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Earth Science and climate change conference

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"Effect of heavy metal ion on the antioxidant properties of *Mentha spicata*"

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An Introduction

Antioxidant-mechanism and how they destroy radical's

Recent year there is an increasing interest in antioxidant.

Main reason for this is the protection of cells,their organelle and metabolic pathways against oxygen free radicals and their derivatives (ROS).

ROS produced in biological system due to exposure of various physical and chemical toxin.

During metabolism intermediate metabolite(ROS) are generated and overload of these ROS leads to oxidative stress to macromolecules like DNA, lipids, Proteins, chromosome break, alteration in signal transduction and gene expression occur and they are implicated to various disorder like cataract, birth defect, reproductive anamolies neuro degenerative diseases and other asssociated with oxidative stress such as cancer. cardiovascular and neuro degenerative diseases

- The balance between production and removal of reactive species Is required to maintain normal physiological function
- Biological system is endowed with various antioxidants like superoxide dismutase, catalase, glutathioneS- Transferase
- The in-vivo system of defense may not be adequate to neutralize all the ROS's there is a need for an external source of antioxidants to neutralize the free radical load in the body.
- Fruits vegetable, spices and various herbal resources recognized as external sources of antioxidants like phenolic acid, flavonoid etc
- These acts as a free radical scavengers or activator of antioxidative defense system to supress radical damages in system
- There fore there is a growing interest toward natural antioxidants

Plants are more prone to heavy metal stress due to manmade or natural activities

- Keeping in mind the antioxidant potential of mint and role of abiotic stress in activating plant defense, the effect of heavy metal ion stress on antioxidant potential of *M.spicata* has been studied.

- Plants are subjected to a number of abiotic stresses, like drought, temperature , radiation, salinity, soil pH, heavy metals, lack of essential nutrients, air pollutants, etc.
- Metal toxicity can cause a redox imbalance and induce the increase of ROS concentration, activating the antioxidant defense mechanisms of plants.
- Plants have developed different strategies to cope with these stresses. Some use an avoidance strategy to reduce trace element assimilation while others use internal defense mechanisms to cope with the increasing levels of the toxic species.
- Antioxidants detoxify, neutralize, and/or metabolize reactive species and hence reduce the incidence and/or severity of some degenerative conditions.

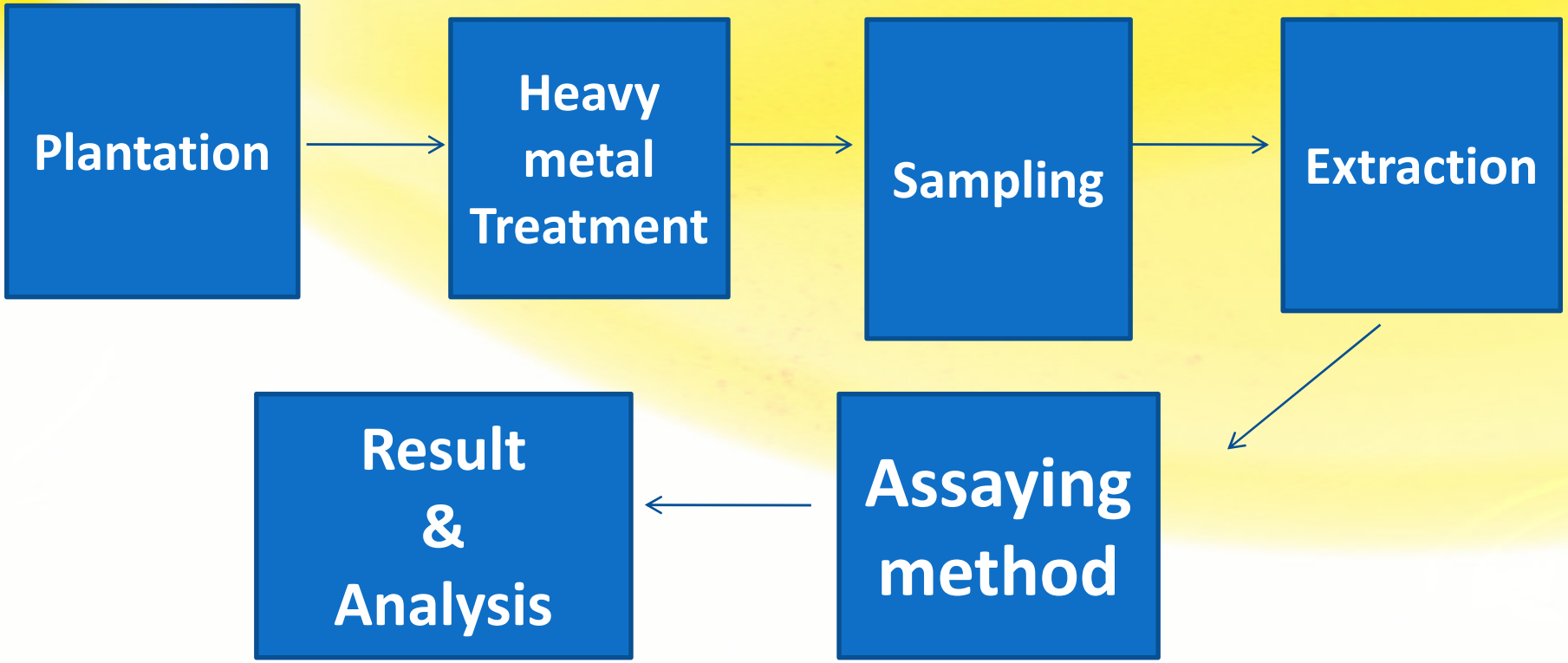
Objective

- Is to determine the change in the total polyphenol contents, TFC and characterize the free radical scavenging, ferric ion reducing capabilities of herbal plants ***Mentha spicata after treating with heavy metal.***

- Comparison of antioxidant activity of the plants, given heavy metal stress on the basis of days.

- Validation of result by performing various assays for the same source.

Experimental Design



Plantation

- Experimental Plant Variety(EVP)

Mentha spicata

- Number of EVPs - 30 units
- Area - Department of Biotechnology, Punjab Agricultural University(PAU) Ludhiana, INDIA





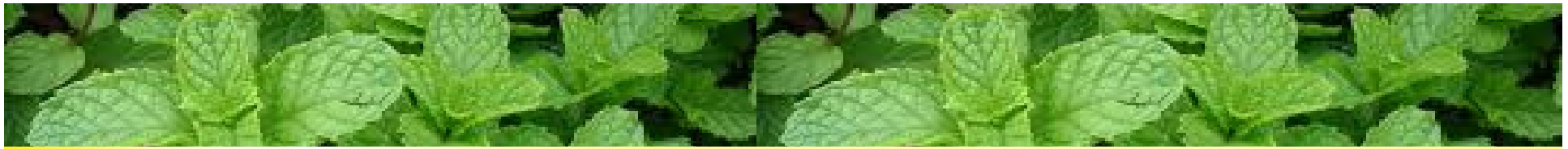
Why M.spicata?

