements of tree

The basic information of the infected trees was recorded and measured including (1) the species name, (2) dates of record, (3) locations, (4) presence of wood decay fungi, (5) height, (6) crown spread, (7) DBH. Tree height and DBH measurement were using standard methods for maintaining the accuracy of the results.

Tree height was measured by using the tangent method for evaluating this parameter which developed by Korning and Thomsen (1994). Alternatively, DBH was measured in aligned with the rules suggested by Agriculture, Fisheries and Conservation Department (AFCD, 2006).

2.4 Soil sampling

Most of the surface soils were collected as the samples randomly in each site from the September 2022 to October 2022, while each composite sample is consisted of 5 sub-samples collected from the surrounding of each site (within 1 m^3). All of the soil samples were sampled and put into the aluminium foil, followed by stored in zip lock bags immediately and transported to the biology laboratory at the Education University of Hong Kong (EDU).

2.5 Fungal disease investigation

In the field study, all of the fungal infected trees were visually identified in the study sites based on the presence of fruiting bodies and mycelia, which were different types of fungi appearance, according to the field guide published by the Development Bureau (2020). The fungal infection rate was calculated by counted number of fungal infected trees and divided by the total number of trees that according to the information given by Leisure and Cultural Services Department (LCSD) and the Education University of Hong Kong (EDU).

2.6 Sample treatment

Soil samples were air-dried for a week. After removing and grinding the large pieces of plant debris and other particles, such as rocks or large particles, they were passed through the sieve with 2 mm.

2.7 Chemical analysis

pH value, electrical conductivity (EC) and redox potential were measured by using pH meter, conductivity meter and redox tester respectively on the soil extracts that obtained by shaking with double-distilled water at 1:5 *(w/v)* sample to water ratio and stored at 4°C refrigerator. On the other hand, total carbon (C), hydrogen (H), nitrogen (N) and Sulphur (S) measurements were analyzed by the Perkin Elmer 2400 series II CHNS/O analyzer. The total organic matter (TOM) and total organic carbon (TOC) were measured by the dry combustion under 550°C in furnace. Nitrate-N was measured by the cadmium reduction method (APHA, 1995).

2.8 Metal pollutant analysis

For the heavy metal analysis, Arsenic (As) (metalloid), Mercury (Hg), Cadmium (Cd), Lead (Pb), Zinc (Zn), Copper (Cu), Iron (Fe) and Potassium (K) were selected for the determination of heavy metal content in the sediment samples and measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) (Optima 3000 DV; Perkin Elmer). Since the first 6 metal(loid)s were the emerging pollutants and the last 2 metals were defined as the macronutrients in soil.

Total metals in the sediment samples were extracted by HCl-HNO3-HF microwave digestion (USEPA Standard method 3051A and 3050B) (USEPA, 2007 & 1996), while the water extractable metals in the sediment samples were extracted by double-distilled water that following the extraction method of measuring pH value, EC and redox potential (APHA, 1995). The Standard Reference Material (SRM) from National Institute for Standards and Technology (NIST) 2711 Montana soil was used for verifying the accuracy of the determination of metal and the recovery rates were within 90% ± 10%. All the metal contents were expressed as milligrams per kilogram (mg/kg).

2.9 Statistical analysis

Variables input for the Principal Component Analysis (PCA) included fungal infection rate, pH, EC, redox potential, C, H, N, S, NO_3-N, As, Hg, Cd, Pb, Zn, Cu, Fe, K in terms of total and water extractable metals. In total, 2250 (25 parameters x 18 sites x 5 samples) raw data were included. The data were first examined by Kaiser–Meyer–Olkin (KMO) statistics and Bartlett's test for suitability for PCA, before they were processed using the Primer 6 software. Those tests are measures of sampling adequacy that use the proportion of variance. The KMO value must be greater than 0.5, and the significance level of Bartlett's test must be less than 0.05. The number and importance of uncorrelated principal components extracted from the soil quality parameters are presented in a scree plot. When the eigenvalue of a principal component is equal to, or greater than, 1, the result of the principal component analysis is considered significant. To minimize the variations among the variables for each factor, the factor axes were varimax-rotated. Rotating the principal components can produce a meaningful representation of the underlying factors by decreasing the contribution of variables with minor significance and increasing the contribution of those with more significance. All data were subjected to one-way ANOVA Duncan's Multiple Range test and T test (<0.05) and Pearson Correlation test (<0.05) using SPSS 28.0 software for further analysis to check whether the existence of significant differences and relationship among the data. Means and standard errors were calculated based on five replicates in each site. Means were compared by Duncan's Multiple Range test (Little and Hills, 1978).

3. Results and discussion

3.1 Fungal infection rate of study sites

Fig. 2 showed the summary of fungal infection rate recorded in the sampling sites which ranged from 0.78 - 24.3%. The highest percentage of 24.3% was detected in the LRP, while the lowest percentage of 0.78% was detected in the EDU. Due to the state of trees in each site were varied, different symbiotic or parasitic association between tree and fungi may be possible to alter the infection rate.

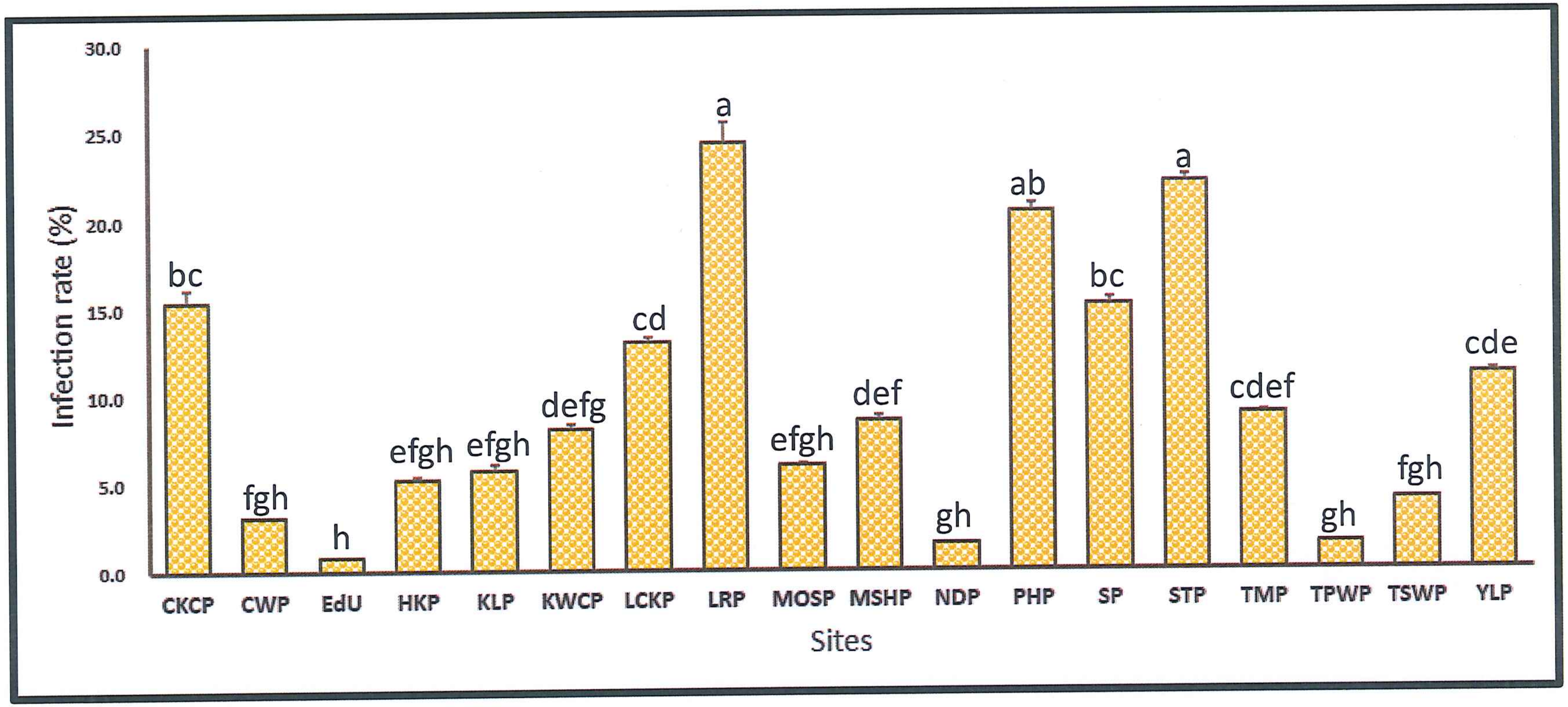

Fig. 2 The fungal infection rate (%) in the various types of trees in the 18 sites

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

3.2 Chemical parameters of soil samples

pH value

Fig. 3 showed that the pH values of all soils in the sampling sites varied from 6.12 to 7.15. The highest pH value of 7.15 was detected in the LCKP, while the lowest pH value of 6.12 was detected in the NDP. Most of the soil was slightly acidic and close to neutral. With reference to the soil improvement guidelines, the optimum range of pH value for the plant grow favorably in 5.5 to 8.0 and most of plants prefer the soil pH around 6.5 to 7.5 for their better growth. Thus, MOSP, NDP, PHP, TMP, TSWP and YLP may suspect to the tree fungal infection as their average pH values were lower than 6.5. The poor growth of certain plants may be presented due to the less available to plant absorption and more strongly bound to the soil particles by the nutrients (Development Bureau, 2022).

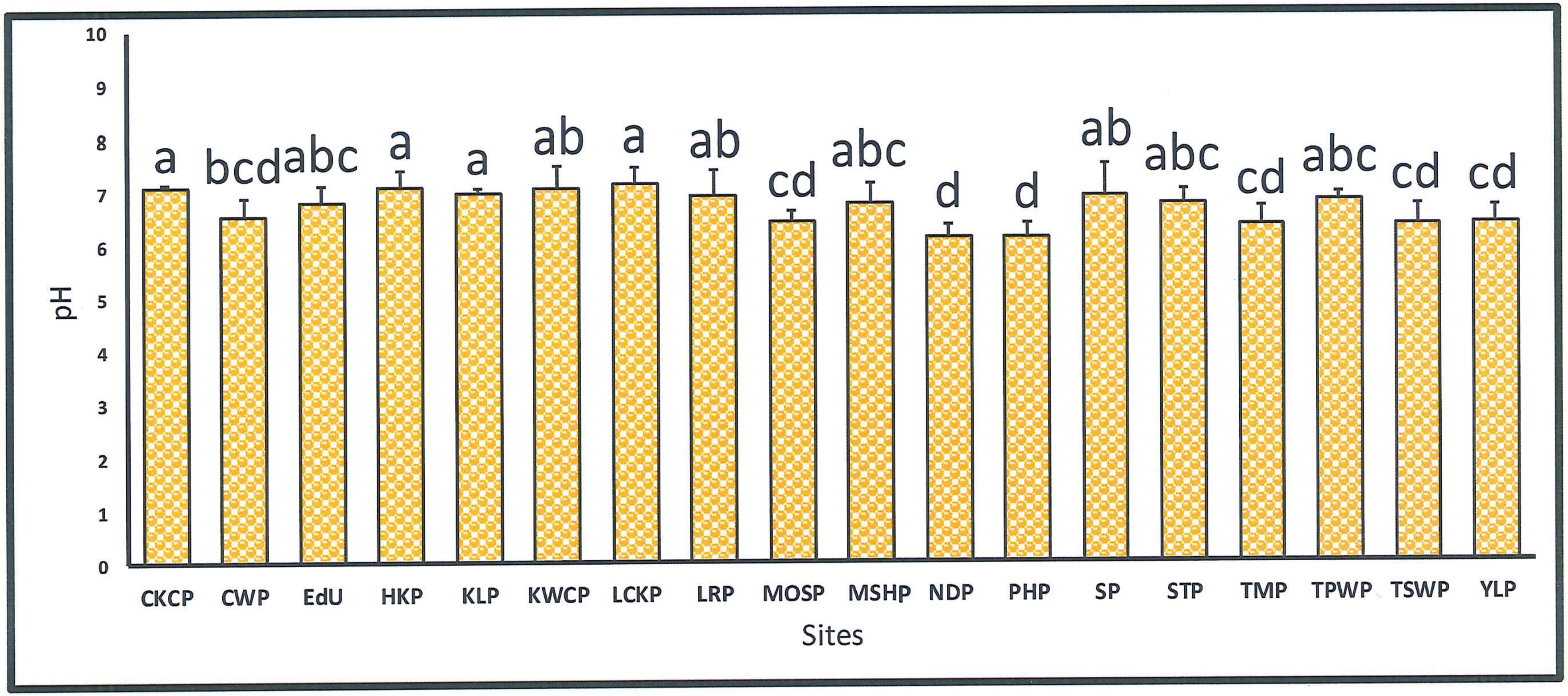

Fig. 3 The pH value in the soils in 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Electrical Conductivity (EC)

According to Fig. 4, EC of the soil samples fell into the range of 334.6-1641.8μs/cm. The highest EC value of 1641.8μs/cm was detected in the HKP, while the lowest EC value of 334.6μs/cm was detected in the PHP. The EC value varied in 18 urban parks due to the varied mobile ions presented in the soil such as cation and anion. Besides, the concentration of mobile ions changed according to its water conductivity and presence of nutrients in the soil. To evaluate the condition of EC value in these 18 sites, only HKP, MSHP, TMP and TPWP were suitable for the optimum EC ranges in soil between 1000 - 5000μs/cm that providing the needs of nutrients in the plant in the form of free ions for their healthy growth (Development Bureau, 2022).

