# Accumulative Study of Heavy Metals in Soils, Water and Uptake by Rhizome of two *Cissus* Species from Various Sites

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# Introduction

- Traditional medicinal plants are used for the treatment of various ailments and primary health care

- WHO has estimated in 1998 that about 75–80% of the world's population use plant - based medicines

- The ecological differences have direct or indirect contact to the medicinal plants and they may be regarded as an index of trace metal concentration in the surroundings

- WHO recommends to check for the presence of heavy metals in the raw materials of the medicinal plants in preparing the finished products

**In Myanmar,**

- **Leaves, stems and rhizomes of *Cissus* species can be used not only as oral drug but also as paste form in abscess, benign and malignant tumors, Gastric cancer for a long time**

- **but these traditional herbs have not been scientifically proved for the absence of heavy metals**

# Aim

- To prove the absence of heavy metals (Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Ni, K, Na and Zn) in rhizome of two *Cissus* species and also in the soils and water from various sites

# Specific objectives

(1) To determine the content of heavy metals in rhizome of *Cissus repens* Lam. and *Cissus discolor* Blume. from Myitkyeena, Moegoke, Aungban and Pyin Oo Lwin

(2) To find out physico-chemical parameters and the content of heavy metals in water and their soils these were grown

(3) To analysze phytochemical constituents of these rhizomes

# Materials

## Chemicals & Reagents

- Analytical grade reagents (Merck),

  Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Ni, K, Na, Zn

- 70% $HNO_3$ & 69% HCl, Double de-ionized water (DDW)

# Instruments

- Atomic Absorption Spectrophotometer (AA 6650, Shimadzu)

- Muffle furnace (LEF 1035), Oven, Analytical balance

# Methods

## Plant authenticity

- Identified & Confirmed by competent taxonomist, Department of Botany, Mandalay University

# Sample Collection

| Sr. No | Medicinal Plants | Place | Sample |
|---|---|---|---|
| 1. | *Cissus repens* Lam. | Site I & Site II<br>Site III & Site IV | Rhizome, their soil, their water samples |
| 2. | *Cissus discolor* Blume. | Site III & Site IV | Rhizome, their soil, their water samples |

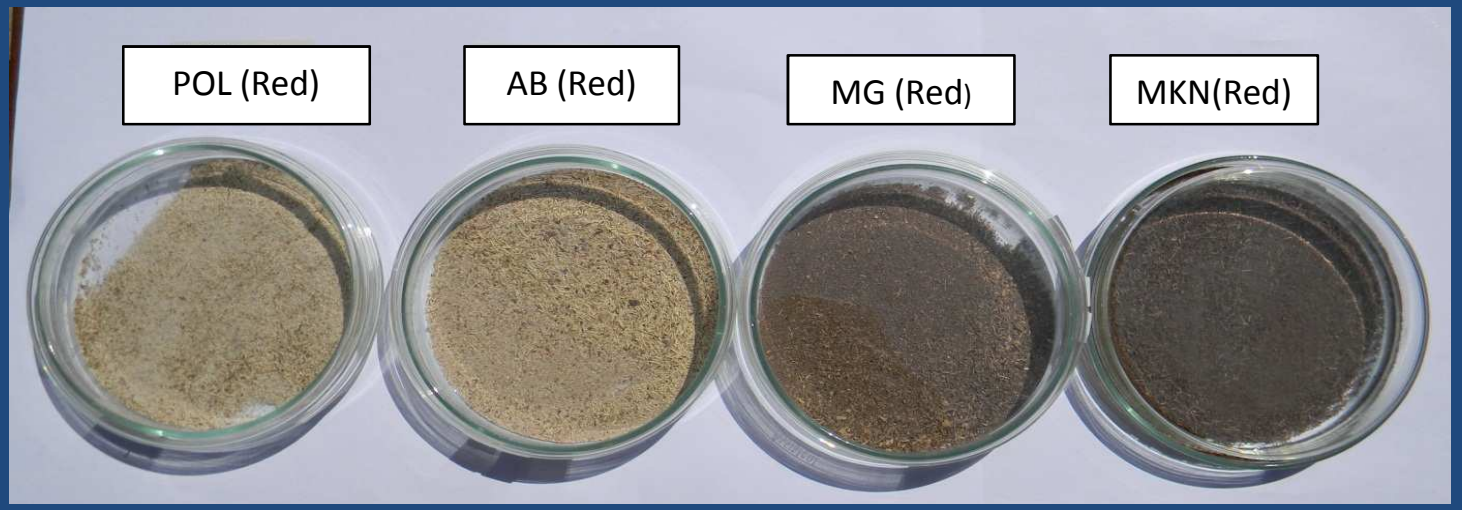
Myitkyeena = site I

Moegoke = site II

Aungban = site III

Pyin Oo Lwin = site IV during December to February

## Powder form of *Cissus repens* Lam. (တပင်တိုင်မြနန်းအနီ)



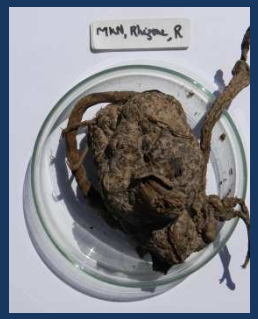

## Powder form of *Cissus discolor* Blume. (တပင်တိုင်မြနန်းအဖြူ)


**POL= Pyin Oo Lwin**
**AB= Aungban**
**MG=Moegoke**
**MKN=Myitkyeena**

# Sample Collection of Soils

- About 8-10 cm depth of the soil were placed in the polyethylene sampling bags
- Soil samples were dried in an oven at 110 ˚C for 2hrs until brittle and crisp

# Sample preparation



Rhizome sample

Wash thoroughly with tap water & doubled de-ionized water

dried in shade at room temperature

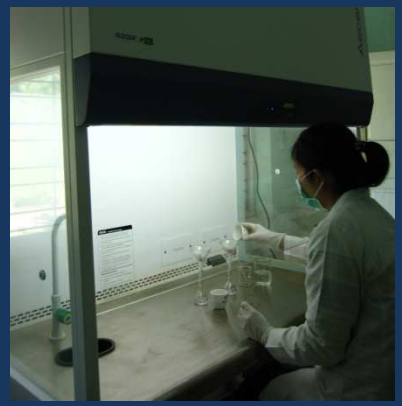
powdered & homogenized with motor & pestle

# Digestion of rhizome sample

Powder ( 2.5 ) g

Heating 110 °C, 2hrs, in an oven to remove moisture

Dried sample in furnace, at 550℃ for 4 hrs, to obtain grey ash, & cool



5 ml of 6M HNO3, to dissolve digest & filter

Made up with double de-ionized water ( 50cm$^3$ Volumetric flask )

# Digestion of soil samples

**Dried soil (1) g**

↓

**Digest with 12ml $HNO_3$-HCl (1:3 v/v)**

**Dryness in an oven at 110℃ for 3 hr, cooled**

↓

**20 ml 2% $HNO_3$ boil 10 mins, cooled & filtered**

**Made up with double de-ionized water ( 100cm³ Volumetric flask )**

# Digestion of water samples

50 ml of water sample

2.5 ml of conc: $\mathbf{HNO_3}$
covered with watch glass

heated for 10 minutes
and cooled

filtered

50 ml volumetric flask,
diluted with de-ionized
water

# Statistical Analysis

- Microsoft Excel v. 2007

  Results were presented as mean ± SD

# Results & Discussion

## Plant Authenticity

**Fig (1)** *Cissus repens* Lam.

(တပင်တိုင်မြနန်းအနီ)

**Fig 2.** *Cissus discolor* Blume.

(တပင်တိုင်မြနန်းအဖြူ)

## Table1. Level of heavy/toxic metals (ppm) in rhizome samples from different sites

| Metal | Site (I) *Cissus repens* Lam. | Site (II) *Cissus repens* Lam. | Site (III) | | Site (IV) | | Reference value |
|---|---|---|---|---|---|---|---|
| | | | *Cissus repens* Lam. | *Cissus discolor* Blume. | *Cissus repens* Lam. | *Cissus discolor* Blume. | |
| Cd | ND | ND | ND | ND | ND | ND | 0.3* |
| Ca | 1604.11 ±31.87 | 1523.15 ±18.05 | 1554.53 ±6.98 | 944.08 ±23.05 | 2978.93 ±167.98 | 1843.13 ±42.98 | 44-614** |
| Cr | ND | ND | ND | ND | ND | ND | 2* |
| Cu | 0.38 ±0.12 | ND | 5.01 ±1.08 | 5.25 ±0.27 | 5.27 ±0.61 | 5.18 ±1.05 | 20* |
| Fe | 284.96 ±7.63 | 112.27 ±4.37 | 150.51 ±25.05 | 101.53 ±3.95 | 102.91 ±3.82 | 65.89 ±11.29 | 20* |
| Pb | 2.28 ±0.21 | 1.86 ±0.36 | 0.37 ±0.57 | 1.15 ±0.64 | 2.91 ±0.73 | 3.94 ±1.3 | 10* |