✓Short life cycle

✓Perennial herb

✓Easy availability and maintenance

✓Having high phenolic compound



- ✓ Plant were raised in poly bags, containing 1 kg of garden soil.
- ✓ Plants were divided into 6 groups, each group having five plants.
- ✓ Two group of plants were kept as a control (given normal watering)
- ✓ Other four group of plants were treated as a test plants (given the stress of heavy metal)



Heavy Metal Treatment

- Different heavy metal at the rate of **1O**ppm concentration were given to plants,
  - Lead as a lead acetate
  - Cadmium as Cadmium Sulphate
  - Arsenic as Sodium Arsenate
  - Nickel as nickel sulphate
- Treatment with heavy metal was not repeated

- 500 ml solution of each heavy metal was prepared
- 100 ml of heavy metal solution was given to each group of test sample containing five plants each.
- Each group of plant sample was then watered(200 ml) two times for 30 days.
- The leaves were then plucked after every fifteen days of time for experimentation.

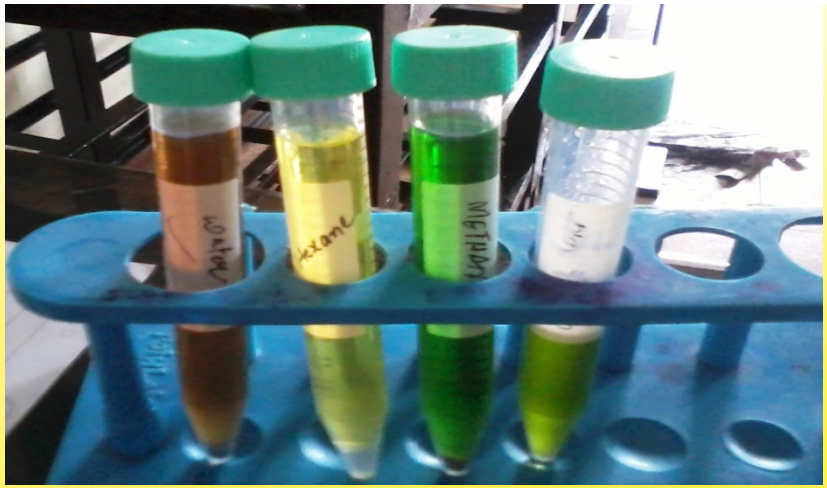
Sampling

Leaves sampling

- First generation leaves were plucked from the plants groups (treated with different heavy metal stress) after 15 days of heavy metal treatment.

- Second generation leaves were plucked after 30 days of heavy metal treatment.

Extraction



Preparation of extract

- 5g wet wt. of *Mentha spicata* leaves were grinded in a pestle motor and mix with 50ml of methanol.
- Allowed for 1 hr incubation at room temperature.
- Centrifuged at 4000 rpm for 15 minutes
- Mixture then filtered by using muslin cloth.
- Filtrate is kept for further experimental purposes.

Assaying Method

- Standardization
- Total Phenolics Content
- Total flavonoid content
- Total reducing assay
- Ferrous Ion Reducing Antioxidant Potential Assay (FRAP)
- DPPH Scavenging Assay
- Superoxide Scavenging Assay

Standardization

- Gallic acid as a standard phenolic compound for TPC evaluation of test sample
- Quercetin as a standard flavonoid compound for TFC evaluation of test sample
- Ascorbic acid as a standard antioxidant for evaluation of antioxidant potential

Phenolic and flavonoid estimation

TPC

10µL test sample

↓

490µL of dist water

↓

1Hr incubation

↓

500µL of **folin reagent**

↓

2 mL **Na2CO3**

↓

Absorbance at765nm

TFC

100µL test sample

↓

500µL of dist water

↓

500µL of methanolic **AlCl3**

↓

2ml **K2CO3**

↓

2mL Dist. **H2O**

↓

Absorbance at 415 nm

Reducing potential estimation

Total Reducing Assay

100µL sample + 500µL PO_4 buffer+500µL K[fe(CN)4]

↓ incubation 50°, 20 min

500µL trichloro acetic acid

↓ 10min centrifuge, 2500rpm

Supernatent+distH_2O(2.9mL)

↓

Soln made to 5ml adding H_2O

↓

Absorbance at 700 nm

FRAP Assay

2.5mL acetate buffer+2.5mL TPTZ +2.5mL $FeCl_3.6H_2O$

↓

FRAP Solution +100µL sample

↓

Solution made to 5mL Dist H_2O

↓

Absorbance 593 nm

Scavenging Capacity

DPPH scavenging activity

10µl sample + 2.5mL **Tris HCl**
+ 1mL DPPH

30 min
incubation

Absorbance at 517nm

Superoxide scavenging activity

100µL of **NBT**+100µl **NADH(prepared in 2.6mL PO4 Buffer)**

100µL of sample

Add 100µL **PMS**

25°C

5 min

Absorbance at 560nm

Calculation

Sample values inTPC, TFC, TRA, FRAP method are calculated in mg/g which is gram equivalent to the standard compound, obtained by the regrsession equation of standard compound

$$y = mx + C$$

y=absorbance of sample

m= absorbance of standard compound

x= value to be determine (mg/g)

C= constant value

Calculation for DPPH and superoxide scavenging capacity

Formula used

$$\text{Inhibiton\%} = \frac{[(\text{A Blank-A sample})]}{\text{A blank}} \times 100$$