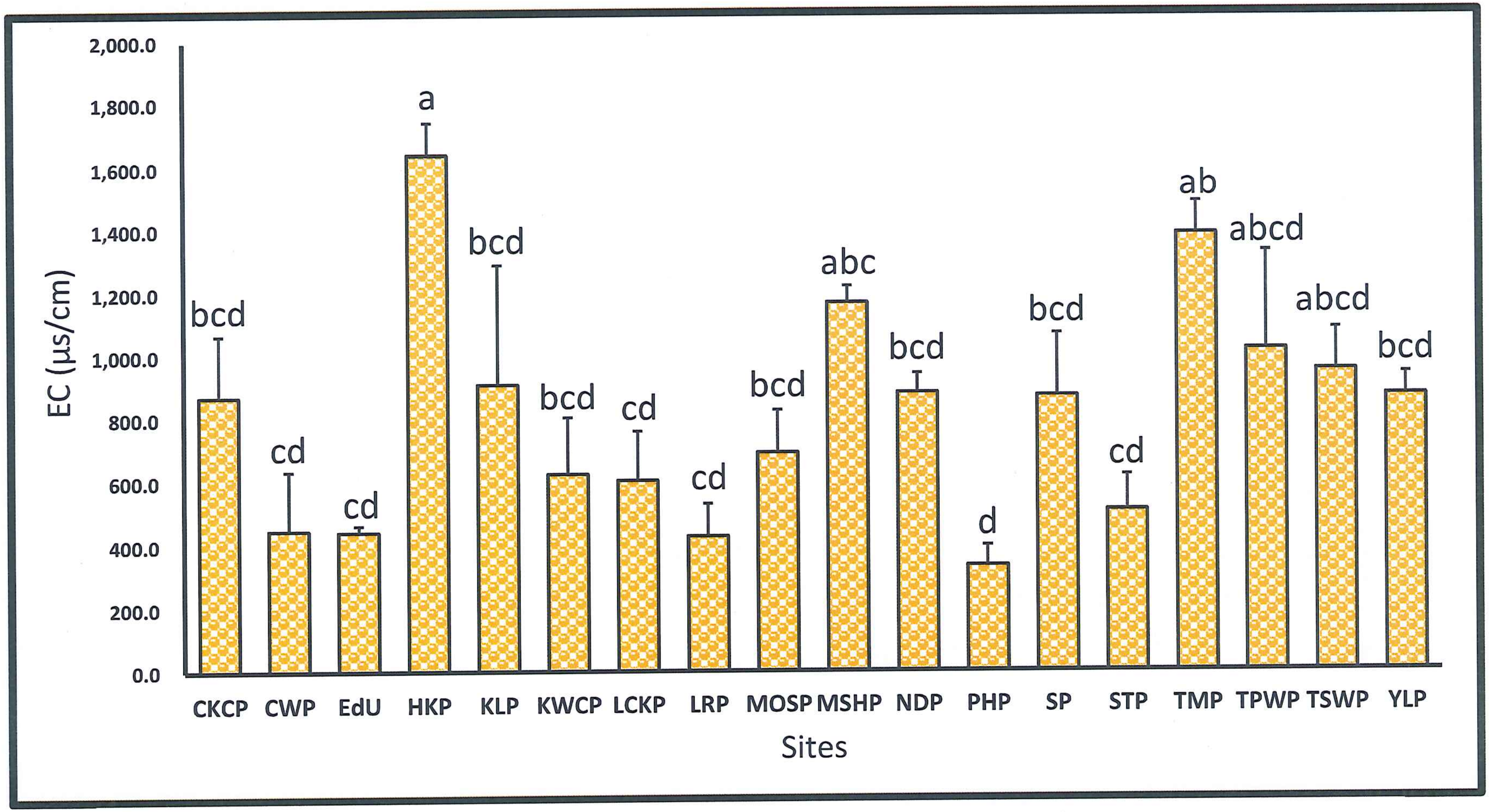

Fig. 4 The EC value (µs/cm) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Redox Potential

Fig. 5 showed that the values of redox potential in all soil samples ranged from +2 to +61.2mV. The highest redox potential of 61.2mV was detected in the EDU, while the lowest redox potential of 2 was detected in the STP. The redox potential of all sites fell on the normal range of −300 to +900mV. However, the optimum redox potential for the plant growth in the range of +400 to +450 mV, meaning that they were lower than 350 mV which are particularly limiting the growth for many plants and even decreased their growth speed (Husson, 2013). Since the states of cation and anion changed frequently according to the external environment in each site. Thus, the further analysis should be conducted to examine the relationship between redox potential and tree health under the controlled condition. Based on this result, the trees grown on these 18 sites may lead to the infection of fungi as the slow speed of plant growth resulted by the low redox potential in soil.

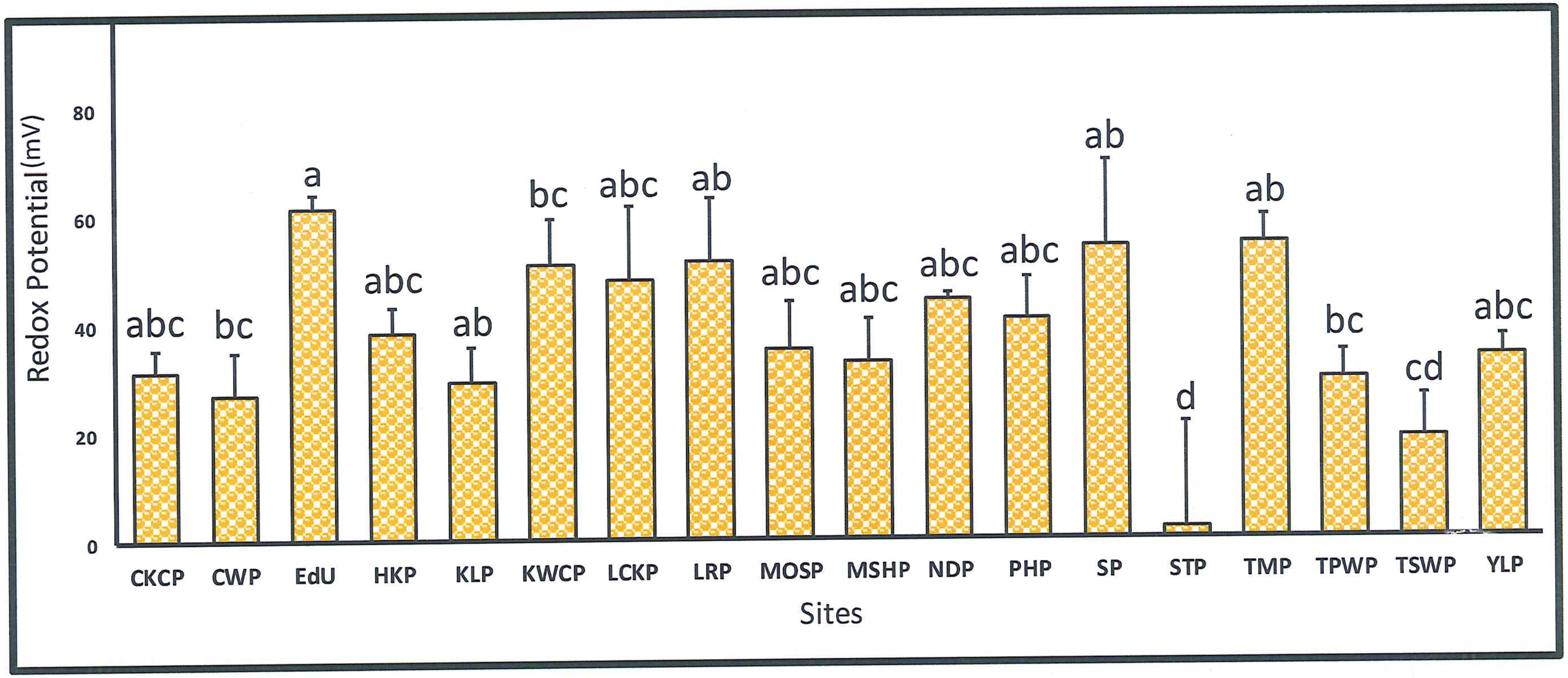

Fig. 5 The redox potential (mV) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Total C and H

Figures 6 to 9 showed that the concentration of total C, H, N and S of all sediment samples ranged from 0.85-4.51%, 0.36-1.81%, 0.14-1.33% and 0.05-0.46% respectively. For total C, the highest percentage of 4.51% was detected in the SP, while the lowest percentage of 0.85% was detected in the LRP. For total H, highest percentage of 1.81% was detected in the SP, while the lowest percentage of 0.36% was detected in the NDP.

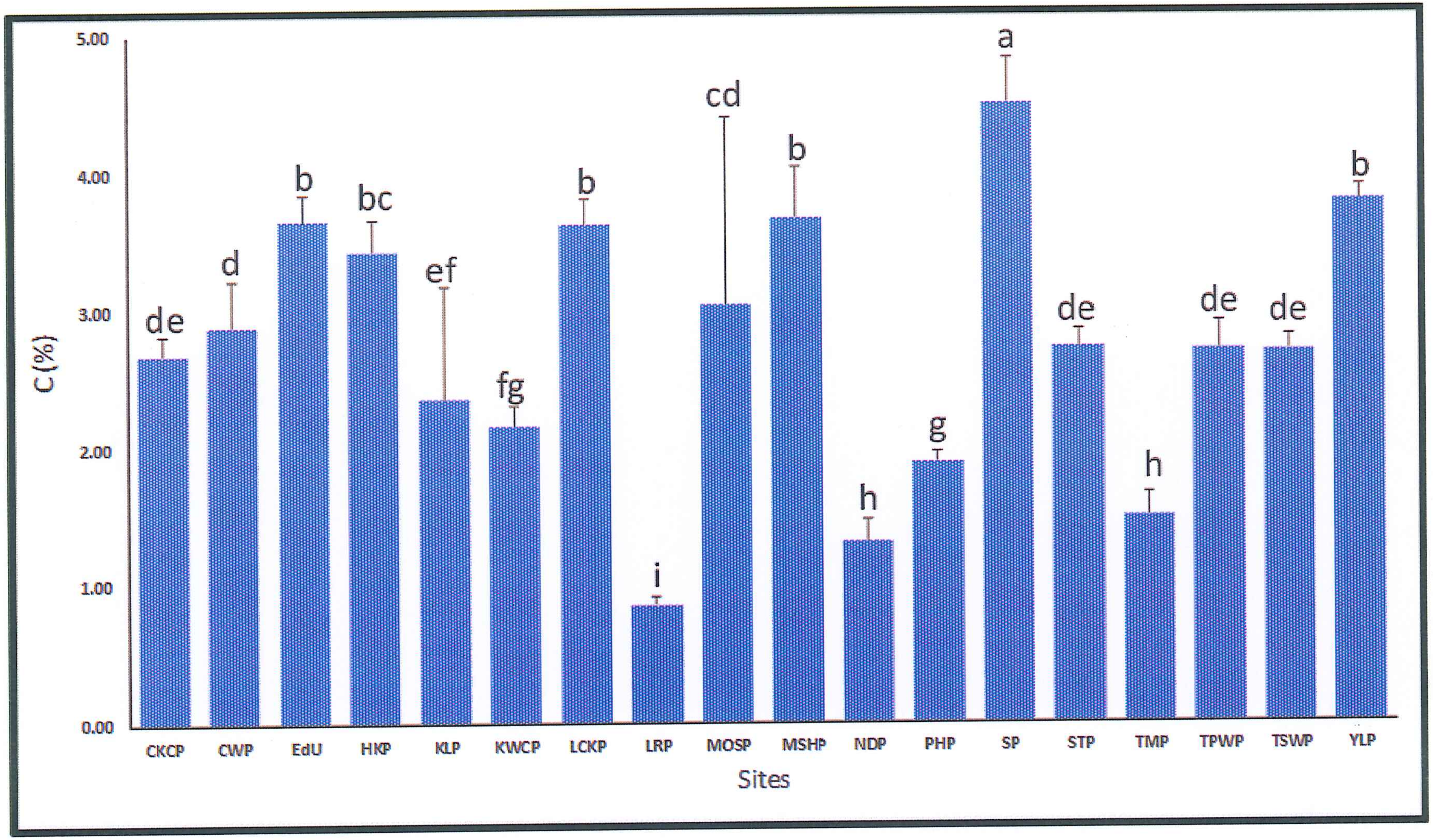

Fig. 6 Total C concentration (%) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

