



A RESEARCH ON IDENTIFICATION OF HEAVY METALS IN A SAMPLE OF ROAD SIDE PLANTS

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ABSTRACT

Background: Metal mining and waste discharge lead to regional heavy metal contamination and attract major concern because of the potential risk to local resident. In recent year, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals.

Main body of abstract- The majority of the heavy metals are toxic to the living organisms and even those considered as essential can be toxic if present in excess. This research was conducted to determine heavy metals in a sample of roadside plants from different locations by using spectrophotometer and to assess their pollution level and also highlights the poisonous impact of heavy metals like- Lead, Chromium, arsenic, zinc, cadmium, copper etc. on soil Human health by using various literature surveys. However, research was based on different methodology including sample collection, size reduction, sample digestion, wet acid digestion using nitric acid, dry ash method, and analysis of sample using spectroscopy.

Short conclusion- With the help of various identification test for heavy metals we Examine heavy metal by using UV spectrophotometer to regulate, measure, and process data for the purpose of identification of heavy metals in a sample of road side plants. By executing successive dilutions of the solutions obtained

after performing digestion of sample being examined with spectral peak reports for serial dilutions of 1µg/ml,2µg/ml,3µg/ml,4µg/ml, are each recorded using a spectrophotometer (Spectrum peak pick report).

Keywords: Heavy metals: Literature surveys: Methodology: Identification test: UV spectrophotometer

1. INTRODUCTION

The appearance of any unwanted foreign substance in something is referred to as "pollution." Pollutants are the name given to these unwanted alien chemicals. In addition to degrading the quality of the air, water, and soil, they also contribute to certain very harmful environmental circumstances like global warming, ozone depletion, and glacier melting. From single-celled microbes to enormous mammals like blue whales, all living creatures rely on the resources that nature provides in order to survive. All life forms are threatened when these resources are contaminated. The development of technology and the industrial age have imprisoned and alienated humans from their natural environment. Both the scope of urbanization and the destruction of nature have grown. According to statistics, there are currently less than 10 billion trees on the planet per year, pesticides and other dangerous chemicals have been discovered in the Antarctic ice sheet, and the Great Pacific Garbage Patch is a collection of microscopic plastic debris in the centre of the Pacific Ocean. According to the US AQI

survey from 2021, India is the fifth most air-polluted nation in the world. The cause is that industry accounted for more than 50% of all pollution, with cars coming in second with 27%, followed by agricultural burning at 17%, and residential cooking at 7% [14]. Each year, millions of people worldwide lose their lives as a result of pollution. Burning fuels to power cars and trucks results in the production of carbon monoxide, which can be deadly if present in large quantities. Nitric oxide (NO) and nitrogen dioxide (NO₂) are the most significant phytotoxic pollutants connected to vehicle transportation in exhaust emissions. In recent years, heavy metal pollution from autos has received a lot of attention. Numerous contaminants found in exhaust gases can harm plants when they are present in high concentrations, according to prior studies [7-11]. However, a large portion of this study has focused only on the individual elements of exhaust emissions, and there is very little data on the effects of the specific combination of contaminants that is unique to metropolitan environments. Large amounts of heavy metals are

"received" by roadside soils from a number of sources, such as car emissions, coal-burning waste, and other activities [10, 11]. Traffic from cars contaminates the roadside. Fuels, the walls of gasoline tanks, engines, and other parts of vehicles, as well as tyres, brake pads, and road surface materials, all contain heavy metals [12, 13].

1.1. Heavy metals

A metallic element with a density of 4-5 g/cm³ is referred to as a heavy metal [1, 2]. The poisonous heavy metals lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), and copper are frequently encountered (Cu). It has been stated that several heavy metals, such as Fe, Zn, Ca, and Mg, are important to human biology, and it has been advised to consume a daily suggested amount of these substances. [2] Others, however, including As, Cd, Pb, and methylation forms of mercury, have been reported to have no recognized biological significance in human physiology and biochemistry and to be dangerous even at extremely low quantities [2]. A very low Pb concentration may prevent some essential plant processes like photosynthesis, mitosis, and water absorption with toxic symptoms like dark green leaves, wilting of older leaves, stunted foliage, and brown short leaves and brown short roots [3]. High Pb

levels in soils may also reduce soil productivity. Given the potential effects on the food chain, the uptake of metals by plants from soil at high quantities may pose a serious health danger. As heavy metals are not biodegradable, their uptake by plants and subsequent accumulation along the food chain pose a potential harm to human health [4]. Consuming foods contaminated with heavy metals can drastically deplete the body of important minerals, which in turn lowers immune system resistance. Growth retardation, malnutrition-related impairments, and a high incidence of upper gastrointestinal cancer are all common [5].