Result and analysis

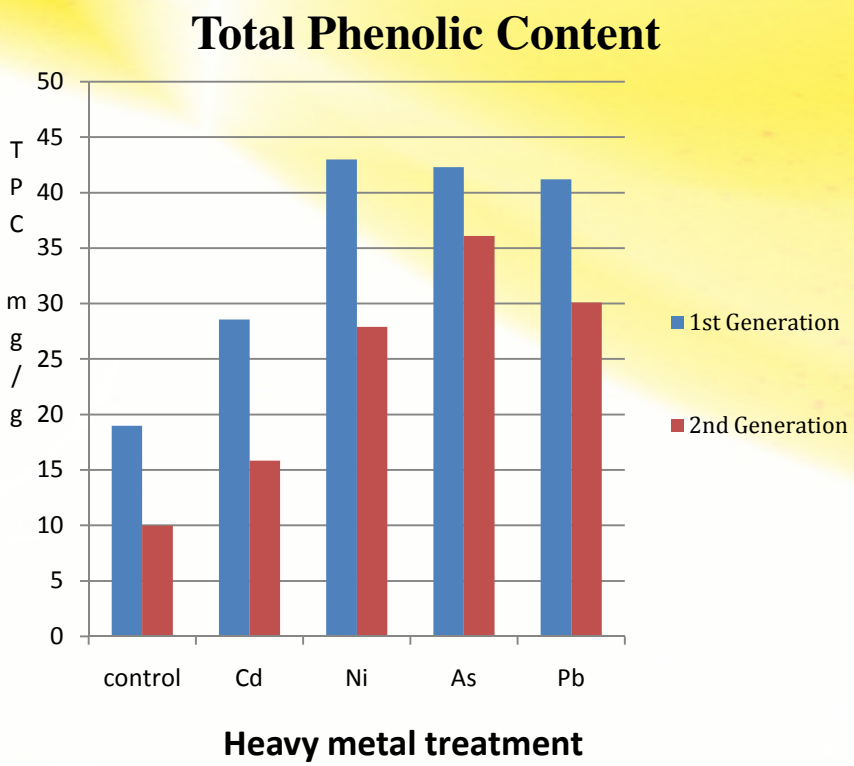
Various assaying method were performed by using ascorbic acid as a standard

- TPC, TFC were measured as gallic acid and quercetin equivalent.
- Total reducing assay, FRAP were measured as ascorbic acid equivalent.
- DPPH and superoxide scavenging assay were examined by calculating % inhibition and also examined by seeing % change of test plants over the control.

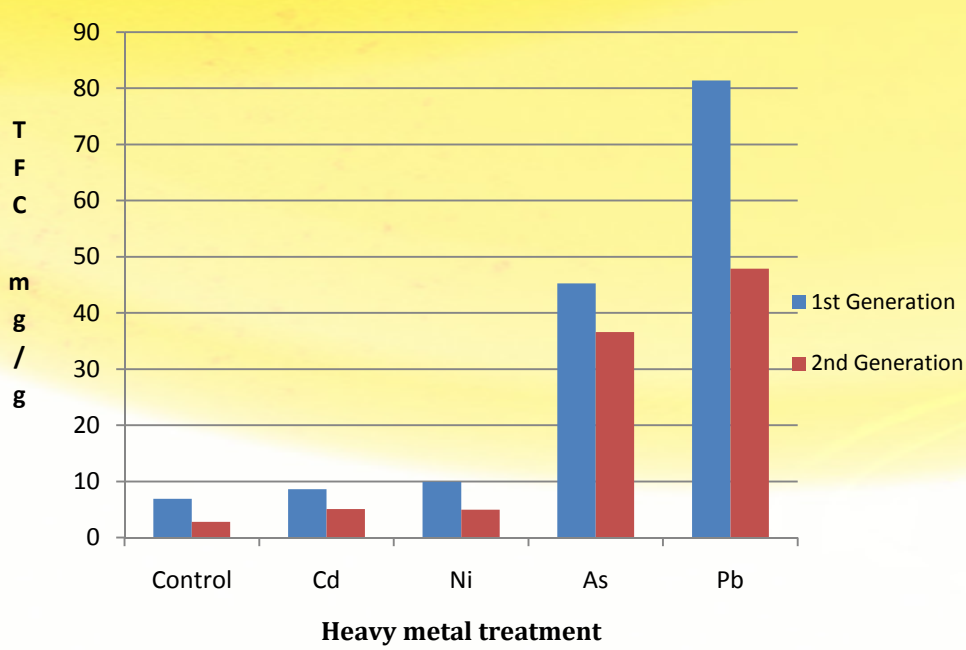
Table 1:-**values in parenthesis represent change over the control*

Treatment	Total Phenolics content		Total Flavonoids Content	
	1st Generation leaves	2ndGeneration leaves	1st Generation leaves	2ndGeneration leaves
Control	21.5	16.9	6.9	2.80
Cd	28.5(32.5)	20.8(23.1)	8.6(24.6)	5.1(82.1)
Ni	40.3(87.4)	28.9(71.0)	9.9(43.8)	4.9(75.0)
As	42.3(96.7)	36.1(113.6)	9.5(-37.7)	3.7(32.1)
Pb	41.2(91.6)	30.1(78.1)	8.14(17.4)	4.8(67.8)