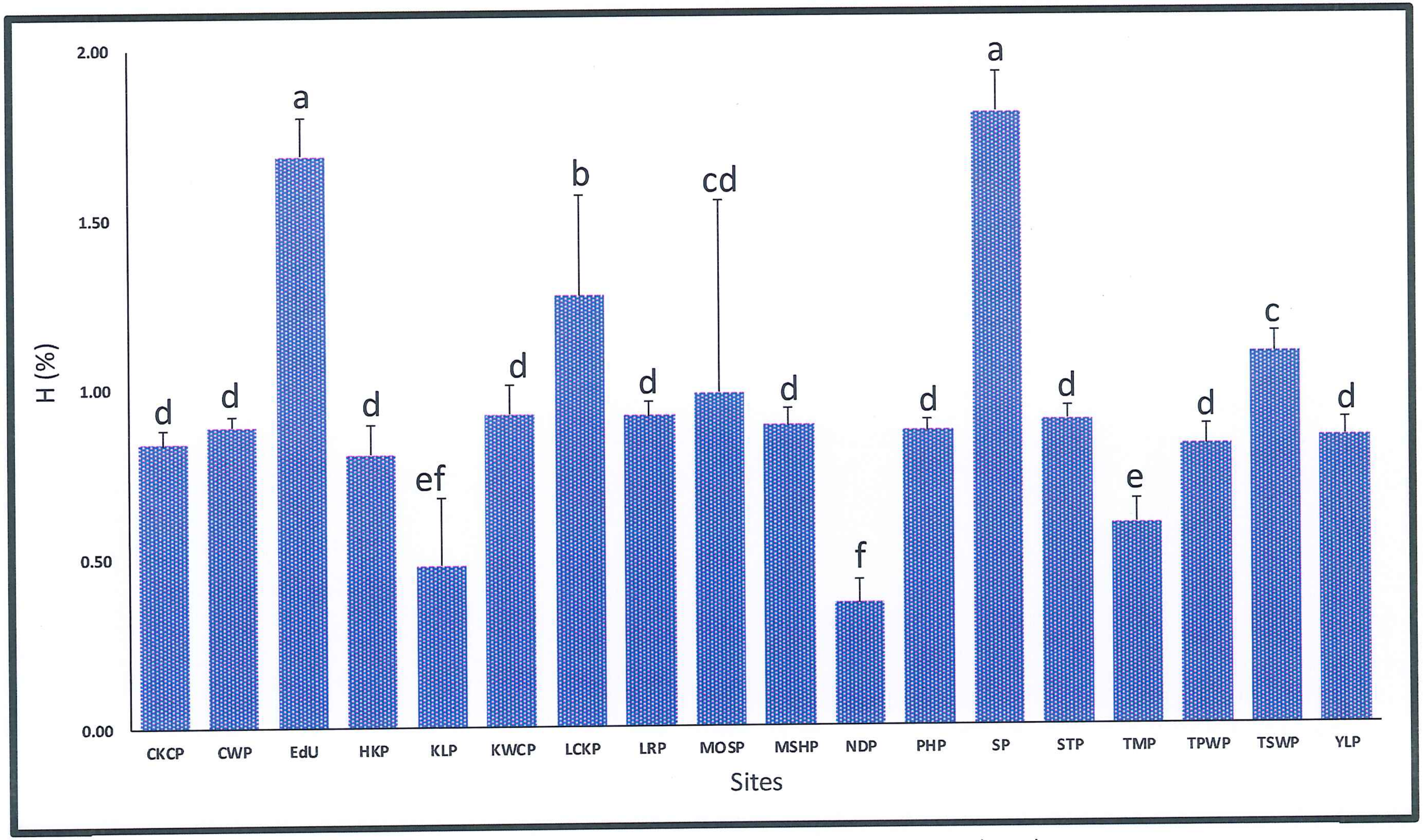

Fig. 7 Total H concentration (%) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Total N

For total N, the highest percentage of 1.33% was detected in the SP, while the lowest percentage of 0.14% was detected in the NDP. The Development Bureau (2022) suggested that the optimal range of N was 40 – 150 mg/kg in soil. This result (1400 – 13300 mg/kg) was exceeded the range of optimal N level and lead the risk of surface runoff due to the excessive nutrients.

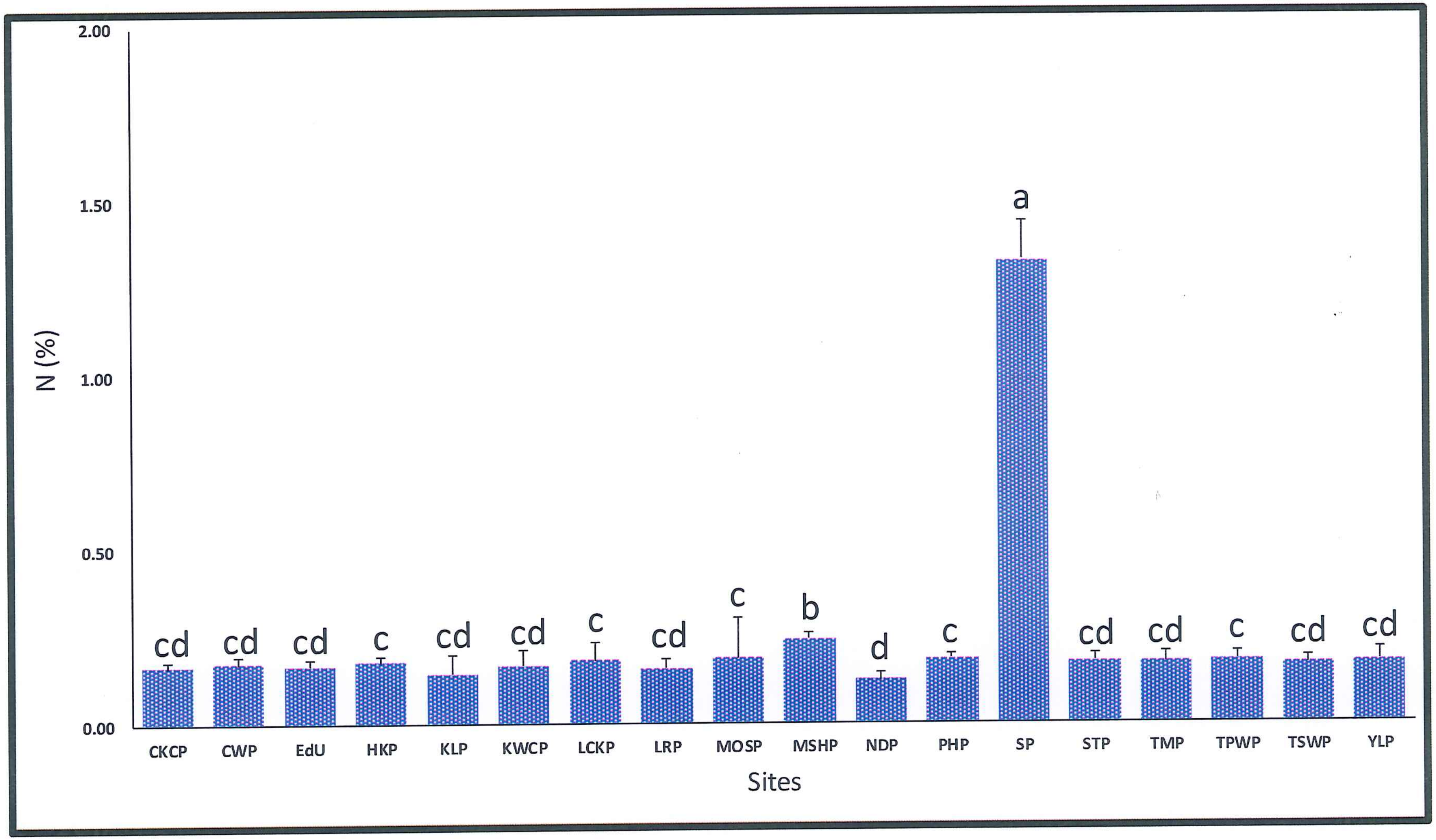

Fig. 8 Total N concentration (%) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Total S

For total S, the highest percentage of 0.46% was detected in the EDU, while the lowest percentage of 0.05% was detected in the KLP. The optimal range of S was 25 – 100 mg/kg in soil. Based on the result study (500 – 4600 mg/kg), the total S content in these 18 sites were exceeded the range of optimum S level. Under the excess S supply, the plant can grow under non saline condition that providing sufficient demand for S for the reduced S compounds synthesis. This element can stabilize and maintain the proteins' tertiary structure and promote the formation of S-bridges of enzyme proteins. It is proved that the photosynthetic capacity improvement correlated with increase of S demand when under the salt stress for dealing with the adverse effects of salt stress (Fatma et.al, 2014). Moreover, Khan et.al (2009) claimed that the higher S assimilation capacity can increase the Cd stress tolerance in the mustard cultivars with high photosynthetic potential. The S supply can also control the roots' ion uptake and the transportation of ions into leaves which related to the antioxidants for the exclusion of Na^+ ions and Cl^- ions content formation.

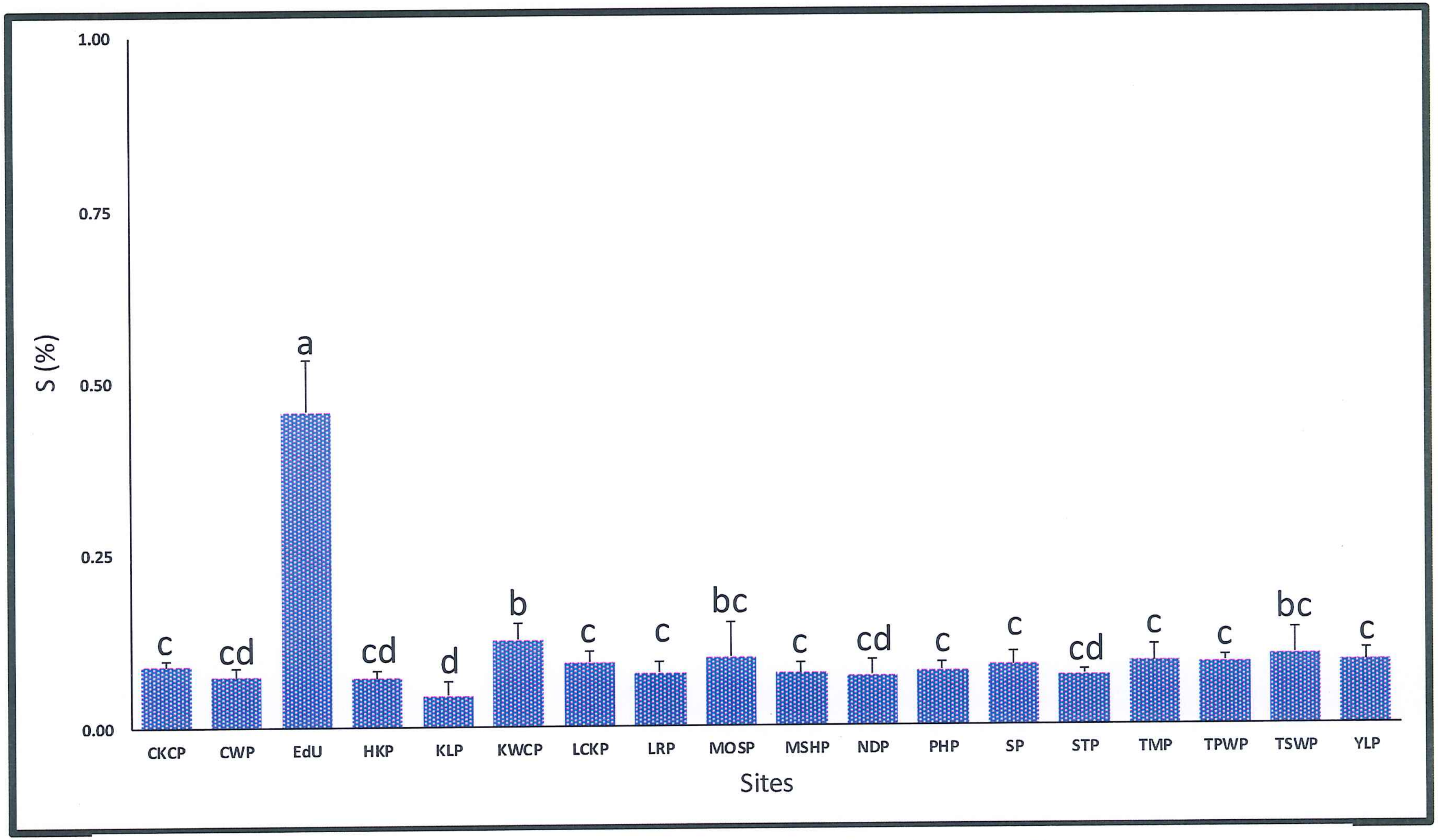

Fig. 9 Total S concentration (%) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Total Organic Carbon (TOC)

With reference to figure 10, the content of TOC ranged from 2.40 to 7.08%. The highest percentage of 7.08% was detected in the YLP, while the lowest percentage of 2.40% was detected in the PHP. The optimum range of soil organic matter (SOM) known as 1 - 6% in the topsoil. Based on the result study, MOSP and YLP were out of the optimum range of SOM that directly correlated with the excess amount of TOC in the soil (Development Bureau, 2022).

When high level of TOC obtained in the soil, it can contribute to the immobilization of some of the nutrients, such as nitrogen. Then the temporary nutrient deficiencies to the microorganisms will be resulted as they tend to compete with crops for the nutrients. Thus, the deficiencies of nutrition lead them die and the nutrients can release back into the nutrient pool of the plant (Bagshaw et.al, 2010).