*WHO, 2005 [10] ** Ajasa, 2004 [11]

## Table2. Level of heavy/toxic metals (ppm) in rhizome samples from different sites

| Metals | Site (I) *Cissus repens* Lam. | Site (II) *Cissus repens* Lam. | Site (III) | | Site (IV) | | Reference value |
|---|---|---|---|---|---|---|---|
| | | | *Cissus repens* Lam. | *Cissus discolor* Blume. | *Cissus repens* Lam. | *Cissus discolor* Blume. | |
| Mg | 50.43±0.37 | 50.03±0.47 | 49.75±0.3 | 49.31±0.55 | 5.29±2.43 | 58.42±1.89 | 2000** |
| Mn | 364.73±7.14 | 228.35±4.76 | 143.94±2.83 | 82.08±1.35 | 23.37±1.36 | 22.83±1.24 | 200* |
| Ni | 1.51±0.07 | 2.16±0.08 | 0.85±0.03 | 0.60±0.06 | 0.43±0.26 | 0.80±0 .06 | 1.5* |
| K | 721.98±5.33 | 707.95±18.61 | 720.43±26.99 | 437.02±15.62 | 582.22±12.27 | 1178.7±19.25 | 6380-36600** |
| Na | 52.03±0.97 | 71.51±2.26 | 81.61±0.74 | 74.4±1.28 | 181.52±6.19 | 93.04±1.40 | 2610-51340** |
| Zn | 8.64±0.61 | 3.29±0.3 | 14.55±2.44 | 5.59±0.38 | 7.76±0.55 | 37.49± 6.74 | 50* |

*WHO, 2005 [10] ** Ajasa, 2004 [11]