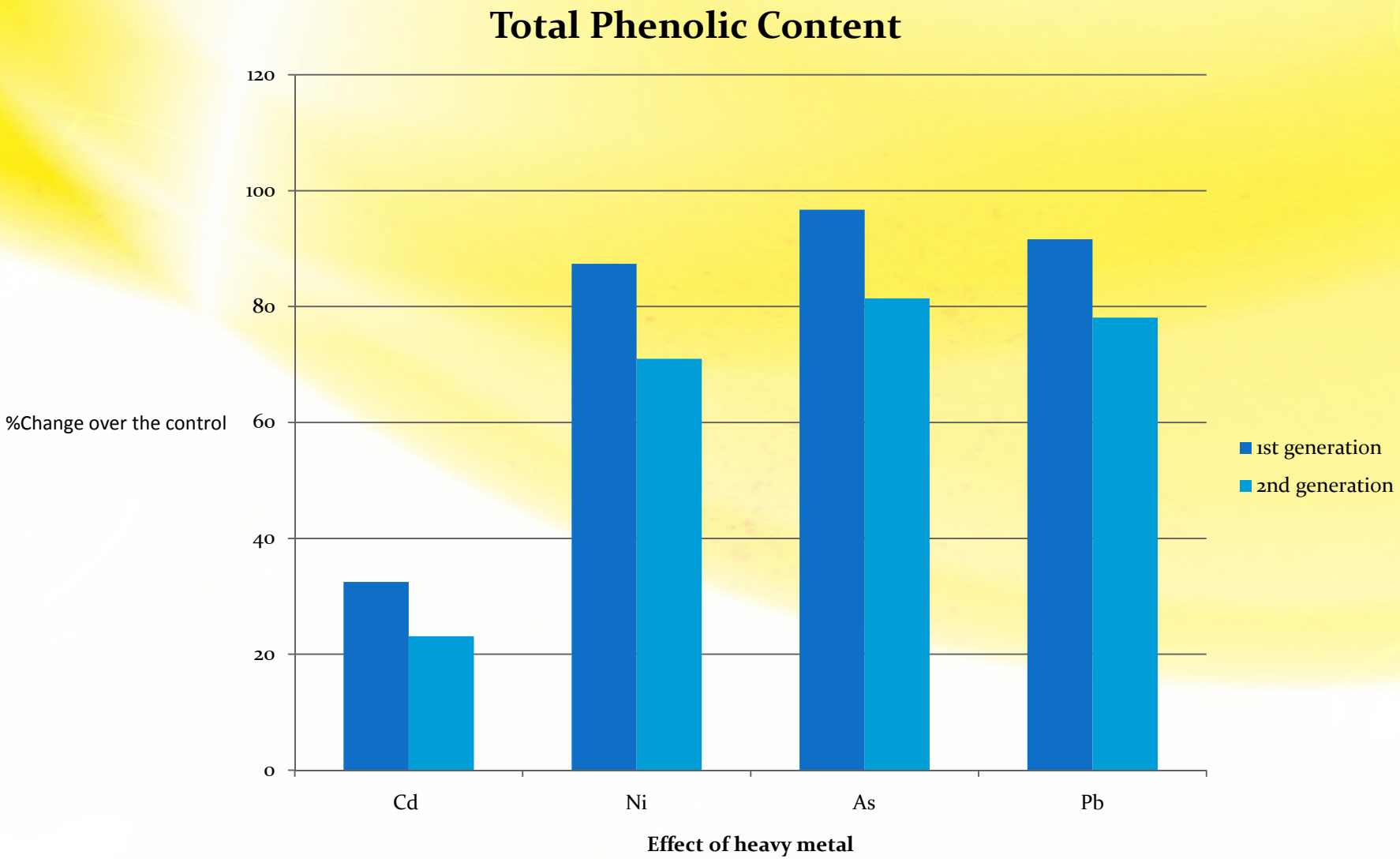
TPC


Total Phenolic Content
TPC mg/g
50
45
40
35
30
25
20
15
10
5
0
control
Cd
Ni
As
Pb
1st Generation
2nd Generation
Heavy metal treatment

TFC


Total Flavonoids Content
TFC mg/g
90
80
70
60
50
40
30
20
10
0
Control
Cd
Ni
As
Pb
1st Generation
2nd Generation
Heavy metal treatment

Graph showing change over the control



Graph showing change over the control

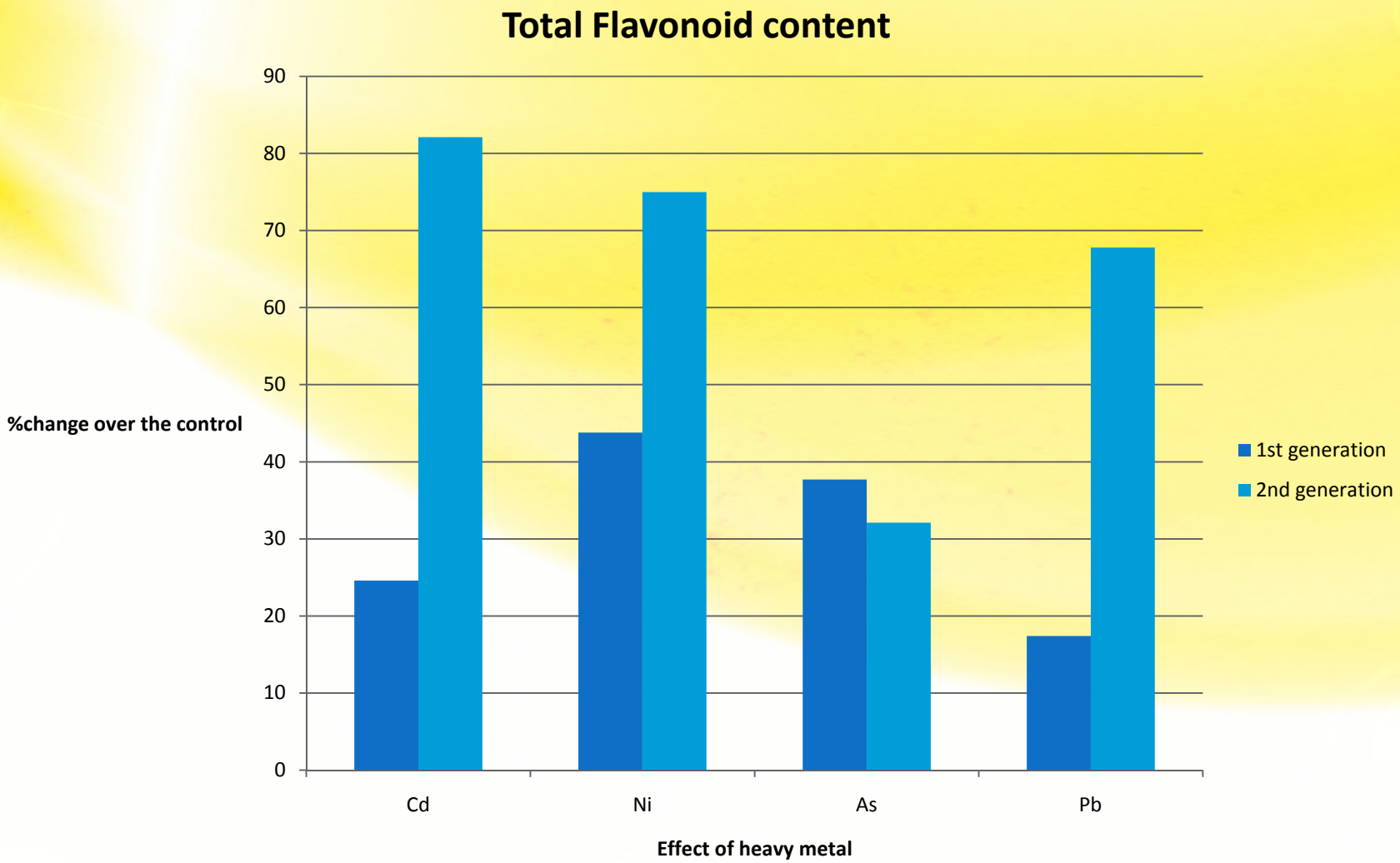
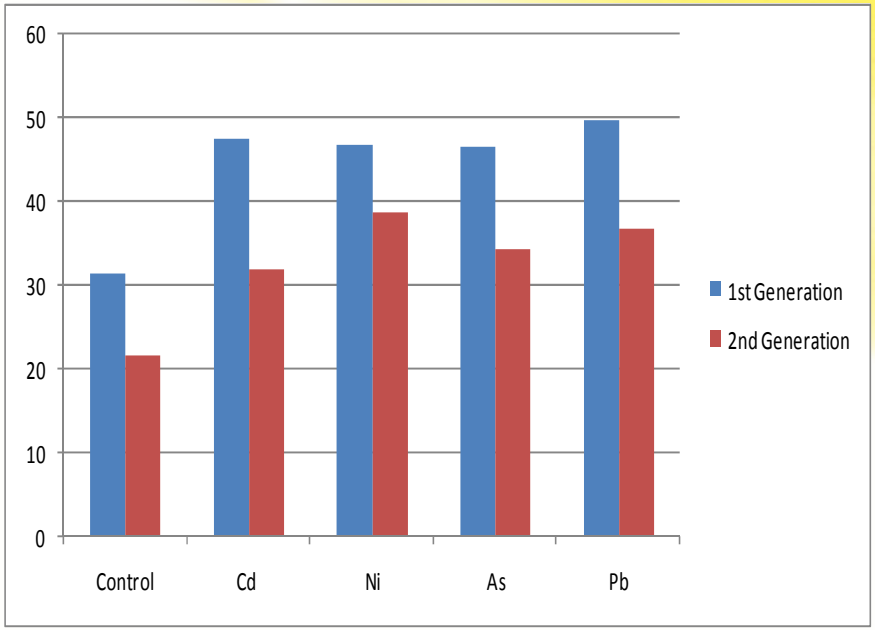