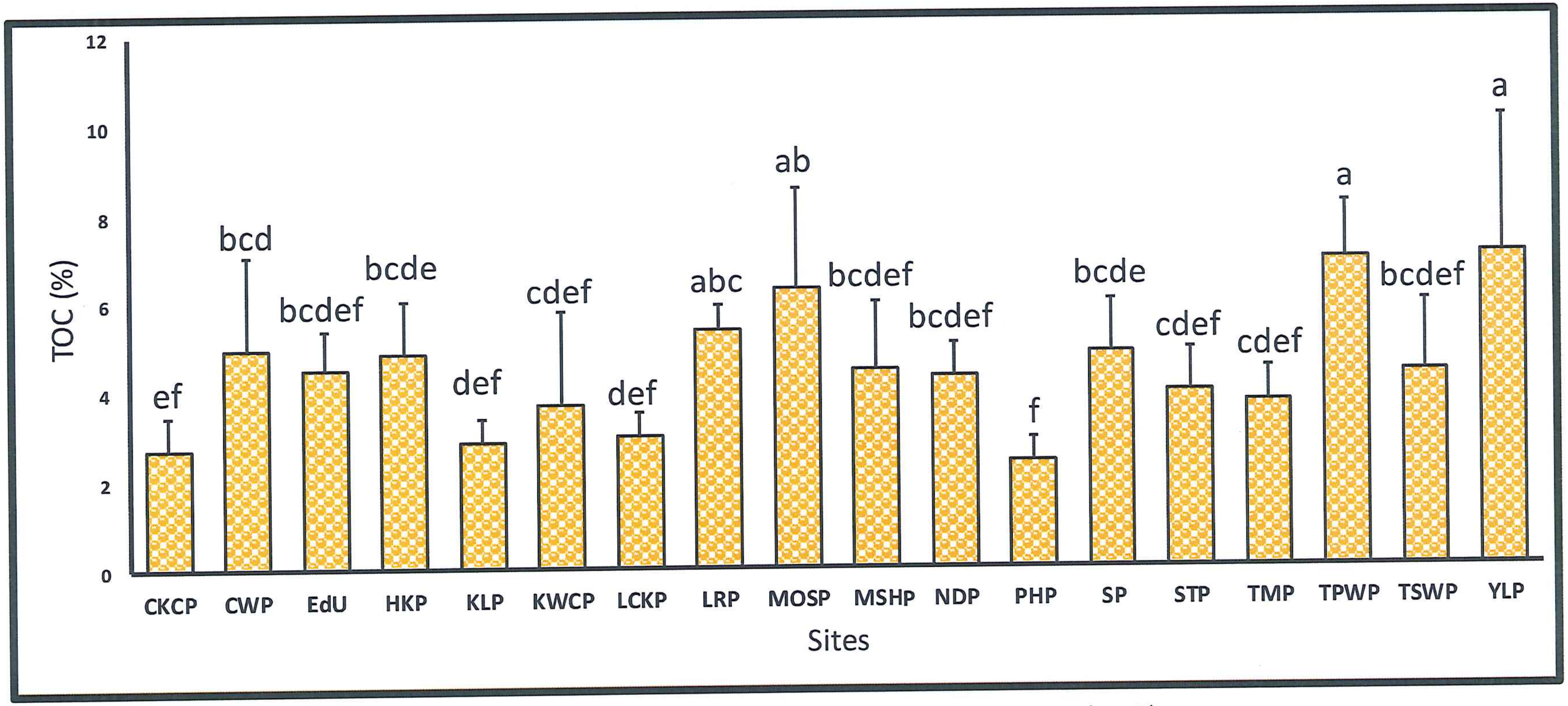

Fig. 10 Total organic carbon (%) in the soils of the 18 sites (n = 5)

Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

Nitrate-N

According to the figure 11, the concentration of nitrate-N ranged from 2.70 to 52.42 mg/kg, the highest percentage of 2.70 mg/kg was detected in the MSHP, while the lowest percentage of 52.42 mg/kg was detected in the CKCP. In this parameter, the amount of the availability of nitrogen in soil that absorbed by plant immediately for making amino acids, proteins and chlorophyll was measured. In general, the nitrate-N level remains the ranged value of 10 – 50 mg/kg (Bagshaw et.al, 2010), only 4 out of 18 sites fell into the optimal level of nitrate-N that ranged from 20 – 40 mg/kg, however, 1 and 5 out of 18 sampling sites were higher than 50 mg/kg and lower than 10 mg/kg respectively. For the site contain the low level of nitrate-N (i.e. CKCP, KWCP, MOSP, TMP and TSWP), the insufficient of nutrients may cause the severe effects to tree health, and hence easily to suffer from fungal infection.

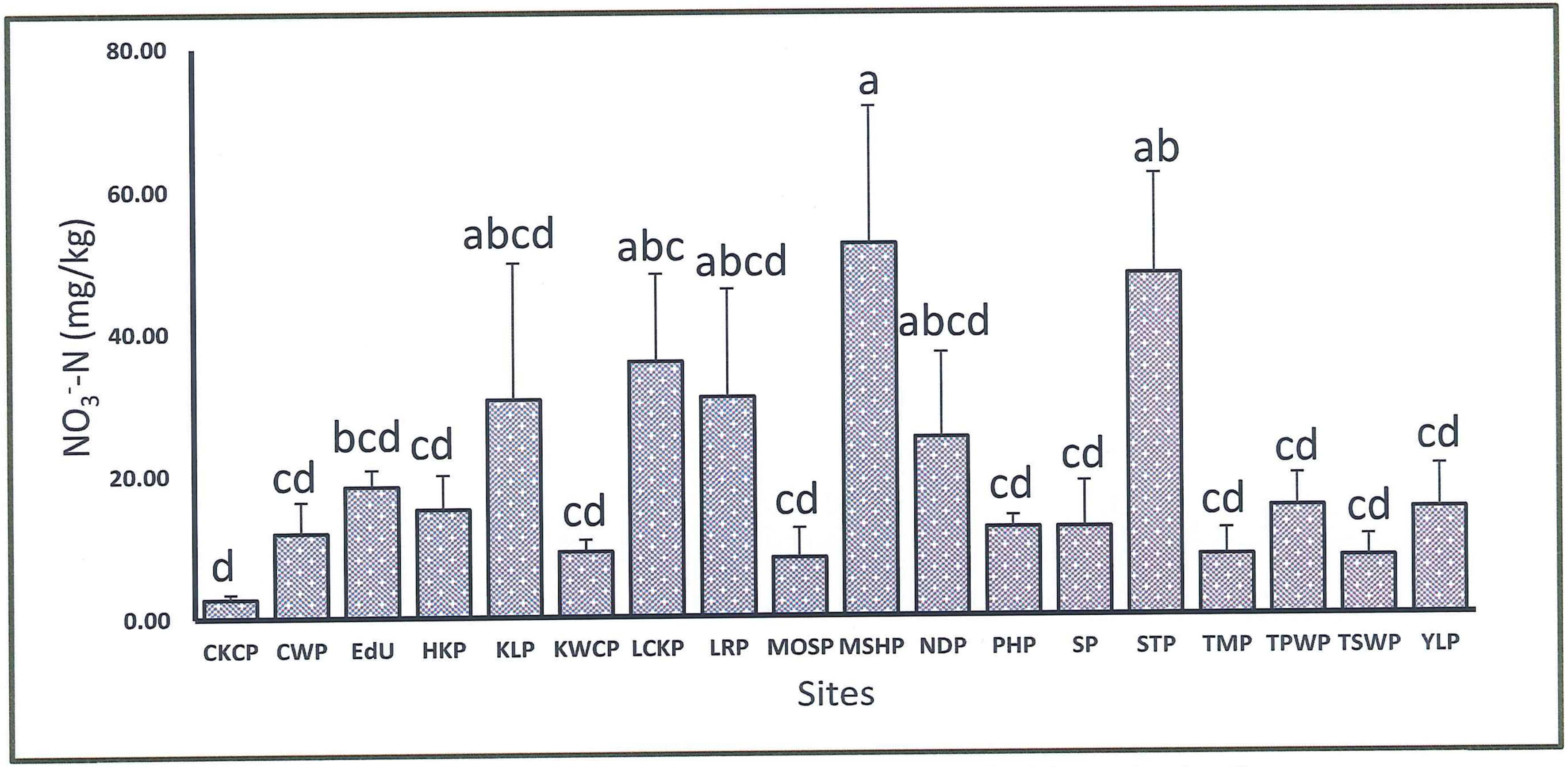

Fig.11 Nitrate-N concentration (mg/kg, dry weight) in the soils of the 18 sites (n = 5)
Mean with different letter are significantly different according to Duncan Multiple Range test at 5% level.

3.3 Heavy Metal Analysis

3.3.1 Water extractable metal in soil

According to the table 2, the range of water extractable metals concentration in soil (mg/kg, dry weight) were the following, Pb <0.01 – 0.07; Zn 0.30 – 1.33; Cu <0.01 – 1.20; Cd <0.01 – 0.02; Fe 1.20 – 50.33; K 13.72 – 123.81; As <0.01 – 1.93; Hg <0.01 – 0.07 respectively. General speaking, the value lower than the total concentration as water extractable metal is a portion of total metal concentration presented in soil.

Table 2 Concentration of water extractable metals (mg/kg, dry weight) in 18 urban parks in Hong Kong (n = 5)

Water extractable metals	Pb	Zn		Cd	Fe	K	As	
CKCP	<0.01	0.302 ±0.084c	0.049 ±0.022b	<0.01	12.859 ±6.047a	21.613 ±5.714b	<0.01	<0.01
CWP	<0.01	0.527 ±0.164ab	0.203 ±0.122b	<0.01	3.918 ±1.137a	123.812 ±54.374a	0.029 ±0.029a	0.037 ±0.037a
EDU	<0.01	0.767 ±0.273ab	0.021 ±0.014b	<0.01	34.451 ±25.558a	13.724 ±3.023b	1.290 ±1.290a	0.149 ±0.094a
HKP	<0.01	0.472 ±0.235ab	0.036 ±0.021b	<0.01	6.989 ±2.839a	47.036 ±14.430b	0.182 ±0.182a	<0.01
KLP	0.045 ±0.013ab	1.329 ±0.370a	1.197 ±0.219a	<0.01	1.197 ±0.219a	63.901 ±20.612b	<0.01	0.076 ±0.037a
KWCP	<0.01	0.627 ±0.246ab	<0.01	<0.01	27.889 ±14.769a	47.146 ±15.690b	<0.01	<0.01
LCKP	<0.01	0.351 ±0.143ab	0.351 ±0.143b	0.011 ±0.002ab	16.731 ±7.233a	52.973 ±14.184b	1.927 ±0.947a	<0.01
LRP	<0.01	0.669 ±0.219ab	0.043 ±0.019b	0.016 ±0.006a	37.938 ±9.862a	61.238 ±31.185b	<0.01	<0.01
MOSP	<0.01	0.484 ±0.206ab	0.046 ±0.030b	<0.01	15.242 ±13.040a	15.731 ±4.656b	1.187 ±0.929a	0.236 ±0.227a
MSHP	0.021 ±0.021ab	0.512 ±0.135ab	0.061 ±0.033b	<0.01	19.933 ±9.414a	45.026 ±19.812b	1.176 ±1.176a	0.013 ±0.009a
NDP	<0.01	0.795 ±0.151ab	0.336 ±0.314b	<0.01	9.924 ±5.676a	36.653 ±10.907b	0.519 ±0.330a	0.077 ±0.051a
PHP	<0.01	0.983 ±0.405ab	0.058 ±0.031b	<0.01	18.172 ±12.207a	18.600 ±6.547b	0.722 ±0.722a	0.040 ±0.030a
SP	<0.01	1.262 ±0.541ab	0.106 ±0.039b	<0.01	50.329 ±46.389a	29.445 ±12.345b	0.837 ±0.525a	0.201 ±0.179a
STP	0.018 ±0.018ab	1.301 ±0.424ab	0.020 ±0.010b	0.018 ±0.008a	25.549 ±5.969a	57.816 ±16.453b	<0.01	<0.01
TMP	0.074 ±0.074a	0.627 ±0.418ab	0.108 ±0.073b	<0.01	17.875 ±11.510a	74.713 ±27.787ab	1.889 ±1.189a	0.042 ±0.040a
TPWP	<0.01	0.663 ±0.176ab	<0.01	<0.01	42.548 ±5.949a	41.737 ±12.651b	0.156 ±0.156a	<0.01
TSWP	<0.01	0.751 ±0.228ab	0.102 ±0.062b	<0.01	6.140 ±1.660a	53.510 ±11.239b	0.032 ±0.032a	<0.01
YLP	<0.01	0.596 ±0.115ab	0.083 ±0.030b	<0.01	24.460 ±15.208a	36.305 ±9.557b	0.037 ±0.037a	0.125 ±0.078a

Pb: Lead, Zn: Zinc, Cu: Copper, Cd: Cadmium, Fe: Iron, K: Potassium, As: Arsenic, Hg: Mercury

Within each water extractable metals, means with the same letter are not significantly different according to Duncan's Multiple Range Test at 5% level.

3.3.2 Total metal concentration in soil

According to the table 3, the range of total metals concentration in soil (mg/kg, dry weight) were the following, Zn 2.65 – 12.38; Pb 12.90 – 45.75; Cu <0.01 – 10.30; Cd <0.01 – 1.59; Fe 1883.10 – 5164.66; K 0.57 – 54.35; As <0.01 – 48.92; Hg 0.59 – 1.91 respectively. Levels of Cd, As and Hg in soil collected from some or all of the 18 sites in Hong Kong were above the values stated by Dutch guidelines (ESDAT, 2000). The Dutch Intervention Values in Dutch guidelines for soil were suggested for determining whether the land that is "already contaminated" causes a serious threat to the health of general public. Thus, the Dutch Target Values were conserving the sustainable soil quality, have the basis of ecological health and evaluating the "uncontaminated" land (ESDAT, 2000). Total Cd, As and Hg soil were exceeded 5, 1.7 and 6 times of those values, thus the regarding sites should conduct the environmental remediation immediately.

In general, as soil is the part of abiotic component in an ecosystem, pollutants may leach to the other component, leading to acute and chronic toxicity in human body.