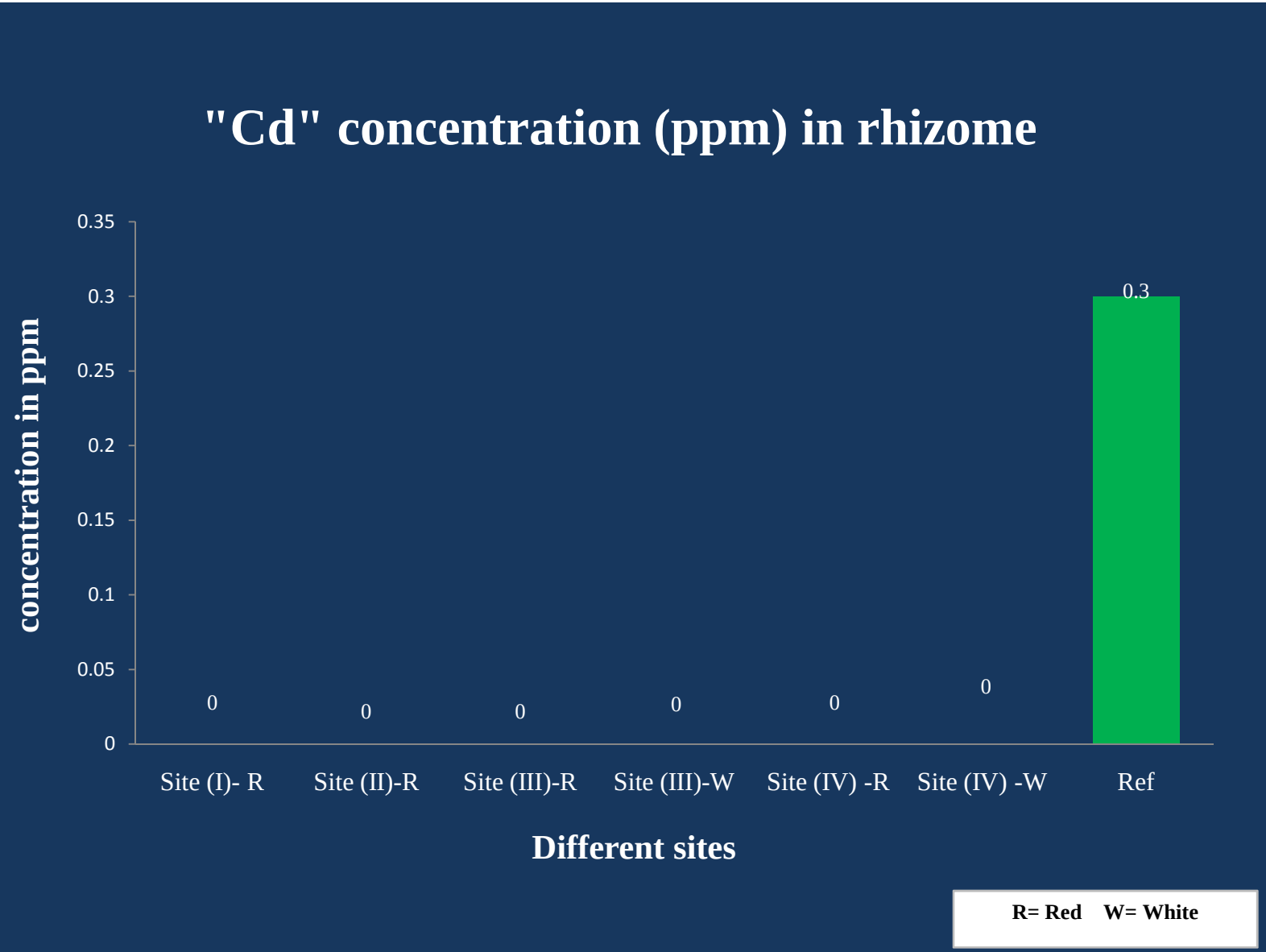



**Figure 1. Cadmium concentration (ppm) of rhizomes in different sites**

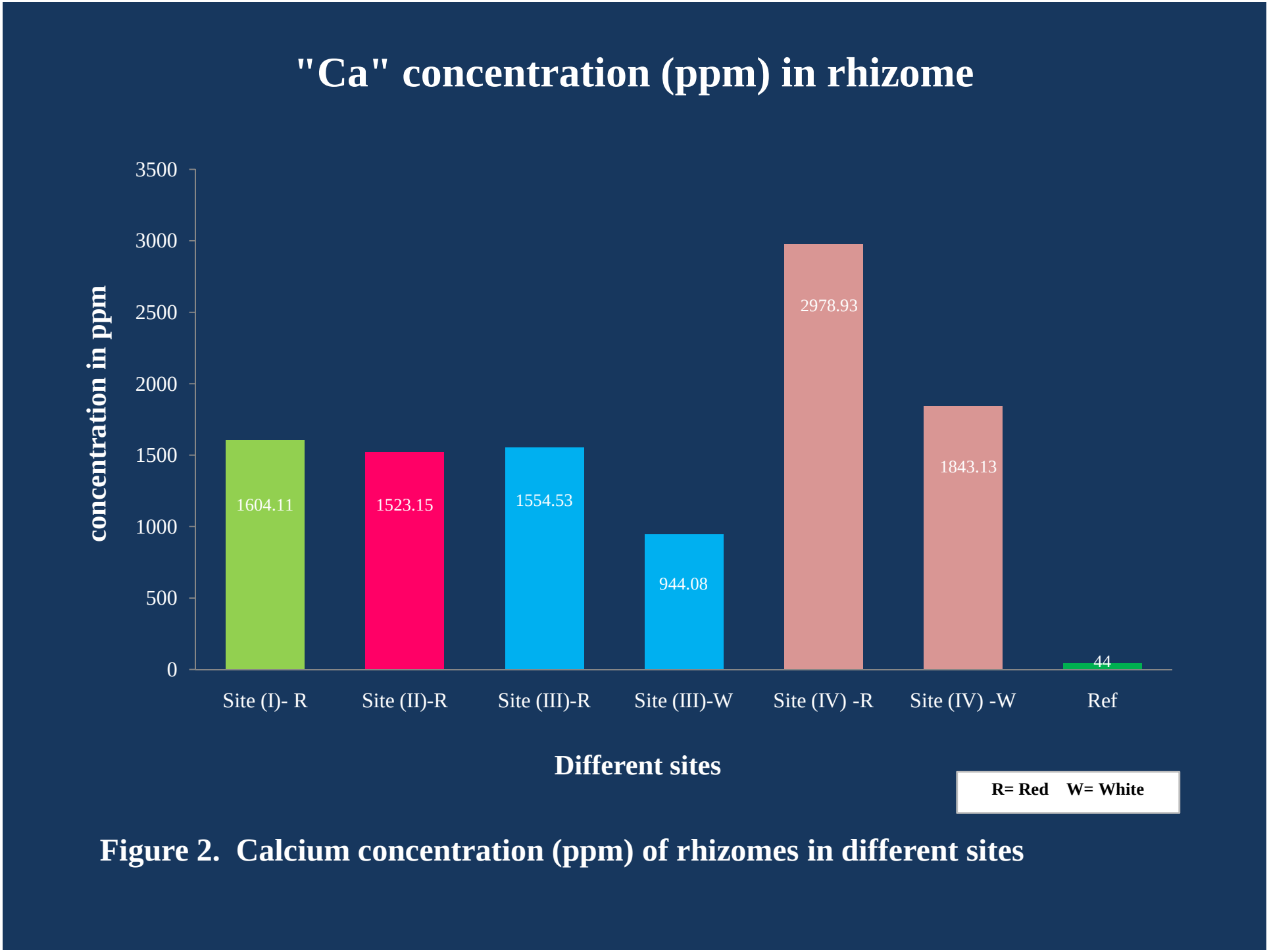



**Figure 2. Calcium concentration (ppm) of rhizomes in different sites**

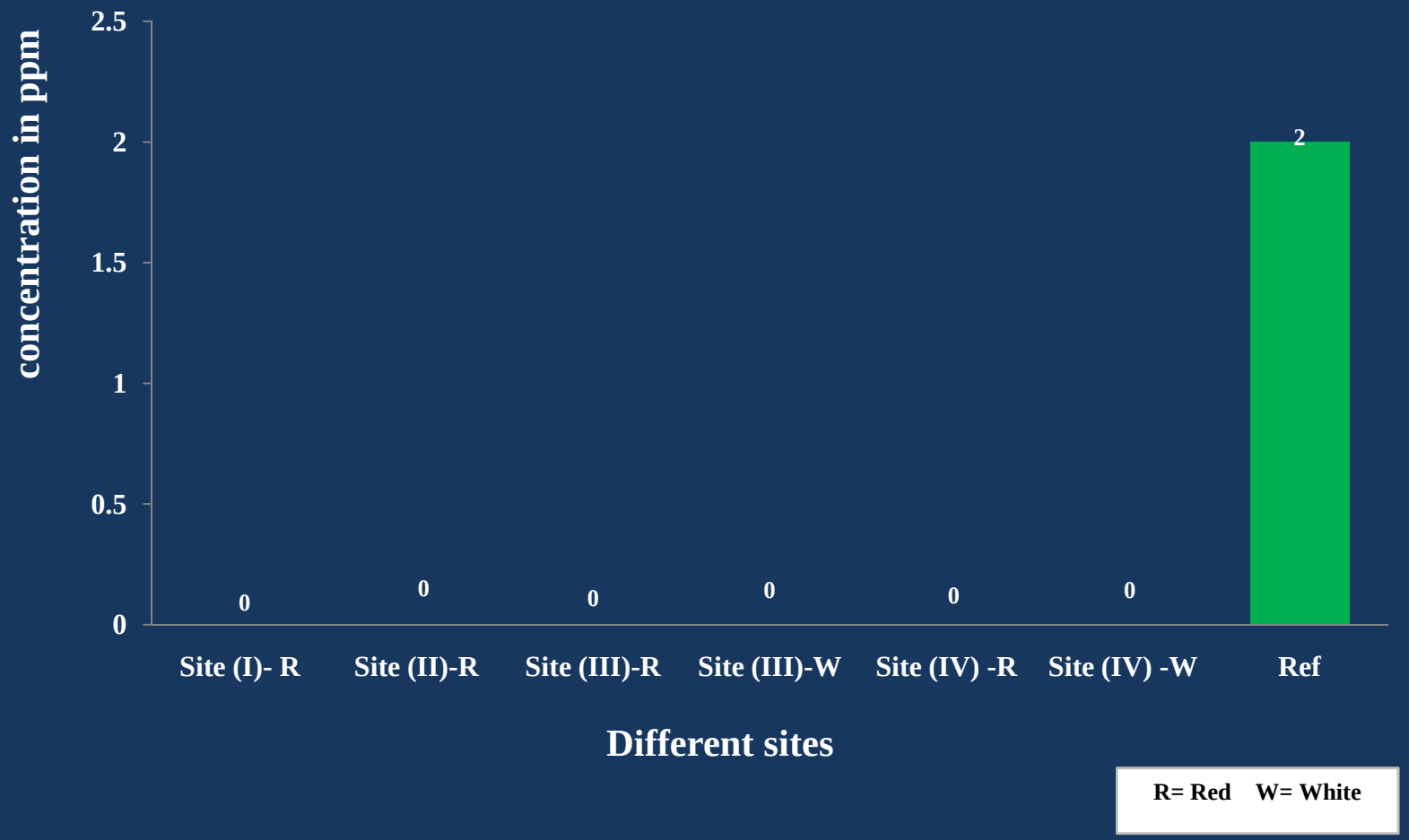



**Figure 3. Chromium concentration (ppm) of rhizomes in different sites**

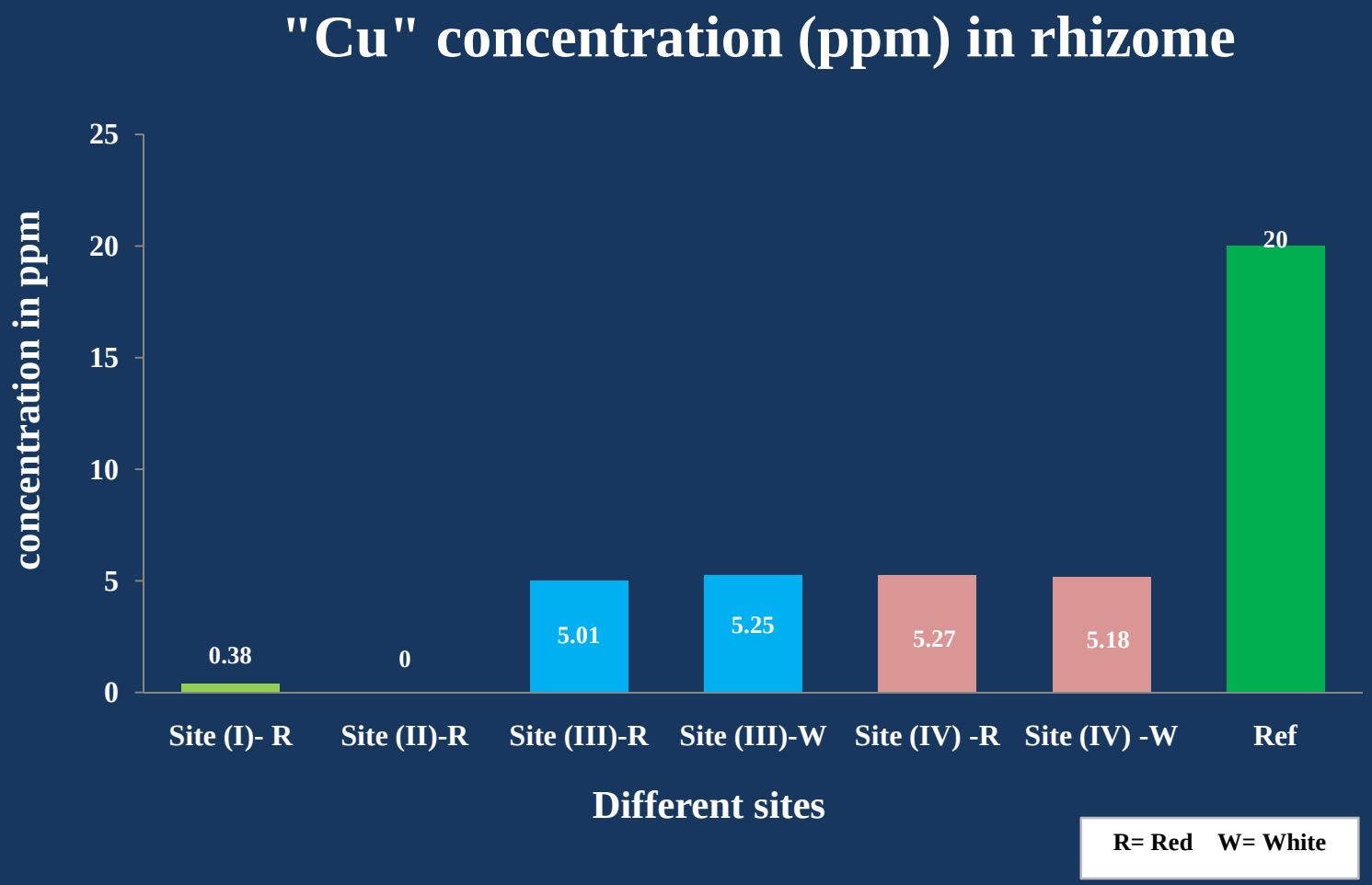



**Figure 4. Copper concentration (ppm) of rhizomes in different sites**

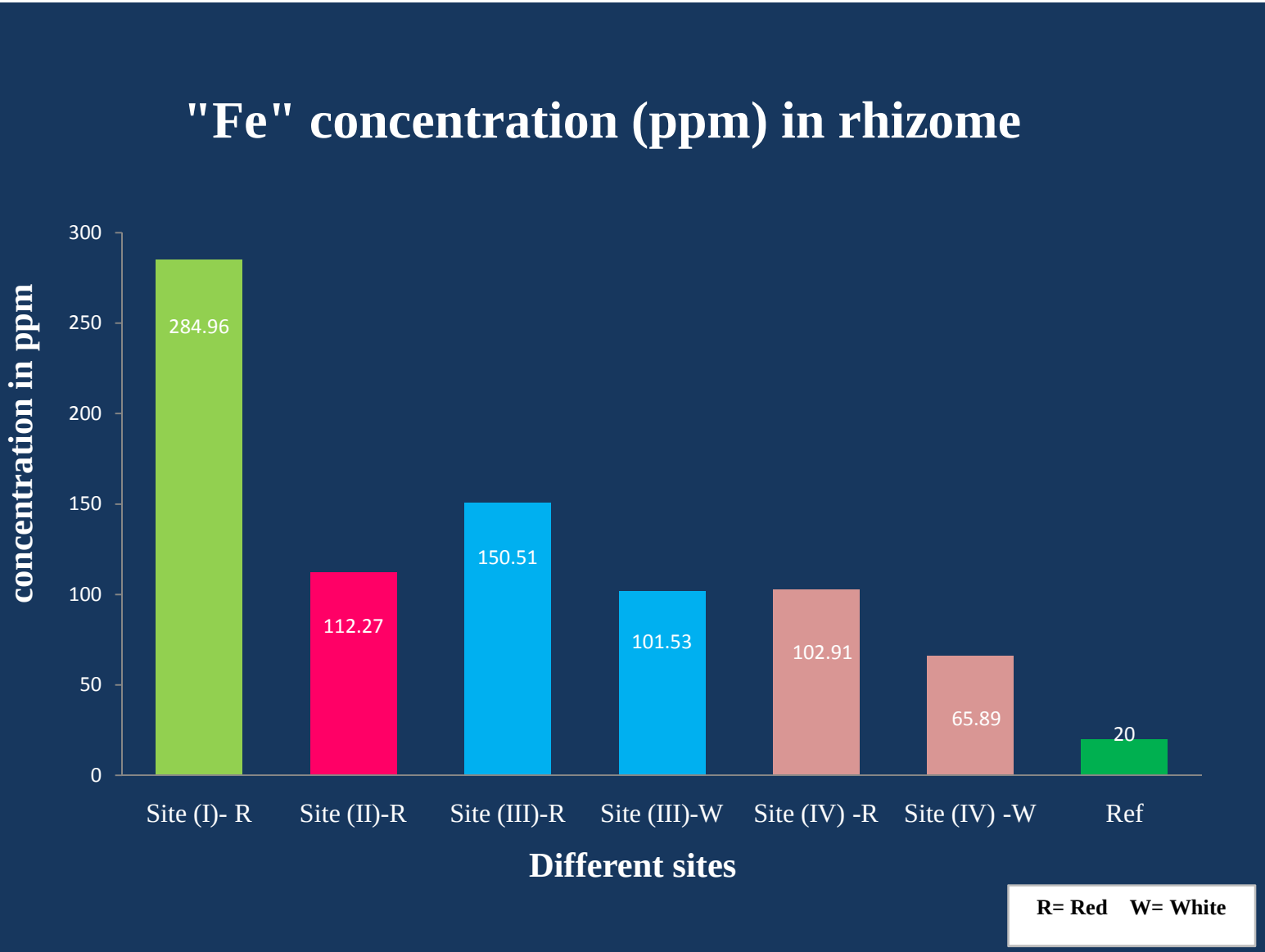