Table 2:-**values in parenthesis represent change over the control*

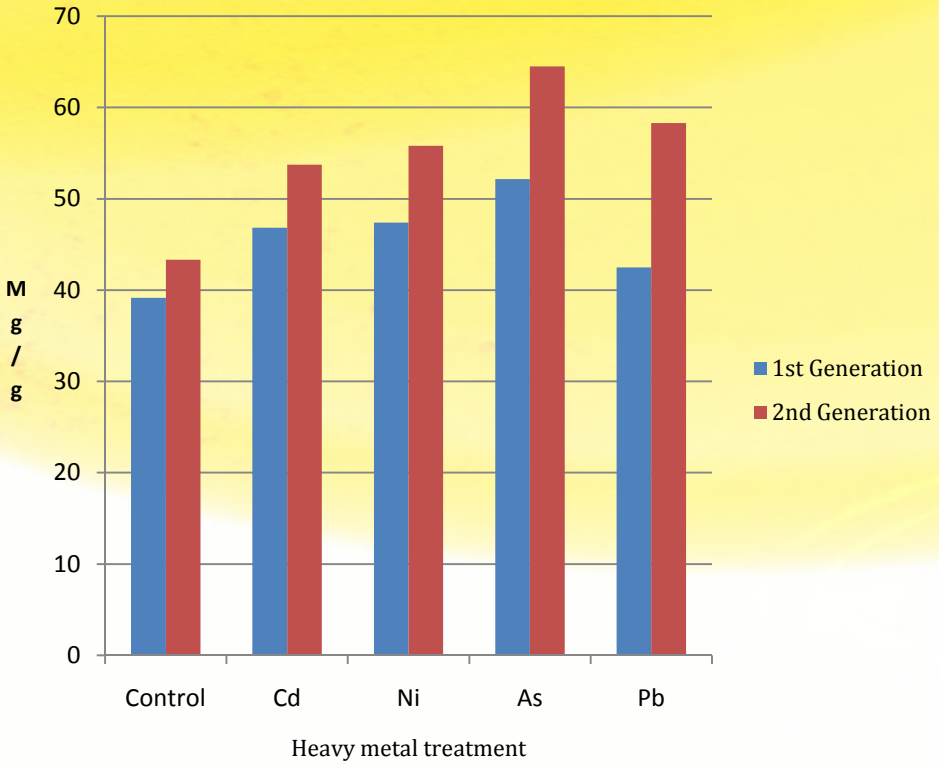
Treatment	Total Reducing Power		FRAP	
	1st Generation	2ndGeneration	1st Generation	2ndGeneration
control	31.4	21.5	39.15	43.33
Cd	47.5 (51.3)	31.8(47.9)	46.8 (19.6)	53.7 (24.2)
Ni	46.8 (49.0)	38.7 (80.0)	47.4 (20.8)	55.8 (28.7)
As	50.5 (60.3)	34.4 (60.0)	52.15 (33.2)	64.5 (48.9)
Pb	49.8 (58.1)	36.7(70.6)	42.5 (8.55)	58.3 (34.5)

Graph showing values in mg/g(gram equivalent to ascorbic acid)