Jakubowski (2003) pointed out that the consumption of food and water drinking were the major routes for human exposure of heavy metals. The air inhalation and soil intake were also the sources of exposure in some of the cases. For Cd, food was the dominant source of Cd exposure, however, then the kidney was the most sensitive and important organ that needed the sufficient protection of this organ. The early disturbance of renal functions will be resulted if the human treated with the long-term occupational and environmental exposure to Cd. For instance, reduce the rate of glomerular filtration or filtration reserve capacity. Cd was also a potential human carcinogen that may causing lung cancer (WHO, 2007).

For As, under arsenic exposure, people may have these symptoms such as abdominal pain, skin lesions and skin cancer. If the people exposed in higher than 0.02 mg/L (WHO limit) from smelting, mining, rock sediment and fossil fuels, they may suffer from bone marrow depression, gastrointestinal symptoms, haemolysis and liver tumours (Momodu & Anyakora, 2010). Besides, As was regarding to the pregnant women that inducing the adverse outcomes and infant mortality. This metal will increase the mortality in young adults because of the lung disease, multiple cancers, kidney failure and heart attacks, even involved the cognitive development, memory and intelligence of youngsters (WHO, 2007).

For Hg, at a high level of exposure Hg vapour (> 1000 μg/m^3), it can cause the severe irritation of the respiratory tracts, pneumonia, pulmonary oedema and other lung damages. For some of the cases, Hg can induce the damage in nerves, brain and kidneys. Coma and death may be resulted in the extreme cases. If the people exposed to Hg under 4-8 hours, the dyspnea, accident experienced chest pains, haemoptysis, coughing, and pulmonary function impairment will be resulted (McFarland & Reigel, 1978). The inorganic Hg (i.e. Hg salts) also have the acute poisoning when the people involved the chronic occupational exposure. The associated effects were mainly related to central nervous system of human (Clarkson, 2002).

Table 3 Concentration of total metals (mg/kg, dry weight) in 18 urban parks in Hong Kong (n = 5)

Total metals	Pb	Zn	Cu	Cd	Fe		As	Hg
CKCP	9.18 ±1.835abc	45.75 ±8.636a	5.84 ±1.380c	<0.01	2907.44 ±396.73bc	2.72 ±1.36bcfg	5.23 ±5.23a	<0.01
CWP	11.12 ±1.367ab	24.51 ±2.037bcd	5.75 ±0.753b	0.01 ±0.01b	3339.61 ±617.29b	20.78 ±5.94cd	<0.01	0.037 ±0.037b
EdU	3.36 ±0.770def	34.63 ±5.894ab	<0.01	<0.01	3458.61 ±359.36b	7.62 ±2.78defg	<0.01	0.149 ±0.094bcd
HKP	8.71 ±1.885abcd	26.66 ±4.898bcd	<0.01	<0.01	2414.55 ±225.63bc	7.96 ±3.64defg	48.92 ±34.40a	<0.01
KLP	6.21 ±1.785bcdef	28.35 ±5.192bc	10.30 ±2.028b	1.59 ±0.500a	1883.10 ±307.95c	2.62 ±1.00efg	<0.01	0.076 ±0.037cd
KWCP	3.19 ±1.408ef	18.16 ±2.722cd	<0.01	<0.01	3619.49 ±492.47b	14.90 ±1.95cdef	23.27 ±23.27a	<0.01
LCKP	11.09 ±1.801ab	37.11 ±7.115ab	14.12 ±4.426a	<0.01	2798.58 ±162.71bc	0.57 ±0.46g	<0.01	<0.01
LRP	7.60 ±1.260abcdef	17.07 ±2.631	2.69 ±0.784c	<0.01	2995.76 ±291.25bc	4.28 ±1.98def	13.58 ±8.50a	<0.01
MOSP	7.13 ±1.714abcdef	12.90 ±1.814d	2.53 ±0.413c	<0.01	3109.65 ±350.42bc	16.00 ±3.93cde	17.09 ±8.50a	0.236 ±0.227bcd
MSHP	8.40 ±0.574abcde	18.07 ±1.777cd	2.44 ±0.307c	<0.01	2917.70 ±29.72bc	47.93 ±6.59ab	5.04 ±5.04a	0.013 ±0.009bcd
NDP	2.65 ±0.4845f	13.12 ±0.679d	2.59 ±0.178c	<0.01	1886.84 ±239.68c	36.85 ±5.29b	39.55 ±27.39a	0.077 ±0.051d
PHP	6.21 ±1.357bcdef	16.76 ±1.413cd	2.51 ±0.123c	<0.01	2308.85 ±121.23bc	1.56 ±0.99ef	33.02 ±24.07a	0.040 ±0.030bcd
SP	4.21 ±0.684cdef	38.20 ±4.535ab	5.67 ±0.235c	<0.01	2856.54 ±384.63bc	4.35 ±1.84efg	<0.01	0.201 ±0.179bcd
STP	4.40 ±0.672cdef	13.49 ±2.154cd	2.19 ±0.378c	0.28 ±0.140b	2519.54 ±246.36bc	11.75 ±4.71cdefg	13.86 ±13.86a	<0.01
TMP	12.38 ±3.890a	13.72 ±1.026d	2.78 ±0.167c	<0.01	2878.01 ±209.53bc	3.76 ±1.62efg	1.26 ±1.26a	0.042 ±0.040bcd
TPWP	6.21 ±2.277bcdef	16.67 ±2.451cd	2.61 ±0.354c	<0.01	3247.50 ±311.02b	22.60 ±7.31c	12.96 ±12.9a	<0.01
TSWP	6.03 ±0.469bcdef	25.98 ±8.026b	5.79 ±1.544c	<0.01	3385.52 ±562.64b	54.35 ±8.09a	1.03 ±1.03a	<0.01
YLP	3.28 ±1.176def	13.16 ±1.671d	3.44 ±0.340c	<0.01	5164.66 ±786.73a	7.01 ±2.58efg	33.82 ±33.82a	0.125 ±0.078a

Pb: Lead, Zn: Zinc, Cu: Copper, Cd: Cadmium, Fe: Iron, K: Potassium, As: Arsenic, Hg: Mercury

Within each total metal, means with the same letter are not significantly different according to Duncan's Multiple Range Test at 5% level.

3.4 Study the relationship between fungal infected tree and soil parameters in the field site

3.4.1 Correlation Analysis

The correlation analysis is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in these 18 sites. According to the table 3, the infection rate is negatively correlated with the pH at the 0.05 level and the TOC at the 0.01 level, meaning that when one variable increases, the other decreases.

The possible reason is that the fungal growth is sensitive to the change of pH and TOC due to the form of carbon available to the acidity and alkalinity of soil.

Table 4 Correlation analysis on the soil samples taken in 18 sites (n = 5)

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	-0.243**	-0.24*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

3.4.2 Principal Component Analysis

PCA is performed on the chemical parameters of soil quality collected in these 18 sites. Sites of greater similarities are plotted closer together. The contribution of each principal component and its eigenvalue after rotation are presented in table 5. The result exhibited two principal components (Eigenvalue > 1) control 99.9% of variations in the fungal infection rates of tree in these 18 urban parks. The first component is the most significant principal, which contribute to 93.7% of the variation, and is highly influenced by total Fe and EC. Principal components 2 is responsible for 6.2% of the variance respectively, and is moderately influenced by EC and total As. The possible reason of EC is the nutrients in form of free ions is available for the plant healthy growth (Development Bureau, 2022). Low value of EC may limit the growth plant by inhibiting the plant leaf development and production due to the deficiency of nutrients (Ding et.al, 2018), which may further pose the trees susceptible to the fungal infection. Alternatively, the possible reason of Fe was the finding proved that some of the fungi (i.e. *R. irregularis*) increased the Fe concentration in plants for the better nutrient production. It is suggested that this fungus has a positive effect to the Fe nutrition of trees. Also, many rhizobacteria and fungi released iron chelators which can contribute to enhance the Fe availability to plants (Tekaya et.al, 2017). Moreover, the possible reason of As was regarding to chemical similarity of P. Since As was a chemical analogue of P. The P transport system was activated and acted as a channel that allowing the water movement and neutralizing the solutes in the roots. At a high level of As, it can disrupt the oxidative phosphorylation and ATP production (Leung et.al, 2010). Thus, trees may not have enough energy for the plant defense and easily to suffer from fungal infection.

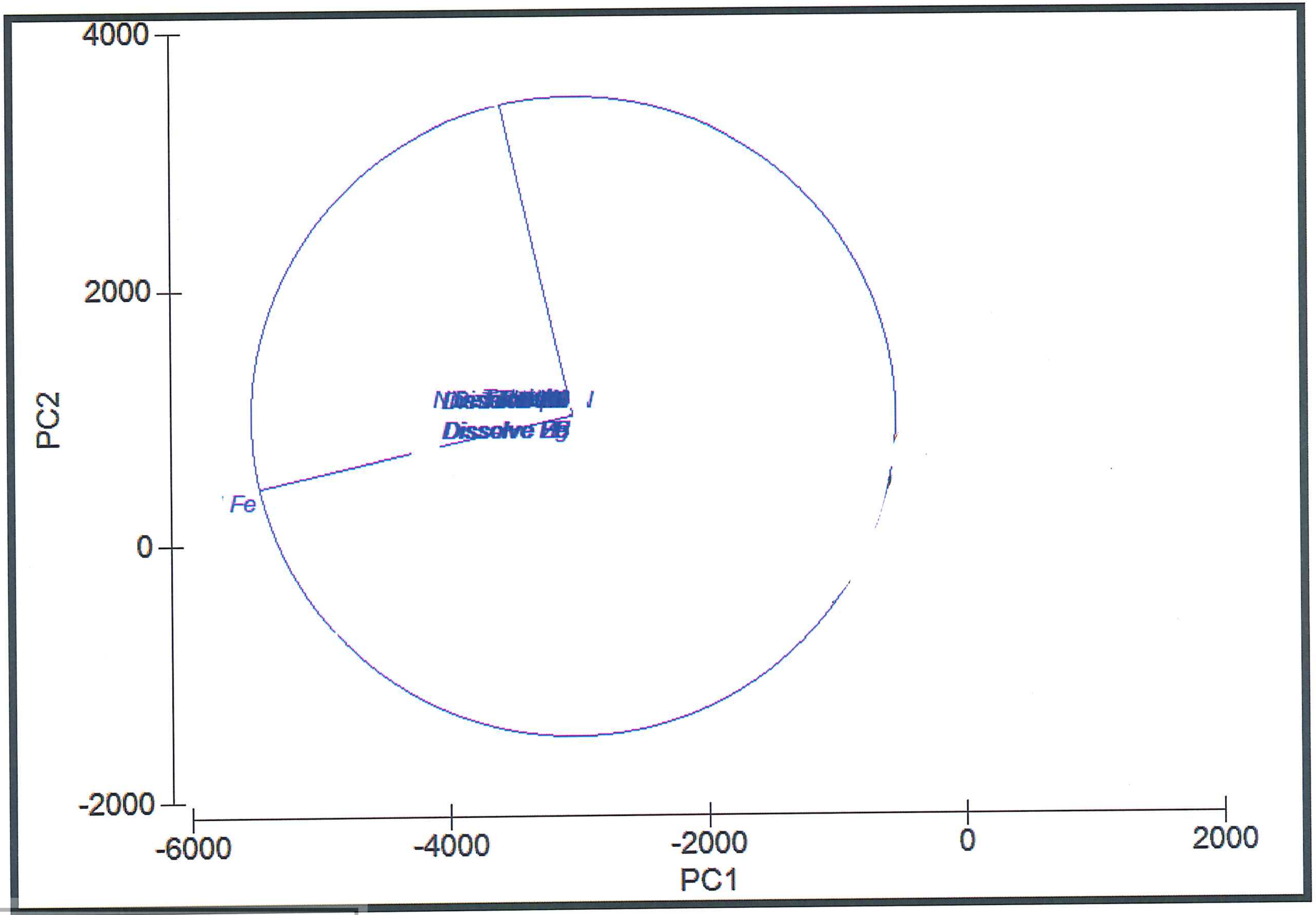

Fig. 12 PCA on the soil samples taken in 18 sites (n = 5)

Table 5 Rotated Component Matrix on the samples taken in 18 sites (n = 5)

Component	Eigenvalues	
	1	2
TOC	-0.001	0.000
pH	0.002	0.001
NO_3-N	-0.223	0.975
Redox	-0.010	0.004
EC	-0.005	0.003
C%	0.000	0.000
H%	0.000	0.000
N%	0.000	0.000
S%	0.000	0.000
Water extractable Pb	0.000	0.000
Water extractable Zn	0.000	0.000
Water extractable Cu	0.000	0.000
Water extractable Cd	0.000	0.000
Water extractable Fe	-0.006	-0.003
Water extractable K	-0.011	0.004
Water extractable As	0.000	0.000
Water extractable Hg	0.000	0.000
Total Hg	-0.002	0.001
Total Zn	-0.006	0.002
Total Cd	-0.001	0.000
Total Cu	0.000	0.000
Total Fe	-0.975	-0.223
Total K	-0.005	0.004
Total As	-0.004	0.008
Total Pb	0.000	0.000
Eigenvalue	2.94	1.96
Contribution	93.7	6.2

3.5 Study the relationship between individual fungal tree species and its soil parameters taken in 18 sites

As different tree species have different genetic variations, with an integration of external environment and soil properties such as chemical parameters and pollutants levels. The relationship between certain species with its corresponding tested parameters can be closely looked its beneficial or detrimental effects specifically. Therefore, it is suggested to examine the correlation analysis and PCA on the soil samples regarding to these 5 tree species that have the severe fungal infection (Table 6):

Table 6 General results of 5 tree species with severe fungal infection in this study

Tree species	Percentage and SD	PC1 factors	PC2 factors	PC3 factors
S.campanulata	10.98% ± 0.15	• EC • Total Fe	• EC • Total As	/
F.microcarpa	7.45% ± 0.20	• EC • Total Fe	• EC • Total As	/
A.confusa	6.67% ± 0.25	• EC • Total Fe	• EC • Total As	/
C.camphora	6.67% ± 0.17	• pH • Total Cu • Total Zn • Total Hg	• Water extractable Pb • Water extractable Cu	• Total S • Total K • Total Cd
L.speciosa	5.88% ± 0.10	• pH • TOC • Total Fe • Total Hg	• Total C • Total N	• Water extractable Pb • Water extractable Cd

3.5.1 The Correlation Analysis and PCA results of *S campanulata, F microcarpa* and *A confusa corresponding to the soil properties*

3.5.1.1 Correlation Analysis

S campanulata

The correlation analysis on *S campanulata* is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in the sites that containing *S. campanulata*. According to the table 7, the infection rate is negatively correlated with the EC value at the 0.05 level, meaning that when one variable increases, the other decreases. The results were in line to the previous section. Therefore, the abundance of mobile ion in soil exhibited the alternative if plant growth.