**Figure 5. Iron concentration (ppm) of rhizomes in different sites**

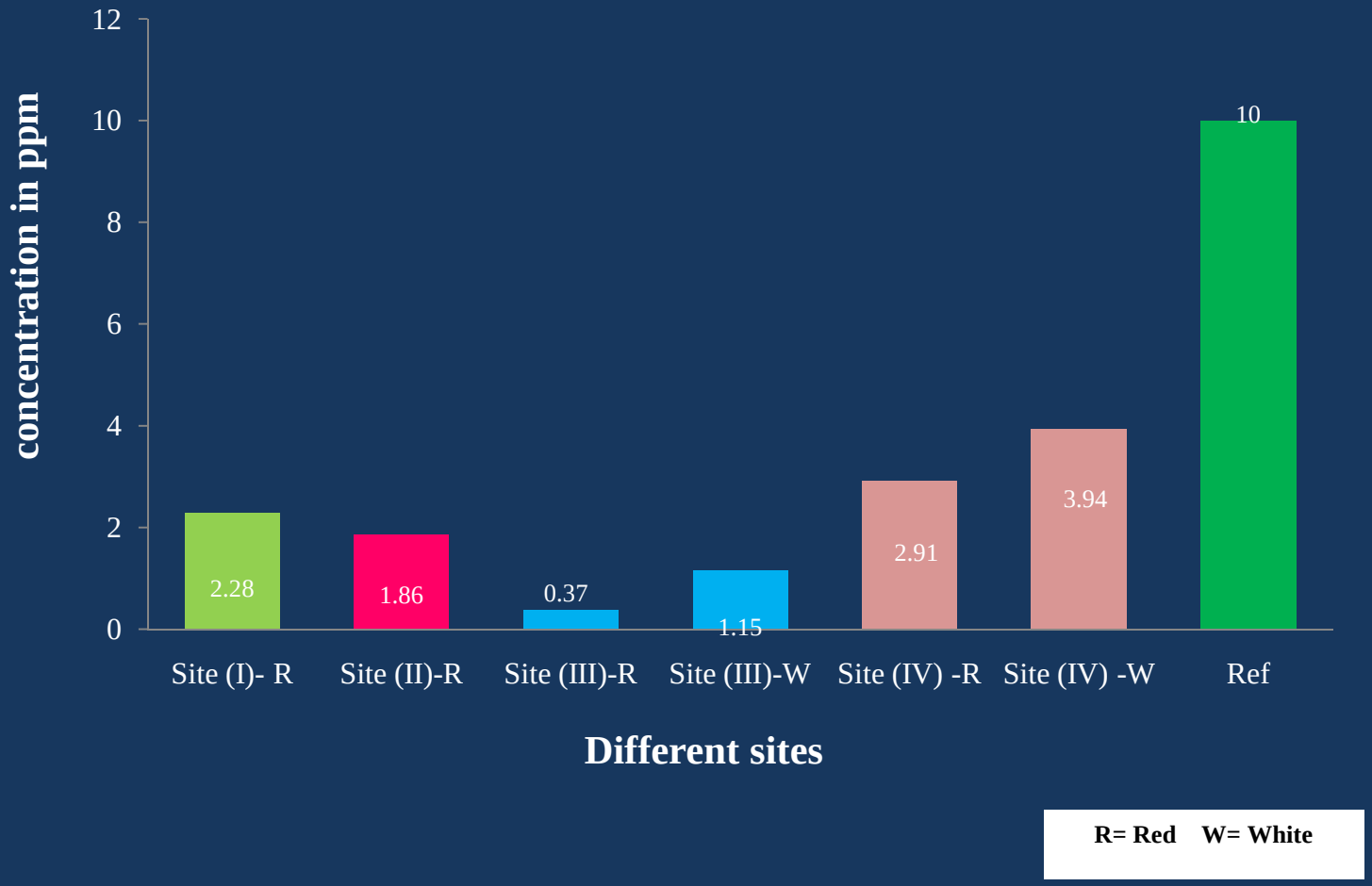



**Figure 6. Lead concentration (ppm) of rhizomes in different sites**

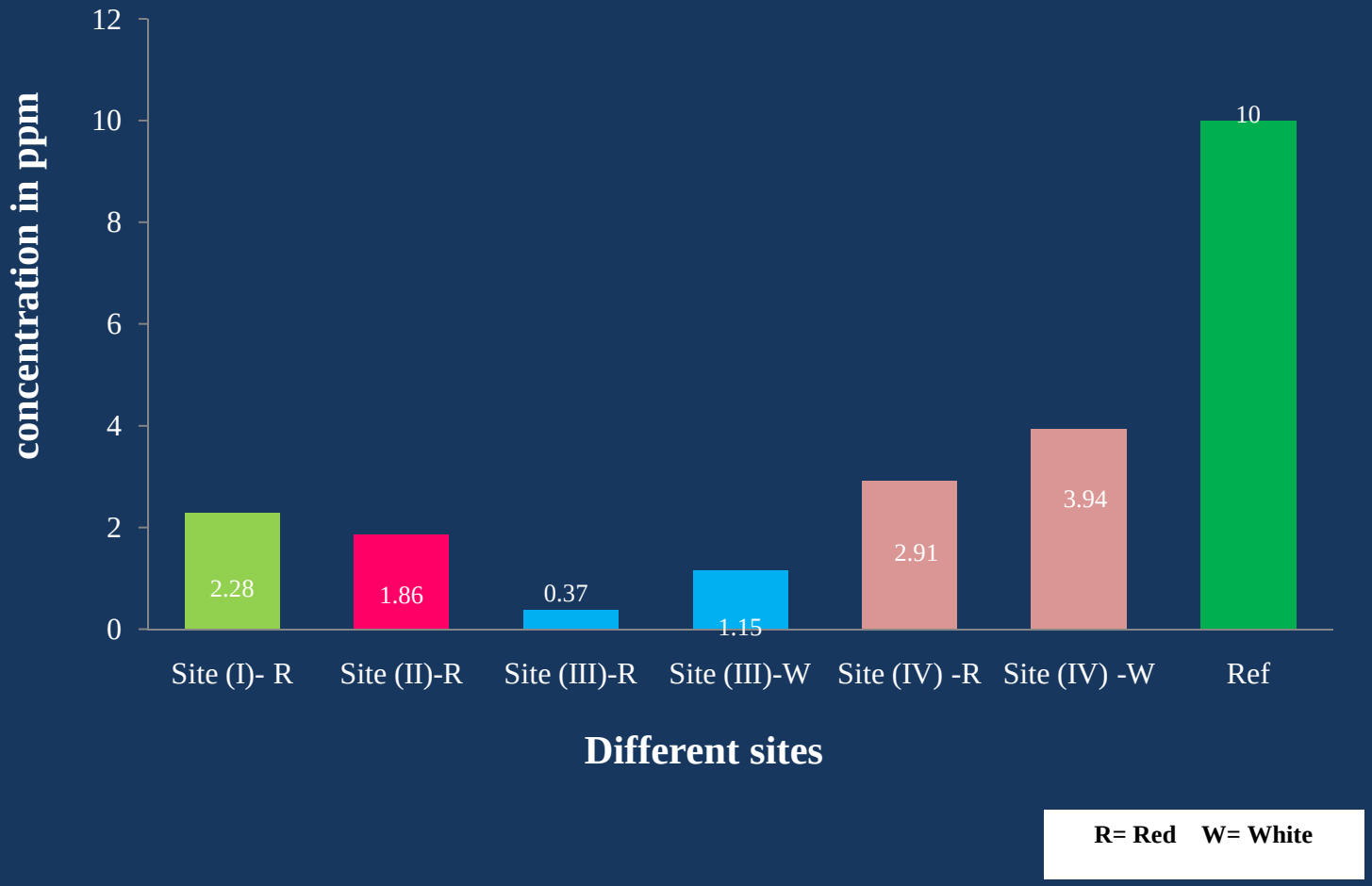



**Figure 6. Lead concentration (ppm) of rhizomes in different sites**

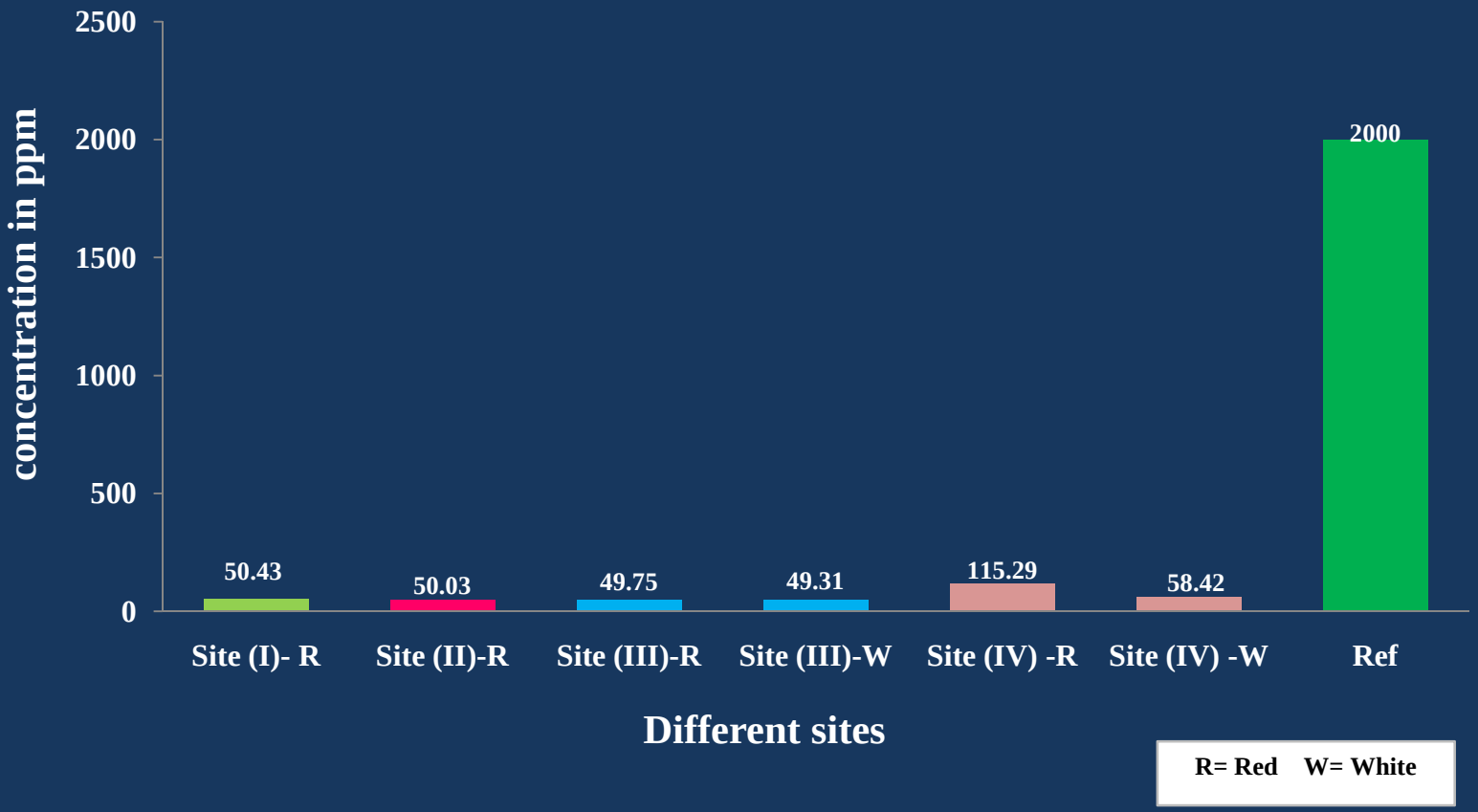



**Figure 7. Magnesium concentration (ppm) of rhizomes in different sites**

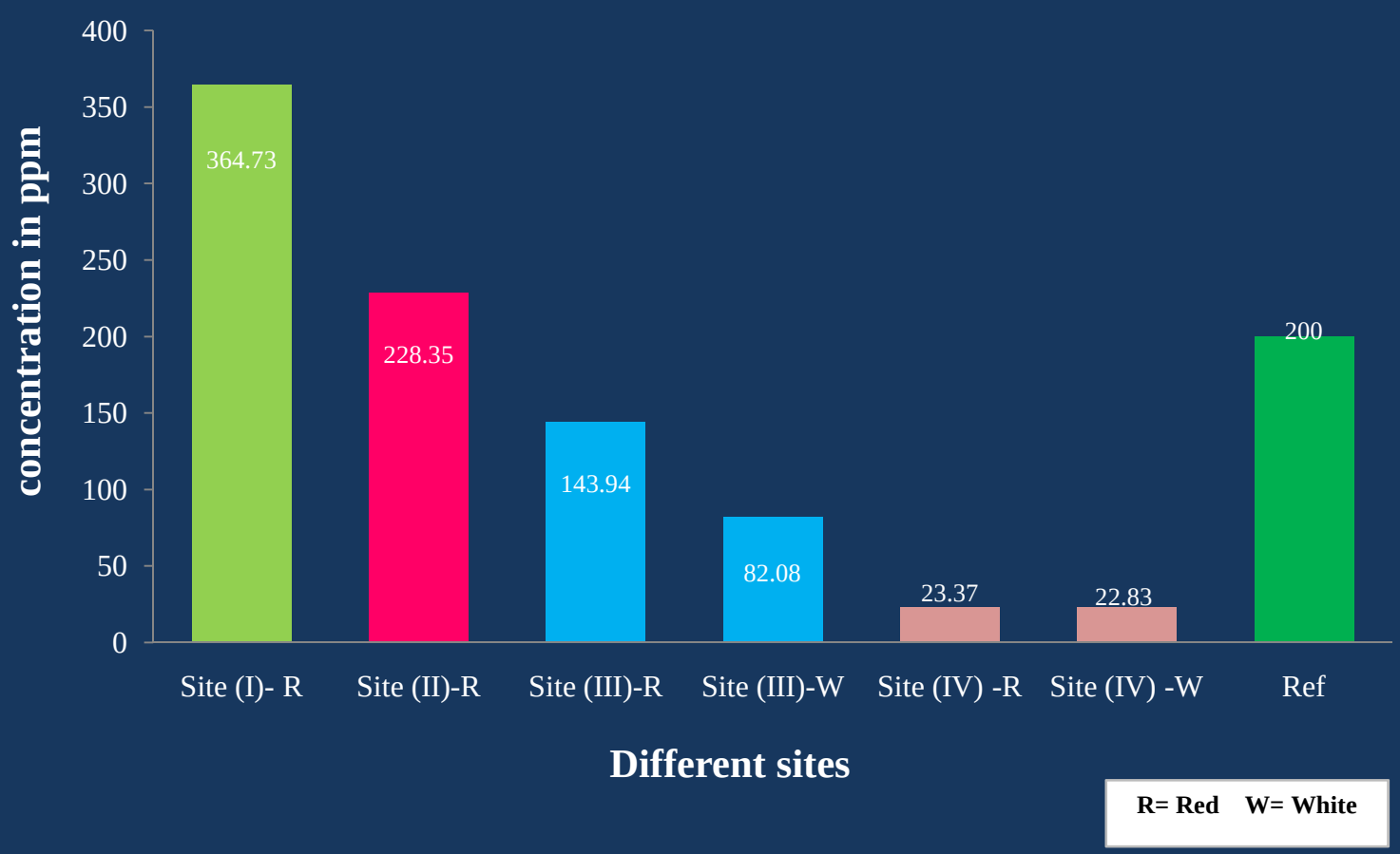