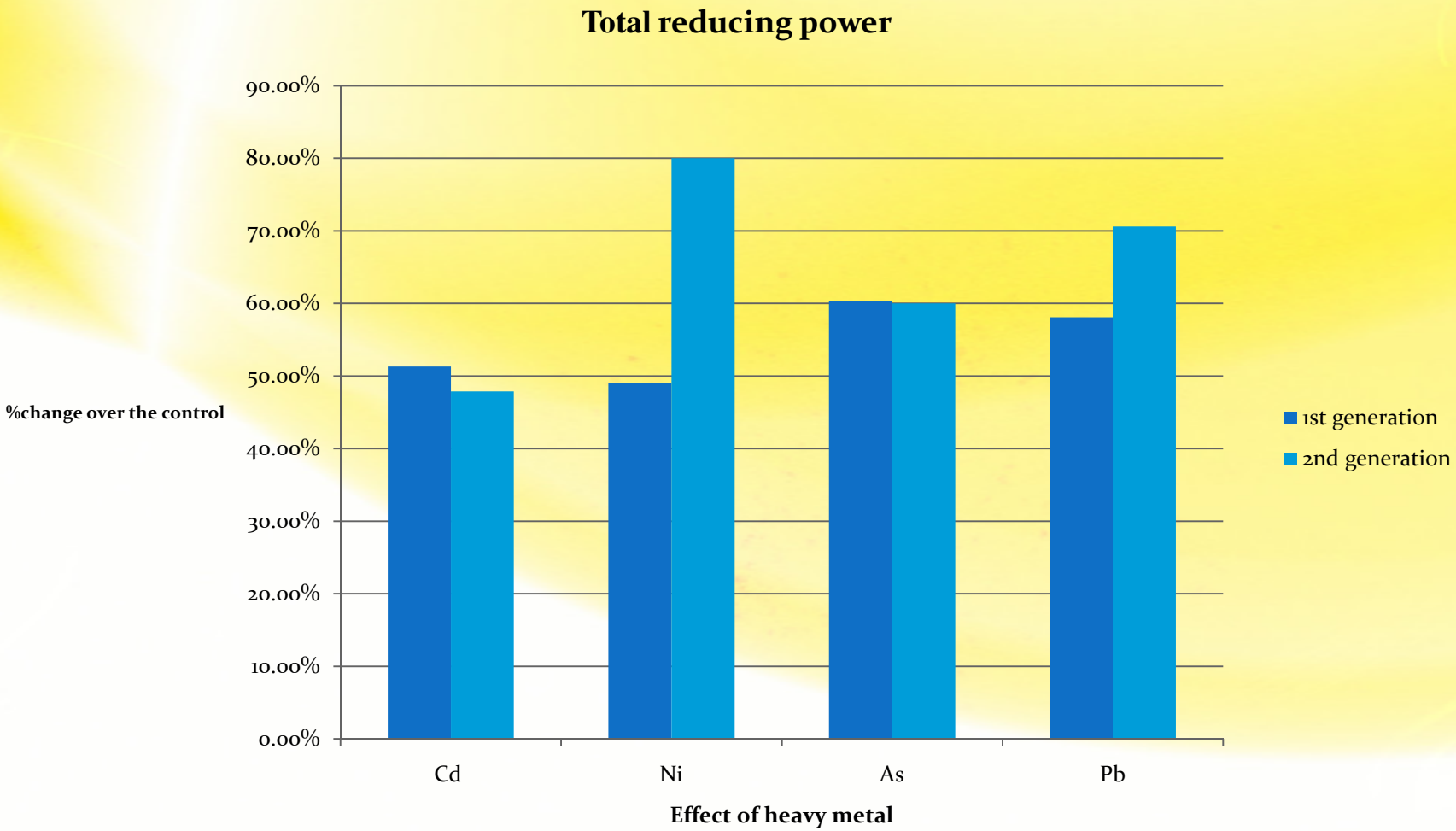
Total reducing assay



FRAP assay



Graph showing change over the control



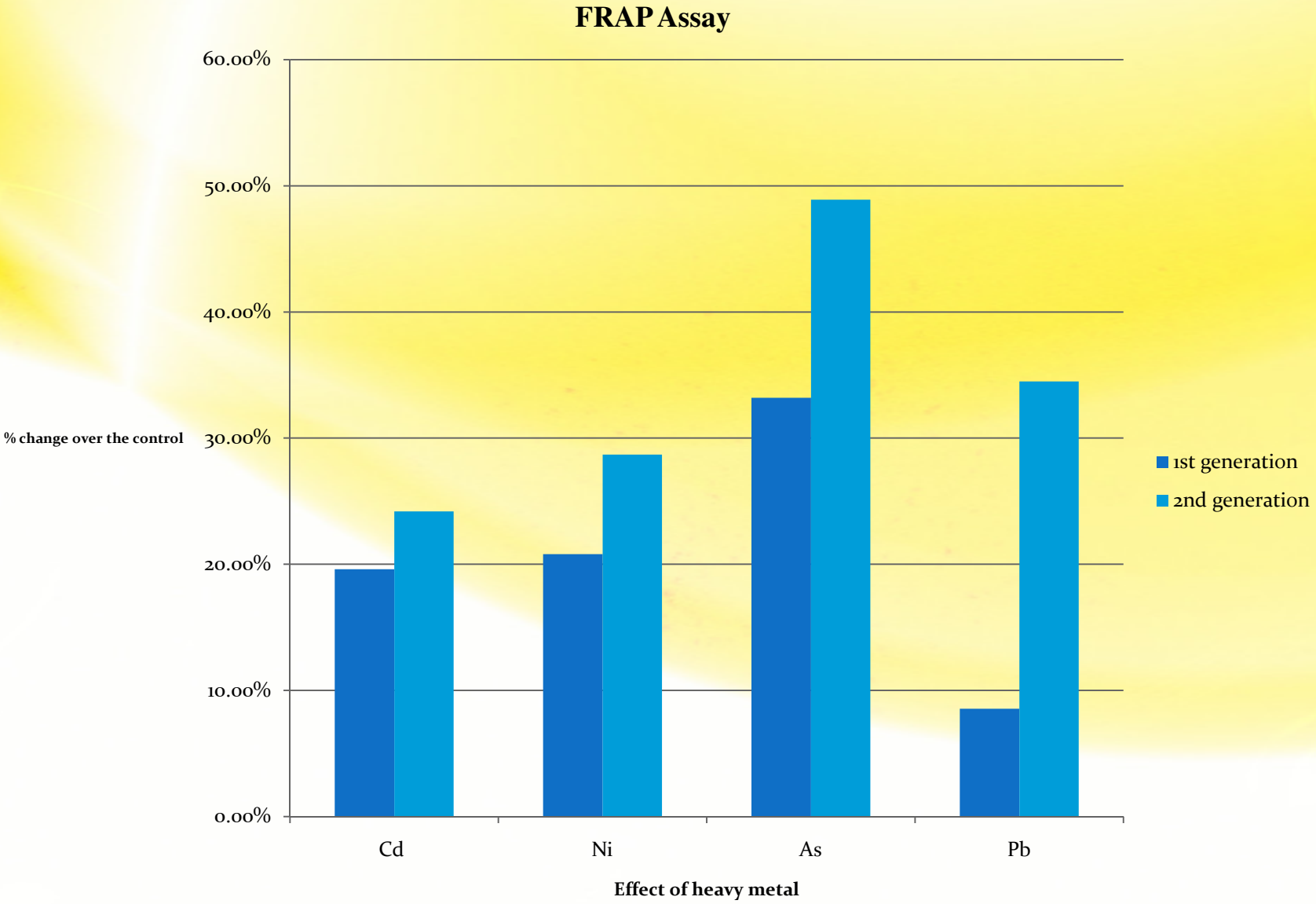