Table 7 Correlation analysis on the soil samples

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.342*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

F microcarpa

The correlation analysis on *F microcarpa* is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in the sites that containing *F. microcarpa.* With reference to table 8, the infection rate is positively correlated with water extractable Hg (Hg_W) at 0.01 level, meaning that both variables tend to increase together. For the Hg toxicity to plant, it will be interfering with the electron transport chain, then declining the mitochondrial and chloroplast activity (Nagajyoti et.al, 2010). The photosynthesis was breakdown by the substitution reaction of the central atom of chlorophyll (i.e. Magnesium (Mg)) when the presence of Hg, and the prevention of photosynthetic light harvesting in the abnormal chlorophyll molecules (Patra & Sharma, 2000). Thus, the plant may reduce the ATP production and become weaken in their defense system, hence the fungi start to be more easily develop on roots and trucks.

Table 8 Correlation analysis on the soil samples

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.422**	n.s.	n.s.	n.s.	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	-0.24*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

A confusa

The correlation analysis on *A confusa* is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in the sites that containing *A. confusa.* According to the table 9, the infection rate is positively correlated with the H, redox potential, water extractable Cd and total Pb at 0.01 level and 0.05 level respectively, meaning that these variables tend to increase together. It was proved that as the heavy metal concentration in soil raised, it may further weaken the plant and the infection rate increased, so fungi were readily to colonize on the tree. Moreover, the infection rate is also negatively correlated with C and TOC at 0.05 level, meaning that these variable increases may lead infection rate decreases due to the host may less dependent on fungi for carbon sequestration. The result speculated that increase the input carbon source into soil may be inhibited the fungal growth on the tree. However, further studies should be conducted to validate the hypothesis.

Table 9 Correlation analysis on the soil samples

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	0.477*	n.s.	n.s.	n.s.	0.422**	0.471*	n.s.	n.s.	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.467*	n.s.	n.s.	n.s.	n.s.	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

3.5.1.2 Principal Component Analysis

S. campanulata

The contribution of each principal component and its eigenvalue after rotation are illustrated in Table 10. As Eigenvalue > 1 in these two principal components, the result of the principal component analysis is considered significant. Two principal components (Eigenvalue > 1) control 99.9% of variations in the fungal infection rates of *S. campanulata*. The first component is the most significant principal, which contribute to 95.3% of the variation, and is highly influenced by total Fe and EC. The second principal component is responsible for 4.6% of the variance respectively, and is moderately influenced by EC and total As.

The results are partially in line to the previous section as EC determined the number of mobile ions including essential and non-essential minerals or nutrients available for plant growth (Development Bureau, 2022).

Moreover, as aforementioned, fungi can increase Fe availability to the plant by releasing the iron chelators (Tekaya et.al, 2017).

Besides, as aforementioned, except hyper-accumulator, As is highly toxic to all plants. Thus, the current finding shown that the fungal infection in the tree can be reflected the toxicity of As to plant, as aforementioned, it is the analogue of P that interfering the absorption of P in the soil (Nagajyoti et.al, 2010).

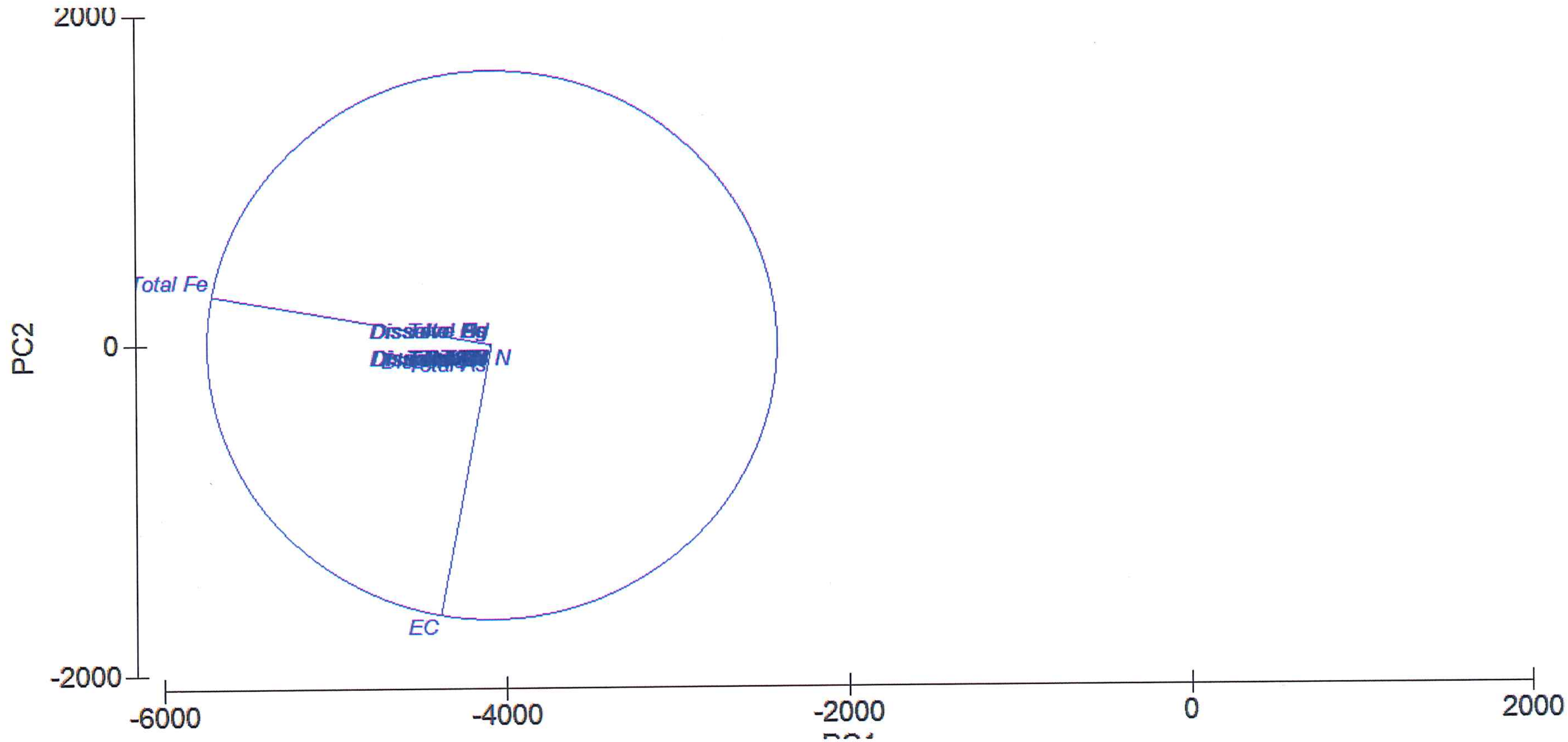

Fig. 13 PCA on the soil samples regarding to *S campanulata*

Table 10 Rotated Component Matrix of *S campanulata*

Component	Eigenvalues	
	1	2
TOC	-0.001	-0.001
pH	-0.002	-0.002
EC	-0.182	-0.983
Redox	-0.008	-0.003
NO_3-N	-0.004	-0.003
C%	0.000	0.000
H%	0.000	0.000
N%	0.000	0.000
S%	0.000	0.000
Water extractable Pb	0.000	0.000
Water extractable Zn	0.000	0.000
Water extractable Cu	0.000	0.000
Water extractable Cd	0.000	0.000
Water extractable Fe	-0.004	0.001
Water extractable K	-0.009	-0.010
Water extractable As	0.000	0.000
Water extractable Hg	0.000	0.000
Total Pb	-0.001	-0.005
Total Zn	-0.006	-0.007
Total Cu	-0.001	-0.001
Total Cd	0.000	0.000
Total Fe	-0.983	0.182
Total K	-0.004	-0.003
Total As	-0.004	-0.024
Total Hg	0.000	0.000
Eigenvalue	3.59	1.72
Contribution	95.3	4.6

F. microcarpa

The contribution of each principal component and its eigenvalue after rotation are illustrated in Table 11. As Eigenvalue > 1 in these two principal components, the result of the principal component analysis is considered significant. Two principal components (Eigenvalue > 1) control 99.7% of variations in the fungal infection rates of *F. microcarpa*. The first component is the most significant principal, which contribute to 73.5% of the variation, and is highly influenced by total Fe and EC. The second principal component is responsible for 26.2% of the variance respectively, and is moderately influenced by EC and total As.

The results are partially in line to the previous section as EC determined the amount of mobile ions including essential and non-essential minerals or nutrients available for plant growth (Development Bureau, 2022).

Moreover, as aforementioned, fungi can increase Fe availability to the plant by releasing the iron chelators (Tekaya et.al, 2017).

Besides, as aforementioned, except hyper-accumulator, As is highly toxic to all plants. Thus, the current finding shown that the fungal infection in the tree can be reflected the toxicity of As to plant, as aforementioned, it is the analogue of P that interfering the absorption of P in the soil (Nagajyoti et.al, 2010).

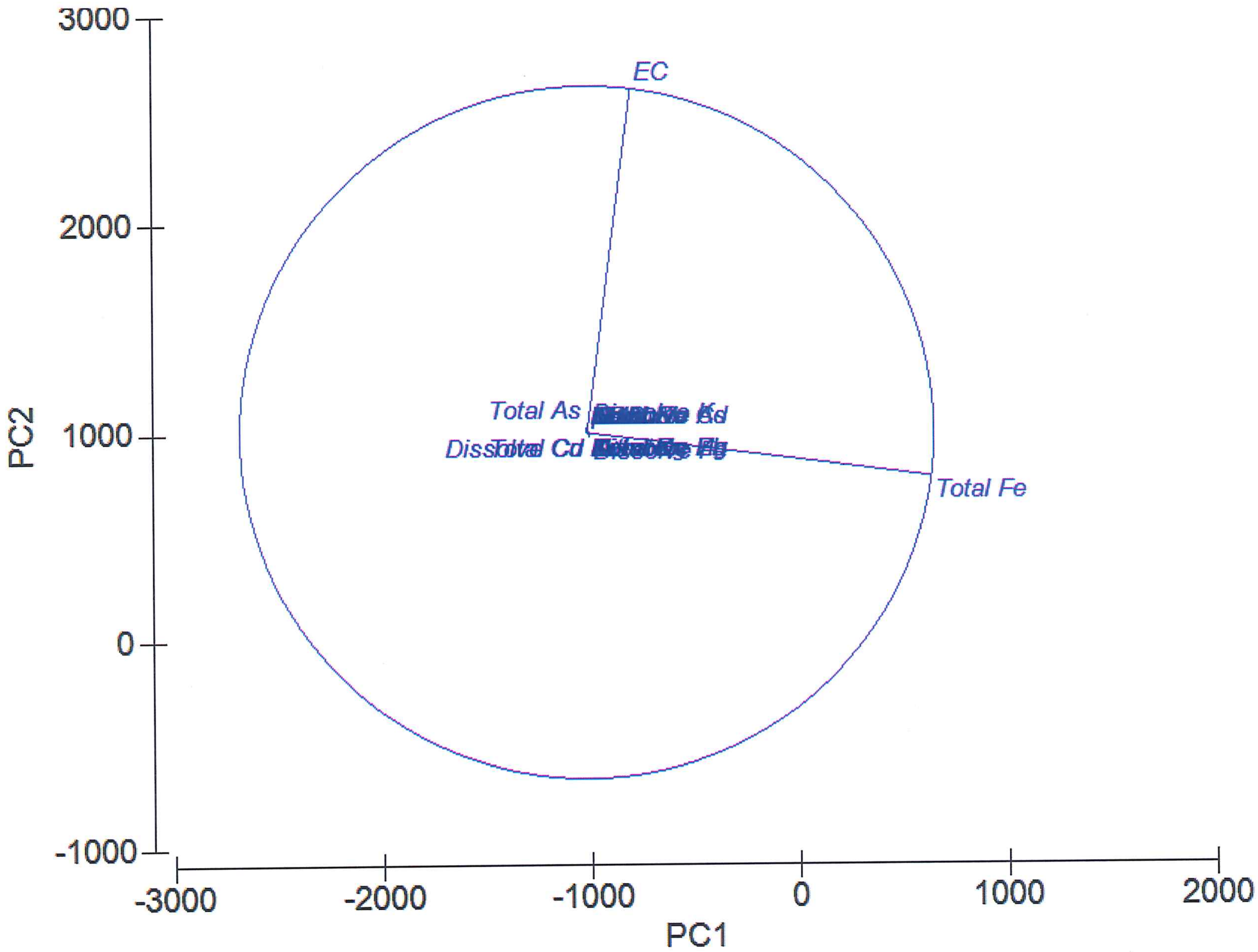

Fig. 14 PCA on the soil samples regarding to *F microcarpa*

Table 11 Rotated Component Matrix of *F microcarpa*

Component	Eigenvalues	
	1	2
TOC	0.001	0.000
pH	0.001	0.001
EC	0.129	0.991
Redox	0.005	0.001
NO_3-N	0.001	0.000
C%	0.000	0.000
H%	0.000	0.000
N%	0.000	0.000
S%	0.000	0.000
Water extractable Pb	0.000	0.000
Water extractable Zn	0.000	0.000
Water extractable Cu	0.000	0.000
Water extractable Cd	0.000	0.000
Water extractable Fe	0.006	-0.009
Water extractable K	0.003	0.007
Water extractable As	0.000	0.000
Water extractable Hg	0.000	0.000
Total Pb	0.002	0.000
Total Zn	0.008	-0.002
Total Cu	0.001	0.000
Total Cd	0.000	0.000
Total Fe	0.992	-0.129
Total K	0.007	0.001
Total As	-0.003	0.016
Total Hg	0.000	0.000
Eigenvalue	1.24	4.43
Contribution	73.5	26.2

A. confusa

The contribution of each principal component and its eigenvalue after rotation are illustrated in Table 12. As Eigenvalue > 1 in these two principal components, the result of the principal component analysis is considered significant. Two principal components (Eigenvalue > 1) control 99.9% of variations in the fungal infection rates of *A. confusa*. The first component is the most significant principal, which contribute to 92.4% of the variation, and is highly influenced by total Fe and EC. The second principal component is responsible for 7.5% of the variance respectively, and is moderately influenced by EC and total As.