**Figure 8. Manganese concentration (ppm) of rhizomes in different sites**

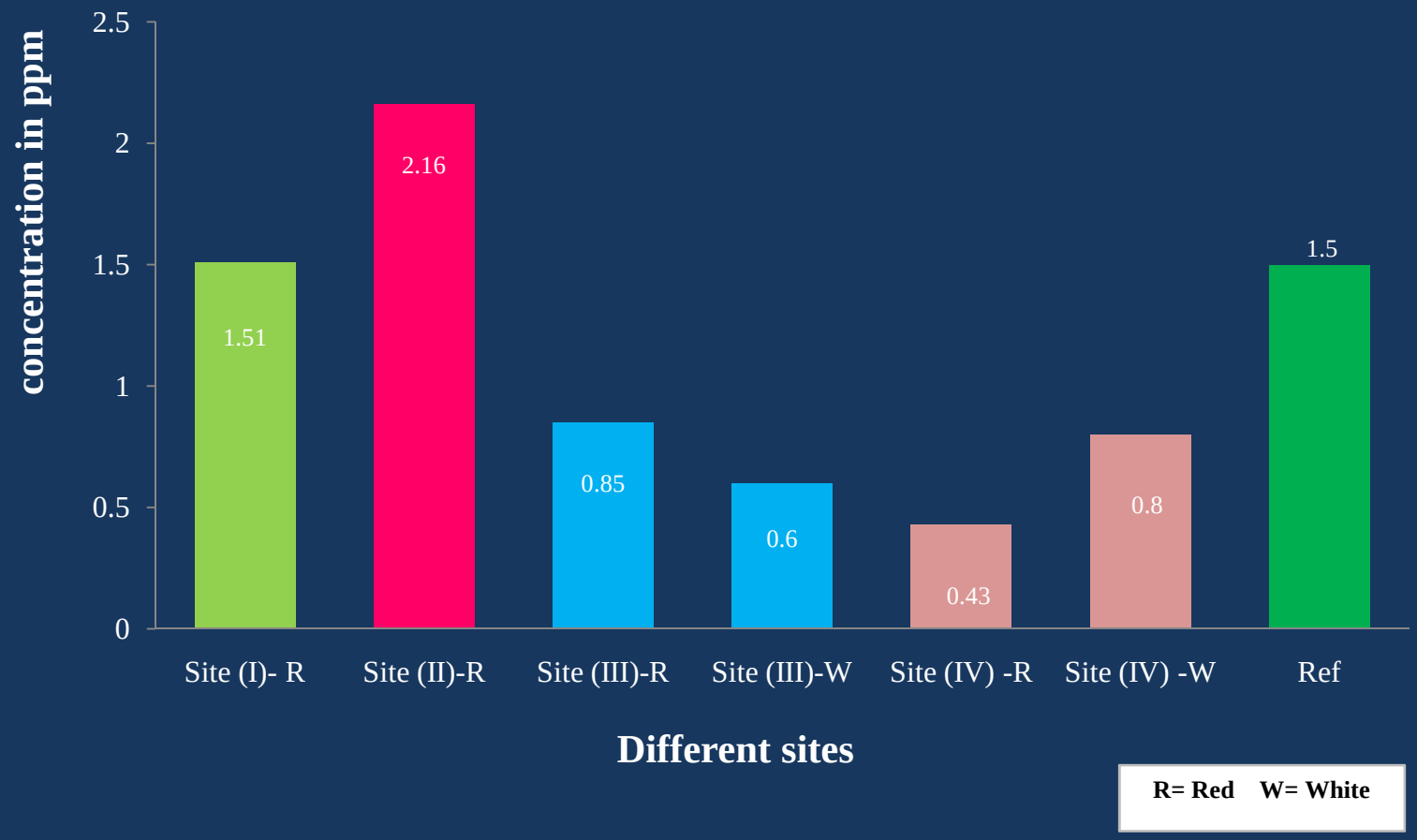



**Figure 9. Nickel concentration (ppm) of rhizomes in different sites**

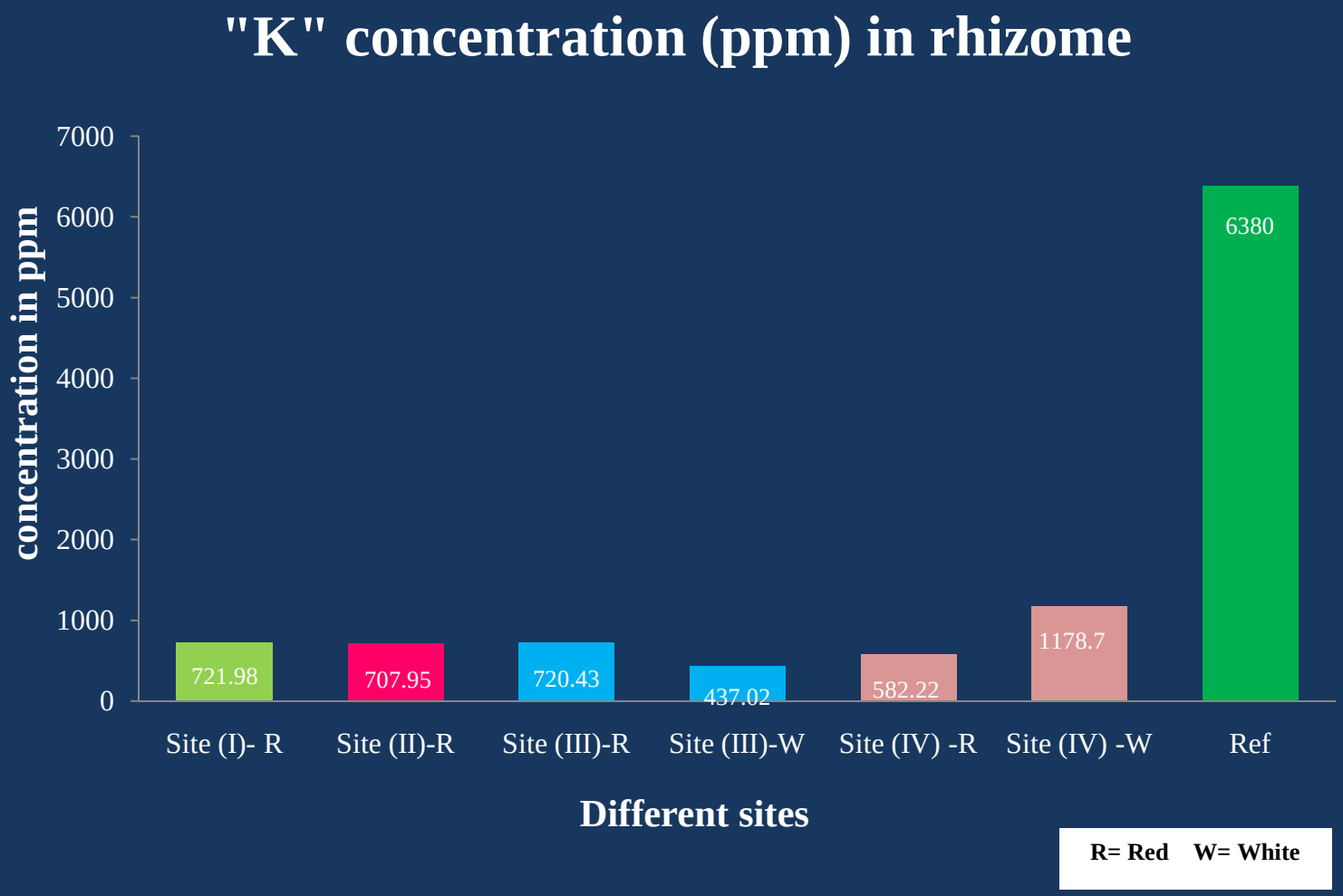



**Figure 10. Potassium concentration (ppm) of rhizomes in different sites**

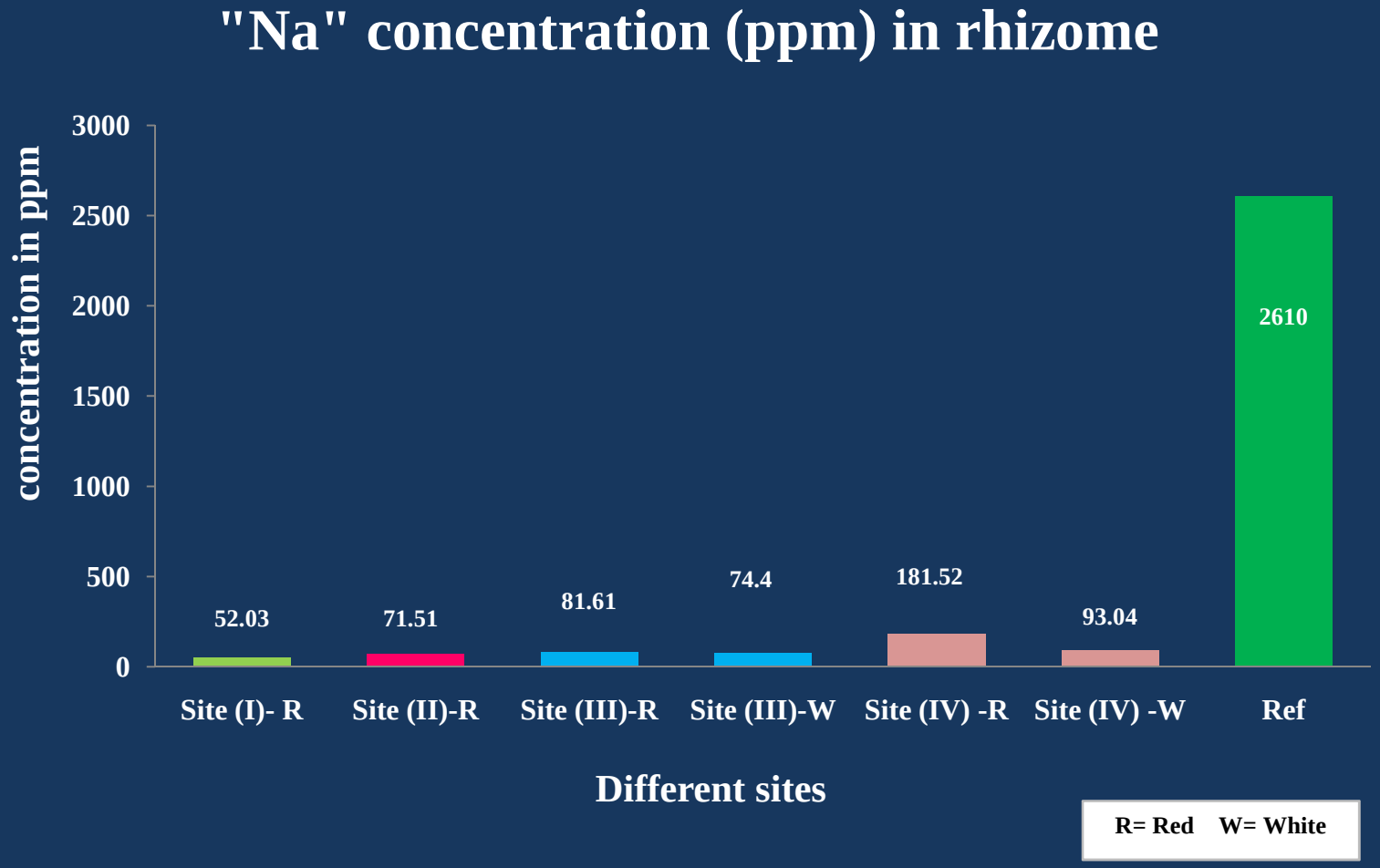



**Figure 11. Sodium concentration (ppm) of rhizomes in different sites**

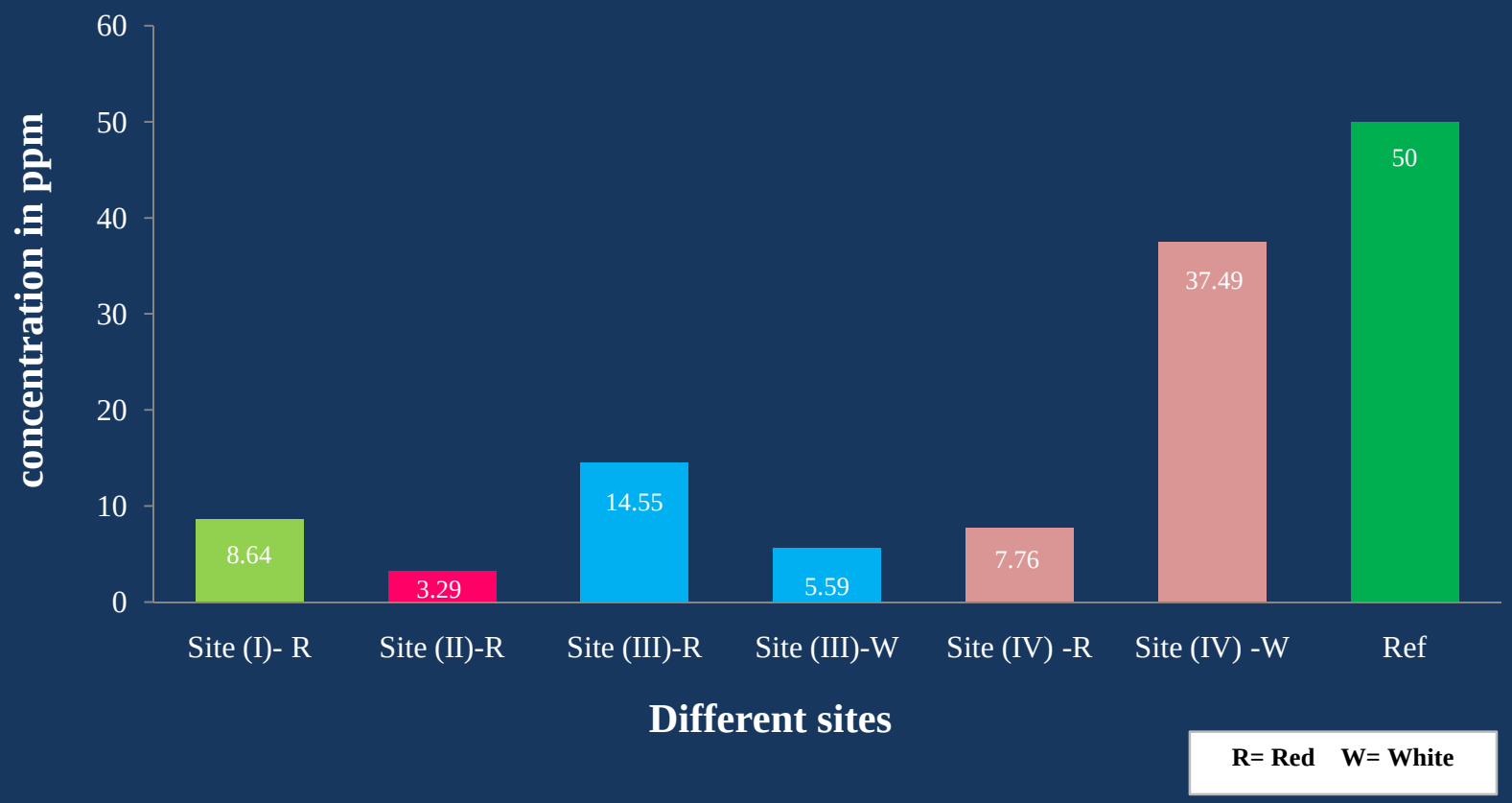