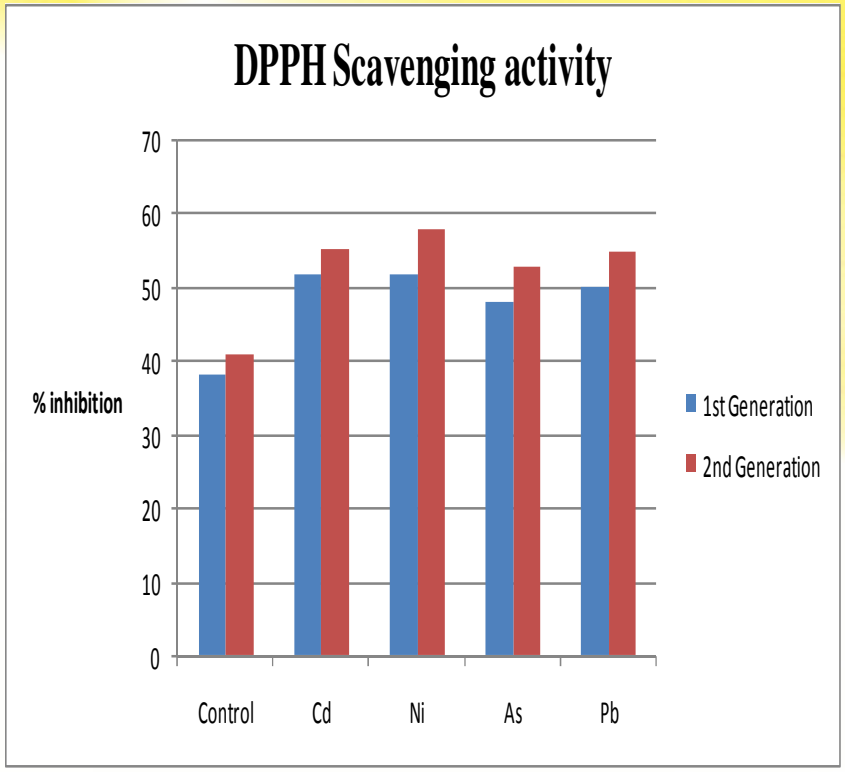
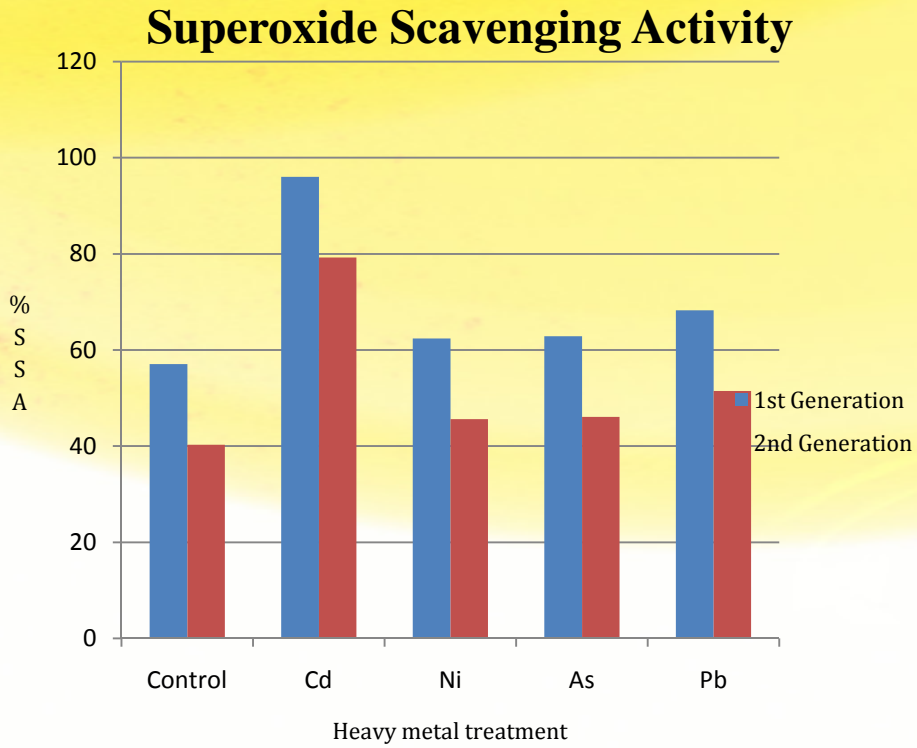
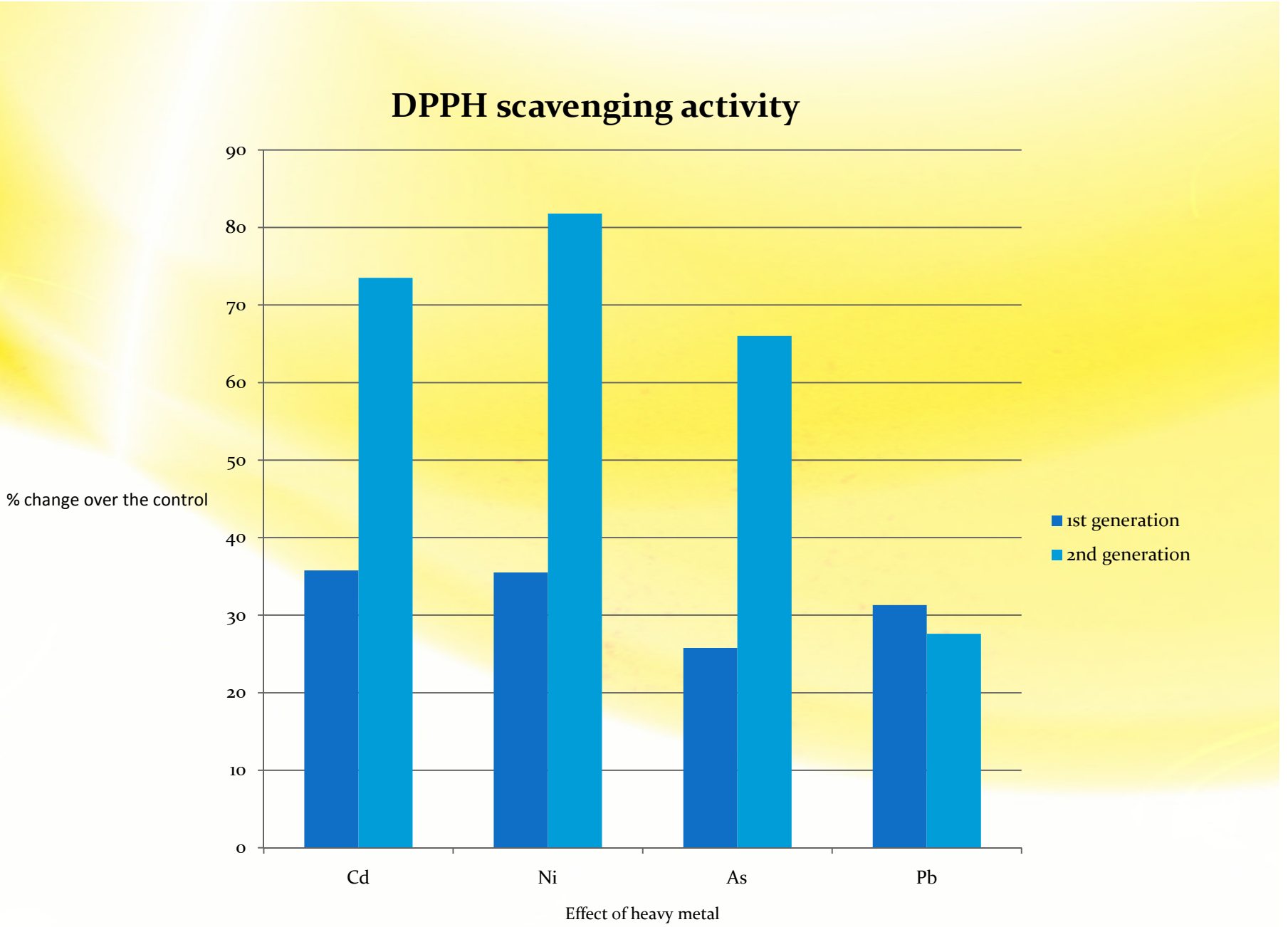