The results are partially in line to the previous section as EC determined the amount of mobile ions including essential and non-essential minerals or nutrients available for plant growth (Development Bureau, 2022).

Moreover, as aforementioned, fungi can increase Fe availability to the plant by releasing the iron chelators (Tekaya et.al, 2017).

Besides, as aforementioned, except hyper-accumulator, As is highly toxic to all plants. Thus, the current finding shown that the fungal infection in the tree can be reflected the toxicity of As to plant, as aforementioned, it is the analogue of P that interfering the absorption of P in the soil (Nagajyoti et.al, 2010).

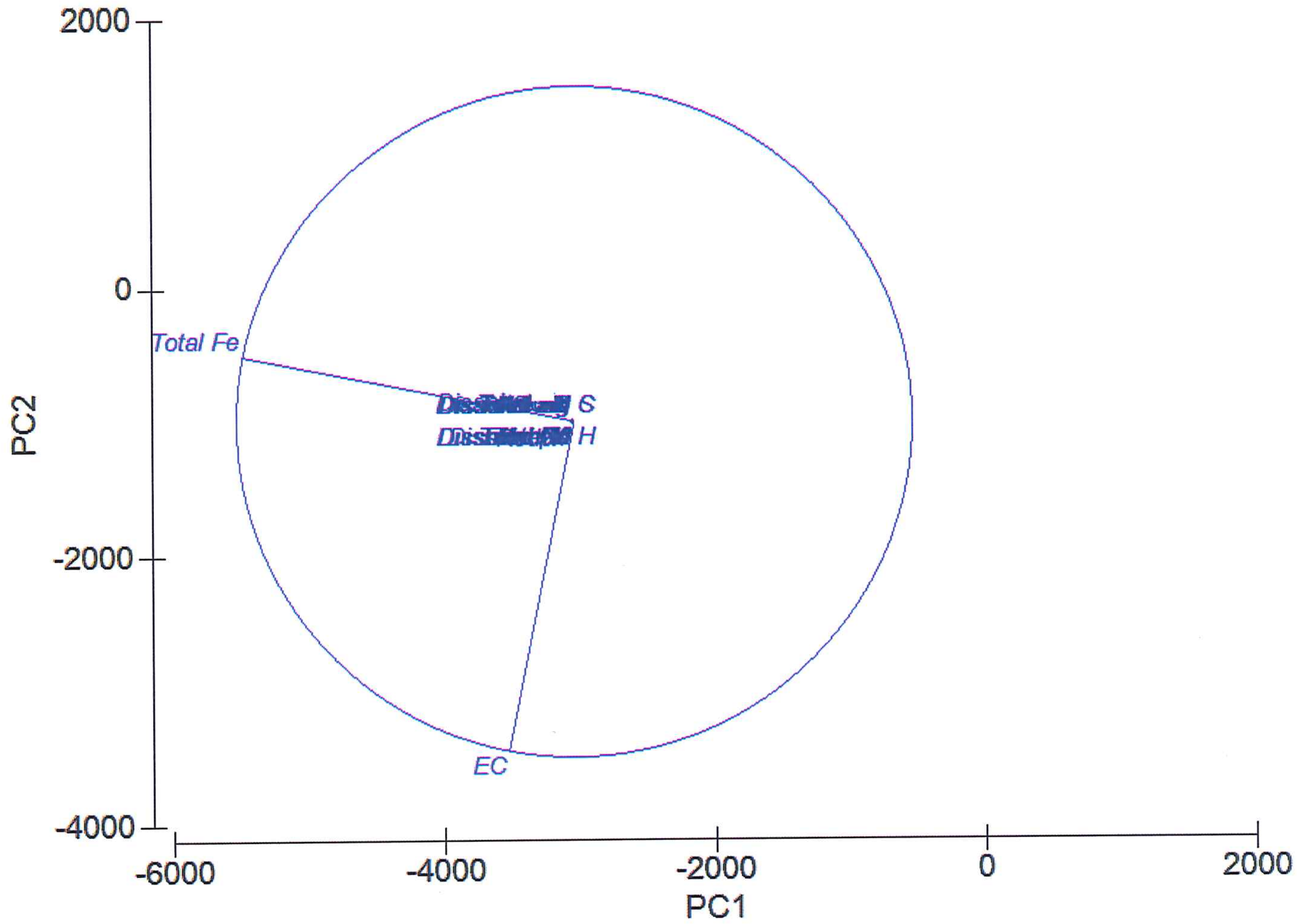

Fig. 15 PCA on the soil samples regarding to *A confusa*

Table 12 Rotated Component Matrix of *A confusa*

Component	Eigenvalues	
	1	2
TOC	-0.001	0.000
pH	-0.001	-0.001
EC	-0.196	-0.981
Redox	-0.009	-0.007
NO_3-N	-0.004	0.003
C%	0.000	0.000
H%	0.000	0.000
N%	0.000	0.000
S%	0.000	0.000
Water extractable Pb	0.000	0.000
Water extractable Zn	0.000	0.000
Water extractable Cu	0.000	0.000
Water extractable Cd	0.000	0.000
Water extractable Fe	-0.007	0.008
Water extractable K	-0.011	-0.002
Water extractable As	0.000	-0.001
Water extractable Hg	0.000	0.000
Total Pb	-0.001	0.000
Total Zn	-0.003	-0.001
Total Cu	-0.001	0.000
Total Cd	0.000	0.000
Total Fe	-0.980	0.196
Total K	-0.002	-0.001
Total As	-0.006	0.006
Total Hg	0.000	0.000
Eigenvalue	4.3	3.5
Contribution	92.4	7.5

3.5.2 The Correlation Analysis and PCA results of *C camphora* and *L speciosa corresponding to the soil properties*

3.5.2.1 Correlation analysis

Correlation Analysis of *C. camphora*

The correlation analysis on *C camphora* is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in the sites that containing *C. camphora.* According to the table 13, the infection rate is positively correlated with the H, S, pH value, total Zn and K at 0.05 level and 0.01 level respectively, meaning that these variables tend to increase together with infection rate. Besides, the infection rate is negatively correlated with C at 0.05 level, meaning that when C increases, the infection rate may tend to decrease.

Yang et.al (2008) discovered that the camphor tree can be resistant to the heavy metal pollution due to its perennial habit. Zn was the trace element for the growth of plant when it is low in soil, whereas *C. camphora*'s transpiration rate decreased when the excessive Zn treated with the plant. For the photosynthetic rate of the plant, it was significantly declined under the stress of Zn in 1500 mg/kg.

Tong & Zhang (2014) discovered that the acid rain induced the inhibitory effects on the growth of *C. camphora* at pH 2.5. Also, plant growth, chlorophyll content and the photosynthetic rate of the seedings of this species were significantly lower than the control setup at pH 2.5. Since the acid rain lead the pH value decrease in the soil and let the pH range lower than the optimum range of pH value (5.5 – 8.0). The high acidity may inhibit the photosynthesis rate of the plant.

Thus, this condition may cause the injury of leaves, the change in enzyme activities, the alternations of water balance, and ultrastructure of mitochondria and chloroplasts.

Moreover, acid rain composed a series of ions such as H^+, SO_4^{2-} and K^+ which were essentially to the plant for providing mineral elements. Acid rain increased the exchange between the H^+, and the cations of nutrient such as K^+ (Deng et.al, 2012). Therefore, pH value, H, S and K may have similar effects to the fungal infection.

Table 13 Correlation analysis on the soil samples

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	0.477*	n.s.	n.s.	n.s.	0.422**	n.s.	0.393*	n.s.	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	0.545**	n.s.	n.s.	n.s.	0.409*	n.s.	n.s.	-0.323*	0.33*	n.s.	0.625**	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

Correlation Analysis of *L. speciosa*

The correlation analysis on *Lagerstroemia speciosa* is performed on fungal infection rate, chemical parameters and heavy metals concentration of soil in the sites that containing *L. speciosa.* According to the table 14, the infection rate is positively correlated with the total Zn and Cu at 0.01 level respectively, meaning that these variables tend to increase together with infection rate.

For the correlation of Zn, it is partially in line to the previous explanation as Zn stress may reduce the rate of transpiration and photosynthesis in *L. speciosa* that similar to the effects of *C. camphora* (Yang et.al, 2008). However, this tree species was not perennial habit that relatively low resistance to the heavy metal toxicity and pollution, then this tree species tends to have higher possibility of the infection by fungi due to the weakening of tree nutrient and energy supports. Nagajyoti et,al (2010) claimed that the toxicity of Zn can induce the accumulation of other heavy metals, such as Cu and Mn, in both roots and shoots which worsen the adverse situation of the tree. The P deficiency also appeared as abnormal color (i.e. purplish red) on the leaf symptom which generate the barrier of the light-dependent reaction of the photosynthesis.

Alternatively, although Cu was the essential nutrient for the plant growth with the suitable amount, the excess Cu lead the toxic effects that reduced the microbial population and altered the rate of key biological processes in the relative ecosystem function. In this condition, along with the high acidity of soil known as toxic to the plant growth because of chlorosis, oxidative stress and root-tip browning, thus the growth of *L. speciosa* become severely retard (Hari Babu & Sudha, 2011). Therefore, the deficiency of nutrients and ATP production may be resulted under the Cu stress, hence the trees increased their possibility to have the fungal infection.

Table 14 Correlation analysis on the soil samples

		Infection	Pb_W	Zn_W	Cu_W	Cd_W	Fe_W	K_W	As_W	Hg_W	Pb	Zn	Cu	Cd
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.507**	0.451**	n.s.
		Fe	K	As	Hg	TOC	pH	EC	Redox	C	H	N	S	Nitrate_N
Infection	Pearson Correlation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Results with asterisk showed significant level at 0.05* and 0.01** respectively; ns, no significant correlation

3.5.2.2 Principal Component Analysis

PCA results of *C. camphora*

The contribution of each principal component and its eigenvalue after rotation are illustrated in Table 15. As Eigenvalue > 1 in these three principal components, the result of the principal component analysis is considered significant. Three principal components (Eigenvalue > 1) control 59.0% of variations in the fungal infection rates of *C. camphora*. The first component is the most significant principal, which contribute to 40.6% of the variation, and is highly influenced by pH, total Cu and Zn. Principal components 2 is responsible for 10.3% of the variance, and is moderately influenced by water extractable Pb and Cu. Principal components 3 is responsible for 8.1% of the variance, and is moderately influenced by total S, total K and Cd.

For the Zn and Cu stress, it is suggested the similar explanation in the correlation part with reference to Yang et.al (2008), Nagajyoti et,al (2010) and Hari Babu & Sudha (2011).