**Figure 12. Zinc concentration (ppm) of rhizomes in different sites**

# Table 3. Level of heavy/toxic metals (ppm) in soil samples of *Cissus* species from different sites

| Metal | site (I) *Cissus repens* Lam. | site (II) *Cissus repens* Lam. | site (III) | | site (IV) | Reference value |
|---|---|---|---|---|---|---|
| | | | *Cissus repens* Lam. | *Cissus discolor* Blume. | *Cissus repens* & *Cissus discolor* | |
| Cd | ND | ND | ND | ND | ND | 3* |
| Ca | 5090.61 ±80.36 | ND | 1019.32 ±21.78 | 119.22 ±2.98 | 261.57±1.33 | 52000** |
| Cr | 44.99 ±1.75 | ND | 53.71±3.49 | 12.58±2.0 | 22.1±2.48 | 100* |
| Cu | 5.52±1.15 | ND | ND | ND | ND | 100* |
| Fe | 2610.68±8.14 | 2668.34±12.34 | 2403.67±16.39 | 2521.01±9.35 | 2493.99±16.34 | 50000* |
| Pb | 57.1±21.03 | 33.82±1.2 | 10.28±2.07 | 7.62±1.15 | 16.91±2.63 | 100* |

*FAO/ WHO, 2001[12], **Pueyo, 2005 [13]