FRAP Assay
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
% change over the control
Cd
Ni
As
Pb
Effect of heavy metal
1st generation
2nd generation

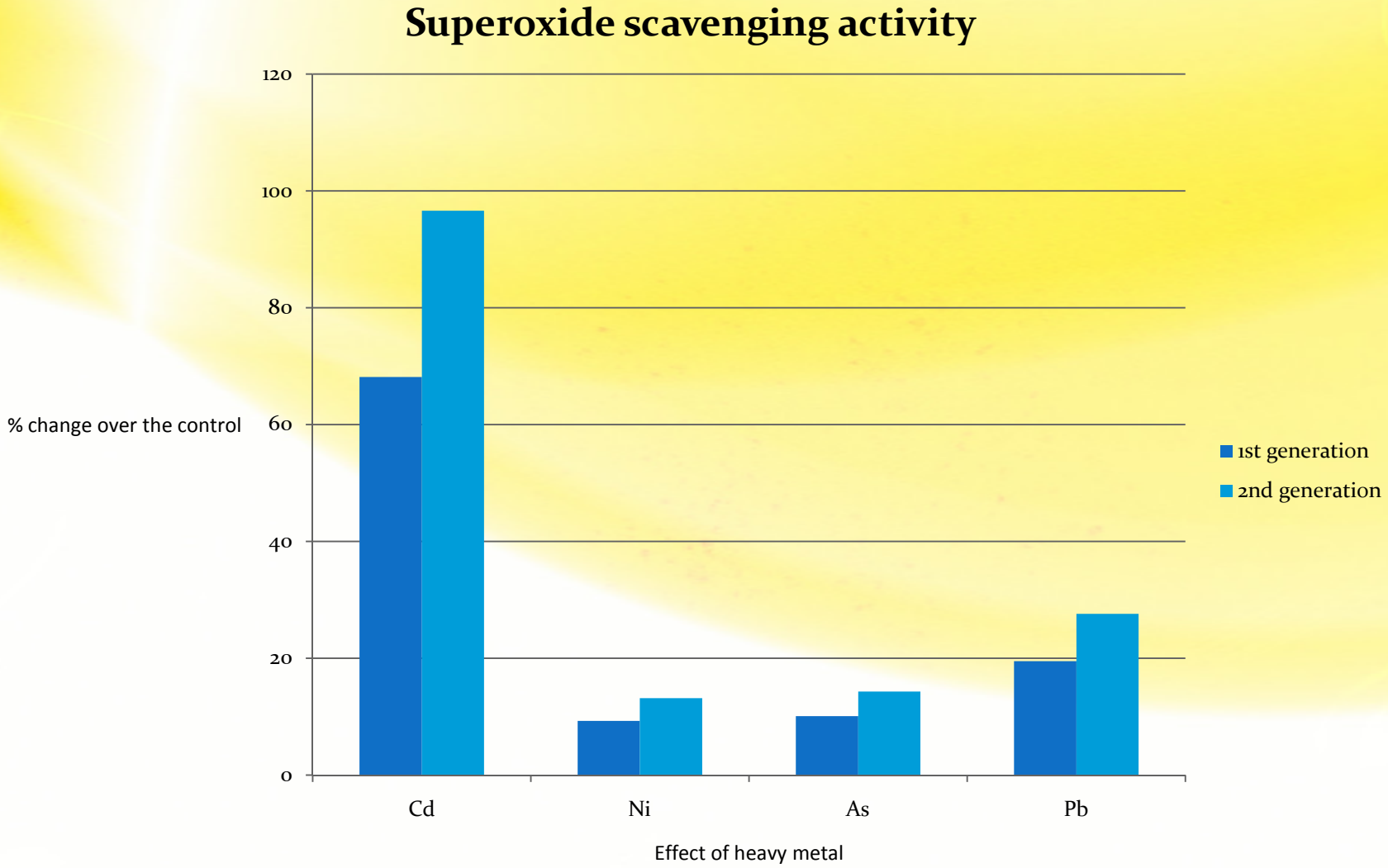
Table 3:-**values in parenthesis represent change over the control*

Treatment	DPPH Scavenging potential (% inhibition)		Superoxide scavenging % inhibition	
	1st Generation	2ndGeneration	1st Generation	2ndGeneration
Control	38.0	40.8	57.1	40.3
Cd	51.6 (35.8)	55.2 (73.5)	96.02 (68.2)	79.2 (96.6)
Ni	51.5 (35.5)	57.8 (81.8)	62.39 (9.3)	45.6 (13.2)
As	47.8 (25.8)	52.8 (66.03)	62.87 (10.1)	46.1 (14.4)
Pb	49.9 (31.3)	54.8 (72.3)	68.24 (19.5)	51.4 (27.6)


DPPH Scavenging activity
% inhibition
70
60
50
40
30
20
10
0
Control
Cd
Ni
As
Pb
1st Generation
2nd Generation


Superoxide Scavenging Activity
% S S A
120
100
80
60
40
20
0
Control
Cd
Ni
As
Pb
Heavy metal treatment
1st Generation
2nd Generation


DPPH scavenging activity
% change over the control
90
80
70
60
50
40
30
20
10
0
Cd
Ni
As
Pb
Effect of heavy metal
1st generation
2nd generation


Superoxide scavenging activity
% change over the control
120
100
80
60
40
20
0
Cd
Ni
As
Pb
Effect of heavy metal
1st generation
2nd generation

Conclusion

- *Mentha spicata* plants test sample treated with different HM ions like Cd, Ni, Pb, As reveals that **it contains antioxidants.**
- The antioxidant activities of the plant sample are due to the presence of **phenolic** compounds containing the hydroxyl group that confers the hydrogen donating ability.
- The strong correlation observed in the present study between antioxidant activity, phenolics, and flavonoid content of different heavy metal on plant suggests a possible use of their parts in making the active ingredients of antioxidant supplement after removing their toxic ingredients.

- The reducing properties are generally associated with the presence of different **reductones.** The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The reductive power of different fractions may be the reason for their antioxidant activity.

Thank you !!

Very much