Besides, the drop of net photosynthetic rate in *C. camphora* leaves was presented from day 28 onward that involved the weakening activity of related enzymes under the stress of Cd and Cu. It is determined that the leaves' stomatal activity and the absorption of carbon dioxide became weak and reduced respectively that may contributed by Cd stress effects (Zhou et.al, 2019)

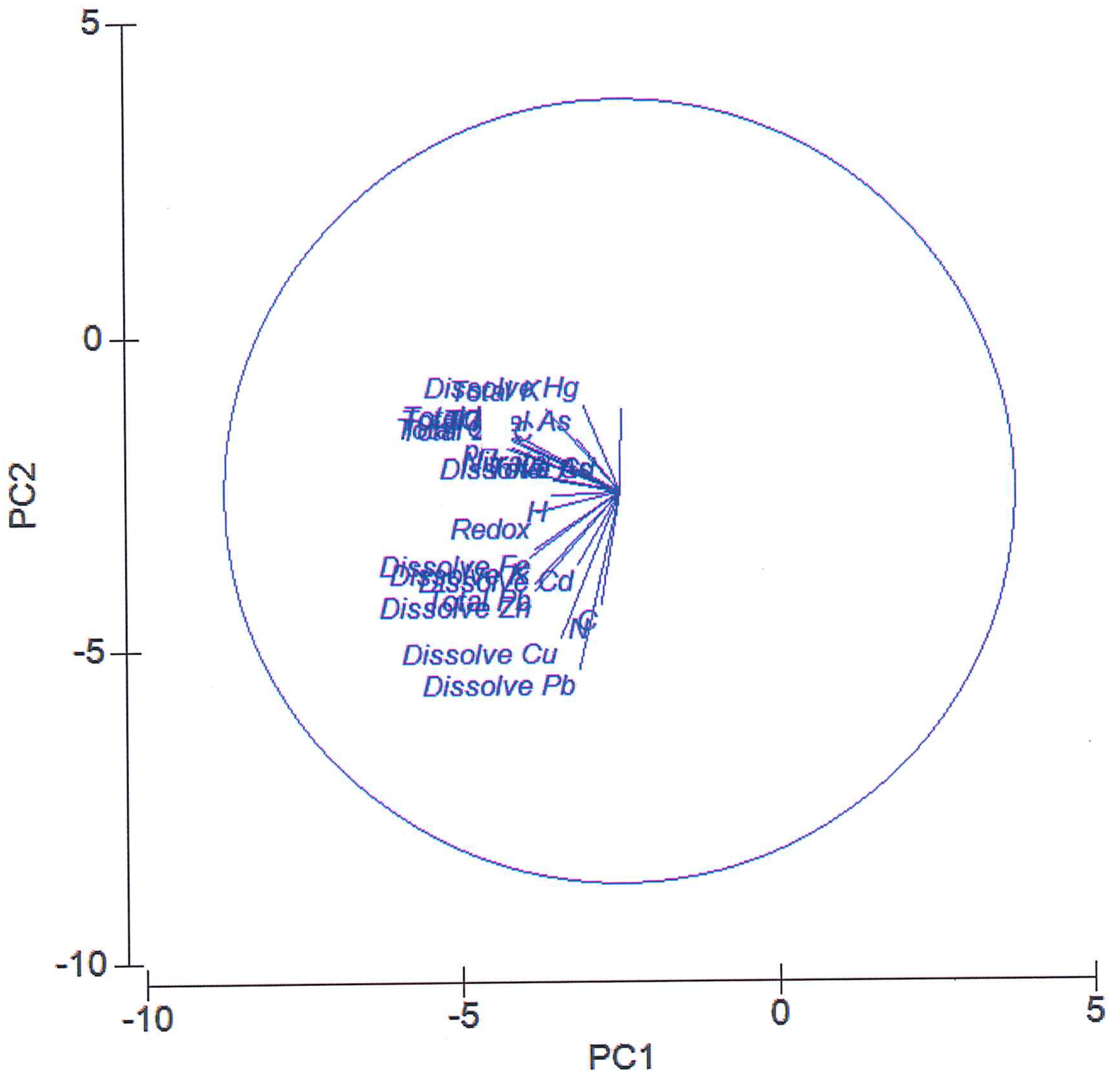

Fig. 16 PCA on the soil samples regarding to *C camphora*

Table 15 Rotated Component Matrix of *C camphora*

Component	Eigenvalues		
	1	2	3
TOC	-0.276	0.140	-0.102
pH	-0.293	0.063	0.053
EC	-0.210	0.108	-0.001
Redox	-0.211	-0.053	-0.126
NO_3-N	-0.170	0.031	0.254
C%	-0.048	-0.282	-0.235
H%	-0.172	-0.010	-0.014
N%	-0.070	-0.304	-0.148
S%	0.004	0.219	0.457
Water extractable Pb	-0.105	-0.452	0.061
Water extractable Zn	-0.215	-0.250	0.021
Water extractable Cu	-0.151	-0.368	0.040
Water extractable Cd	-0.106	-0.184	0.015
Water extractable Fe	-0.214	-0.145	0.076
Water extractable K	-0.227	-0.166	0.146
Water extractable As	-0.061	0.016	-0.122
Water extractable Hg	-0.093	0.222	-0.409
Total Pb	-0.216	-0.233	0.136
Total Zn	-0.283	0.112	0.127
Total Cu	-0.284	0.118	-0.069
Total Cd	-0.051	0.015	0.328
Total Fe	-0.281	0.138	-0.101
Total K	-0.188	0.214	0.353
Total As	-0.109	0.137	-0.298
Total Hg	-0.266	0.149	-0.152
Eigenvalue	10.6	2.69	2.1
Contribution	40.6	10.3	8.1

PCA results of *L. speciosa*

The contribution of each principal component and its eigenvalue after rotation are illustrated in Table 16. As Eigenvalue > 1 in these three principal components, the result of the principal component analysis is considered significant. Three principal components (Eigenvalue > 1) control 59.2% of variations in the fungal infection rates of *L. speciosa*. The first component is the most significant principal, which contribute to 37.5% of the variation, and is highly influenced by pH, TOC. Principal components 2 is responsible for 11.5% of the variance, and is moderately influenced by total C and N. Principal components 3 is responsible for 10.2% of the variance, and is moderately influenced by water extractable Pb, Cd and S.

From the research study, the increase of Pb, Cd and S content and decrease in pH value in the roadside *L. speciosa* as the pollutants produced by the driven of motorcycles. The larger level of Pb accumulated in its leaves and that of Cd accumulated in its barks (Li et.al, 2010). This may lead the tree become debilitate posed by metals' toxicity and adverse effects to their photosynthetic mechanisms. Therefore, the fungal infection become more easily in trees.

On the other hand, as mentioned in the previous points, the TOC is related to the soil organic matter (SOM) that forming the chelate structures with some metals, especially transition metals, it may increase the stress of trees (Development Bureau, 2022). Then the metal toxicity to *L. speciosa* may be appeared when the concentration of heavy metals become excess and stress. It was tending to alter the effectiveness of electron transport chain, transpiration rate and photosynthetic rate, and hence leading the trees susceptible to the fungal infection.

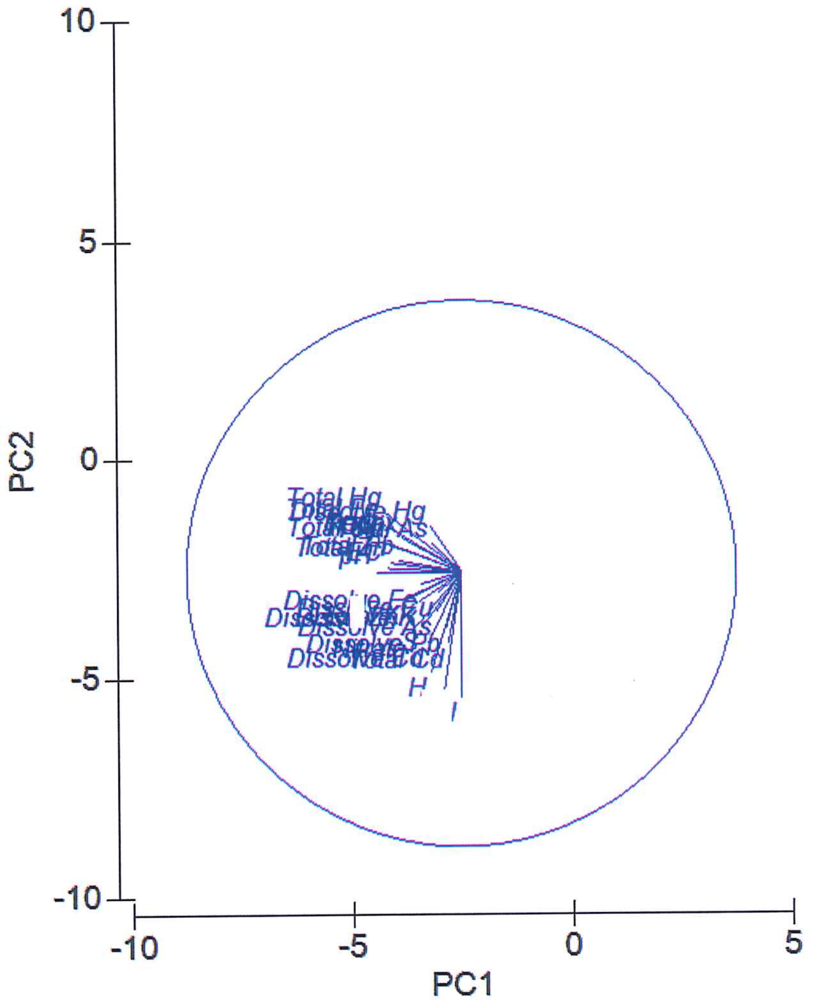

Fig. 17 PCA on the soil samples regarding to *L speciosa*

Table 16 Rotated Component Matrix of *L speciosa*

Component	Eigenvalues		
	1	2	3
TOC	-0.293	0.110	0.019
pH	-0.308	0.000	-0.014
EC	-0.270	0.013	0.028
Redox	-0.215	0.123	0.152
NO_3-N	-0.186	0.218	-0.289
H	-0.065	-0.427	0.215
C	-0.111	-0.361	0.374
N%	-0.002	-0.453	0.012
S%	-0.142	-0.195	0.366
Water extractable Pb	-0.059	-0.205	-0.418
Water extractable Zn	-0.211	-0.105	-0.260
Water extractable Cu	-0.086	-0.071	0.042
Water extractable Cd	-0.121	-0.255	-0.395
Water extractable Fe	-0.144	-0.041	-0.224
Water extractable K	-0.152	-0.103	-0.125
Water extractable As	-0.091	-0.139	0.118
Water extractable Hg	-0.112	0.165	0.115
Total Pb	-0.228	0.038	0.026
Total Zn	-0.259	0.031	0.019
Total Cu	-0.271	0.105	0.022
Total Cd	-0.051	-0.258	-0.081
Total Fe	-0.285	0.175	-0.028
Total K	-0.215	-0.099	0.219
Total As	-0.108	0.102	-0.011
Total Hg	-0.271	0.212	-0.024
Eigenvalue	9.76	2.98	2.65
Contribution	37.5	11.5	10.2

3.6 General discussions

3.6.1 Feasibility of using heavy metals to alter the growth of fungi in the tree

For the positive effects of fungi, some heavy metals may have positive correlation with fungi development. For instance, Mn and Cu required in the catalytic cycle and played as a cofactor role for the ligninolytic enzymes of white rot fungi, *Heterobasidion* species (Yakovlev et.al, 2013). It was suspected some fungal species shown its excellent tolerance to the presence of metal ions in MEA medium. When the Cu concentration increased, their biomass production was keeping the raising trend and did not inhibit the fungal development when compared with other white rot fungi and blue stain fungus (Guillen & Machuca, 2008).

Nevertheless, the essential and non-essential heavy metals were toxic to fungi development under the excess amount of metals, whereas they contain the high tolerance threshold to some essential metals (Baldrian, 2003). Cu can completely inhibit the growth of the majority of the white rot fungi when the concentration of Cu increased to 10 mM, except for *W. cocos* and *L. sulfureus* (Guillen & Machuca, 2008), whereas further verification of these phenomenon is needed in field condition.

3.6.2 Possible mechanism for heavy metals in soil to affect tree growth and its health status

From the results and explanation of this study, it is determined that heavy metals stresses and toxicity are the major sources of poor tree growth and health. Talebzadeh and Valeo (2022) also claimed that heavy metals in leaves can potentially damage the structure of chlorophyll in the chloroplast that interfering the photosynthesis and the process of natural growth. If under the high concentration of heavy metals exposure, the further adverse effects to the functional properties of leaves will be resulted by declining the leaf areas, the width of stomatal pore, stomatal density, and thickness of the cuticle.

3.6.3 Possible management strategies to prevent further deterioration of tree health when infected by fungi

According to the Dutch guideline's reference value, if some of the heavy metals were exceeded the limits, the immediate remediation should be done. In this study, As, Hg and Cd were exceeded the limit in the Dutch guideline (ESDAT, 2000), and tends to increase the risk of fungal disease found in the tree. Thus, it is suggested that the municipal and industrial wastes should be controlled and restricted by the government. For example, the municipal green waste should be composted to benefit the soil quality in vegetable production. Alternatively, the industrial waste should be checked with framework for ensuring the waste contain no content of heavy metals that minimize the contamination of soil and hence prevent the further adverse effects to the tree health (Bagshaw et.al, 2010). Finally, photosynthesis mechanism of tree can resume under the normal situation for getting the sufficient nutrients for their growth and survive.

4.Conclusion

From the results of this study, it is obvious that most of the findings implied that heavy metals were the major source of the weakening of tree's normal mechanism. There was no direct relationship between the heavy metals' concentration and fungi growth. This study illustrated that the possible routes of the fungal infection due to the negative effects to the urban trees by the some of the heavy metals stress, such as As (<0.01 – 48.92 mg/kg), Cu (<0.01 – 10.30 mg/kg), Cd (<0.01 – 1.59 mg/kg), Hg (0.59 – 1.91 mg/kg) , Zn (12.90 – 45.75 mg/kg), K (0.57 – 54.35 mg/kg) and Pb (2.65 – 12.38 mg/kg). They were also the major dominant chemical parameters correlated with fungi. According to PCA results, pH, EC, TOC and S were also the parameters that correlated with fungi. It is recommended that conducting this analysis with a temporal pattern, such as spring, summer, autumn and winter, as the soil properties and the tree status varied in different seasons and climate conditions. Besides, the study should further in depth to examine the physical and chemical parameters for checking the correlation of fungal infection and heavy metals concentration in bulk and rhizosphere soils. Also, the relationship between pest and fungal infections to the tree is also one of the sources of affecting the tree health.

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