# Table 4. Level of heavy/toxic metals (ppm) in soil samples of *Cissus* species from different sites

| Metal | site (I) *Cissus repens* Lam. | site (II) *Cissus repens* Lam. | site (III) | | site (IV) | Reference value |
|---|---|---|---|---|---|---|
| | | | *Cissus repens* Lam. | *Cissus discolor* Blume. | *Cissus repens* & *Cissus discolor* | |
| Mg | 160.68±1.35 | 97.82±0.41 | 149.56±0.82 | 144.88±0.68 | 151.26±1.04 | 9000*** |
| Mn | 1241.42±15.37 | 466.66±7.51 | 583.76±8.94 | 597.32±9.5 | 924.52±13.25 | 2000* |
| Ni | 33.77±0.46 | 13.14±0.44 | 22.61±0.87 | 12.27±0.49 | 16.57±23.31 | 50* |
| K | 4686.4±34.1 | 887.26±6.99 | 1355.14 ±36.88 | 549.12±14.39 | 619.15±12.31 | 37000*** |
| Na | 287.23±4.86 | ND | 45.49±0.53 | 31.78±0.44 | ND | 25000*** |
| Zn | 41.63±1.1 | 28.22±0.73 | 28.98±0.83 | 44.32±1.24 | 14.36±0.42 | 300* |

*FAO/ WHO, 2001[12], **Pueyo, 2005 [13], ***Adriano, 1986 [14]

# Table 5. Level of heavy/toxic metals (ppm) in water samples from different sites

| Metals | Site (I) | Site (II) | Site (III) | Site (IV) | Reference value |
|---|---|---|---|---|---|
| Cd | ND | ND | ND | ND | 0.003* |
| Ca | 11.12±0.46 | 29.47±1.16 | 1.11±0.04 | 37.67±1.6 | 75*** |
| Cr | ND | ND | ND | ND | 0.05 * |
| Cu | ND | ND | ND | ND | 2* |
| Fe | ND | ND | 0.01±0.00 | 0.06±0.01 | 0.3 * |
| Pb | ND | ND | ND | ND | 0.01* |

*FAO/ WHO, 2001[12], **Pueyo, 2005 [13], ***Adriano, 1986 [14]

# Table 6. Level of heavy/toxic metals (ppm) in water samples from different sites

| Metals | Site (I) | Site (II) | Site (III) | Site (IV) | Reference value |
|---|---|---|---|---|---|
| Mg | 2.30±0.03 | 2.29±0.02 | 0.78±0.03 | 2.06±0.04 | 50 *** |
| Mn | ND | ND | 0.07±0.01 | 0.11±0.00 | 0.4 * |
| Ni | 0.02±0.00 | 0.05±0.00 | 0.02±0.00 | ND | 0.07* |
| K | 0.38±0.03 | 2.82±0.26 | 0.31±0.1 | 1.79±0.04 | 10 ** |
| Na | 4.65±0.04 | ND | 1.65±0.04 | 1.38±0.03 | 200 *** |
| Zn | ND | ND | ND | ND | 3* |

*WHO, 2008 [15], **USA, 1976 [16],***WHO, 1994 [17]

# Table 7. Level of some physico-chemical parameters for water samples from different sites

| Sampling Location | Conductivity ( µS/cm) | | TDS (mg/l) | | Salinity (g/l) | | pH | | Temperature (Ċ) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ±SD | Range | Mean ±SD | Range | Mean ±SD | Range | Mean ±SD | Range | Mean ±SD | Range |
| Site (I) | 315.33 ± 0.58 | 314.75-315.91 | 272 ±0.00 | 272-272 | 0.19 ±0.00 | 0.19-0.19 | 6.87± 0.21 | 6.66-7.08 | 19.37 ±0.23 | 19.14-19.6 |
| Site (II) | 292.67 ±11.02 | 281.65-303.69 | 253.67 ±6.66 | 247.01-260.33 | 0.18± 0.01 | 0.17-0.19 | 7.37± 0.68 | 6.68-8.04 | 21.17 ±1.27 | 19.9-22.44 |
| Site (III) | 31.57 ± 0.64 | 30.93-32.21 | 25.07 ±2.32 | 22.75-27.39 | 0.03± 0.02 | 0.01-0.05 | 8.1± 0.1 | 8 - 8.2 | 18.37 ±0.81 | 17.56-9.18 |
| Site (IV) | 292.67 ± 9.07 | 283.58-301.74 | 256± 2.65 | 253.35-258.65 | 0.18± 0 | 0.18-0.18 | 6.43± 0.21 | 6.22-6.64 | 22.43 ±1.59 | 20.82-24.02 |
| Reference value | (300 µS/cm) WHO, 1994 [17] | | TDS<300:Excellent 300-600: Good 600-900: Fair 900- 1,200: Poor Above 1200: unacceptable WHO, 1994[17] | | Non Saline <1 Slightly Saline 1-3 Moderately Saline 3-10 Very Saline >10 Rabinove,1958[18] | | 6.5-8.5 WHO,1994 [17] | | | |

# Table 8. Physico-chemical parameters for soil samples from four different sites

| Sampling Location | | pH | Temperature (Ċ) |
|---|---|---|---|
| Site (I) | | 7.9 | 21.7 |
| Site (II) | | 7.22 | 21.8 |
| Site (III) | *Cissus repens* Lam. | 7.79 | 22.0 |
| | *Cissus discolor* Blume. | 7.55 | 21.6 |
| Site (IV) | | 7.50 | 22.2 |
| References Value | | (6.5-9.2) WHO, 1994 [17] | |

# Table 9. Phytochemical Constituents of Rhizome of *Cissus* species from Various Sites

| Sr. No | Type of compound | Site (I) *Cissus repens* Lam. | Site (II) *Cissus repens* Lam. | Site (III) | | Site (IV) | |
|---|---|---|---|---|---|---|---|
| | | | | *Cissus repens* Lam. | *Cissus discolor* Blume. | *Cissus repens* Lam. | *Cissus discolor* Blume. |
| 1 | Alkaloids | - | - | - | - | - | - |
| 2 | α amino acid | - | - | - | - | - | - |
| 3 | Carbohydrate | +++ | ++ | + | +++ | + | +++ |
| 4 | Flavonoids | - | - | - | - | - | - |
| 5 | Glycosides | +++ | ++ | + | ++ | + | +++ |
| 6 | Phenols | + | + | ++ | +++ | + | ++ |
| 7 | Protein | - | - | - | - | - | - |
| 8 | Reducing sugar | +++ | ++ | + | +++ | ++ | ++ |
| 9 | Resins | - | - | - | - | - | - |
| 10 | Saponins | +++ | ++ | + | + | + | + |
| 11 | Starch | +++ | ++ | + | ++ | +++ | ++ |
| 12 | Steroids | - | - | - | - | - | - |
| 13 | Tannins | - | - | - | - | - | - |
| 14 | Tri-terpene | - | - | - | - | - | - |

(+) = presence (-) = absence

# Conclusion

- Most of the tested medicinal rhizomes and all the tested water and soil samples from four different sites contain the tested metals which are within permissible limit

- **‘Cd’ and ‘Cr’ concentrations were not detected in all studied rhizomes in four sites**

- **‘Pb’ was detected in all rhizome but within the permissible limit**

- **‘Cd’ , ‘Cr’, ‘Pb’ cause both acute and chronic poisoning, adverse effect on kidney, liver, vascular and immune system**

- All studied rhizomes contain Cu", "Mg", "K", "Na" and "Zn" within the permissible limit in all sites

- 'Cu' is one of the essential elements for growth of plants and development of living organisms

- 'Mg' is the fourth most abundant element in the human body and is essential to be good health

- **'K' ions are the most abundant cation in the human body and it is necessary for cell growth and function**

- **'Na' ion is responsible for maintaining normal hydration and osmotic pressure**

- **Deficiency of 'Zn' is characterized by recurrent infections and lack of immunity**

- **“Ca” and “Fe” level of all studied rhizomes were above the permissible limit in all sites which may be due to**

- **the growing of these plants in contaminated as mineral in soil**
- **explosive materials used for mining**
- **the dam water drained from the hilly areas which have also been exposed to mining work**
- **hyper accumulators even though their soil contain the lower elemental level**

- The high concentration of ‘Ca’ contained in all studied rhizomes may be high therapeutic value

- Humans need large amounts of ‘Ca’ for construction and maintenance of bones, teeth and normal function of nerves and muscles

- In all sites, “Fe” levels were shown to be toxic, the reason could be easily dispersible and absorbable in the water or soil or air

- ‘Fe’ is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transport in human body systems

- **"Mn" and "Ni" levels of *Cissus repens Lam.* in site (I) & (II) are above the permissible limit**

- **Their toxicity in human is not very common occurrence because its absorption by the body is very low**

- This study revealed that free from (polluted element) toxic contaminant (Cd, Cr, Cu, Pb and Zn) in water, soils and rhizomes from four different sites were detected.

- all the physico-chemical parameters including conductivity, TDS, salinity, pH and temperature of the water samples are considered to be good.

- It should be collected from areas without contamination of heavy metals
- Special care must be taken during the administration of remedy prepared from the plants
- It must be necessary to have a look on good quality control methods

- This study is to make awareness among the public regarding the importance of collection sites of medicinal plant

# References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