1.2 Plants and heavy metals

There are complicated interactions between heavy metals and plants. Because plants need to have the micronutrients for vital biological processes, heavy metals are needed nutrients in trace concentrations for healthy growth [6]. Because of the fact that they have no recognized physiological functions in plants, certain of these heavy metals, like As, Cd, Hg, Pb, or Se, are not required for plant development. Because of the fact that they have no recognized physiological functions in plants, certain of these heavy metals, like As, Cd, Hg, Pb, or Se, are not required for plant development. Elements, including such Co, Cu, Fe, Mn,

Mo, Ni, and Zn, are essential for the normal development and metabolism of plants, but when their contents are higher than ideal levels, they can easily cause poisoning [7].

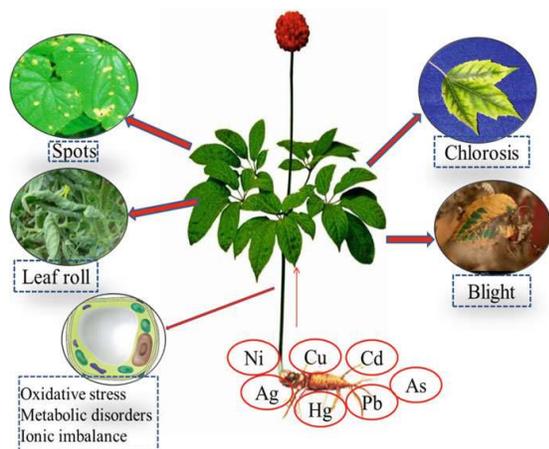


Figure 1: Heavy metals' impact on plants

Heavy metal levels in soil, both high and low, can have an impact on crop growth because they interfere with plant metabolism, slowing down processes like photosynthesis and respiration as well as causing organelle degeneration and even plant death [8]. Heavy metals have the potential to be toxic and phototoxic to plants, which can lead to chlorosis, weak plant growth, depressed yields, and may even be accompanied by reduced nutrient uptake, abnormalities in plant metabolism, and a decreased capacity for leguminous plants to fixate molecular nitrogen. Temperature, moisture, organic matter, pH, and the availability of nutrients are only a few of the many variables that influence the absorption and accumulation of heavy

metals in plant tissue. Temperature, moisture, organic matter, pH, and the availability of nutrients are only a few of the many variables that influence the absorption and accumulation of heavy metals in plant tissue [9, 10].

1.3 The Impact of Heavy Metals on Soil

In the industrialized world, heavy metal poisoning of the soil is a major concern [10, 11]. One of the main factors contributing to soil pollution is thought to be heavy metals. They have harmful effects on the soil biota by interfering with important microbial functions and reducing the quantity and activity of soil microorganisms. The microbiological features of soil, such as respiration rate and enzyme activity, are negatively impacted by an increase in metal concentration and appear to be excellent markers of soil pollution. A small alteration in the soil microbial profile was seen in lead-contaminated soil (Pb).

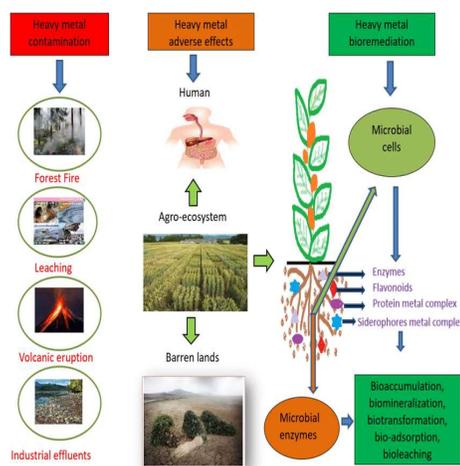


Figure 2: Sources of heavy metals in the environment

Metal pollutants have been released in agricultural areas over the past few decades as a result of growing urbanization, industrialization, and careless and flawed farming methods. Heavy metal contamination in agricultural soils can cause functional problems with the soil, slow plant growth, and even be harmful to human health by contaminating the food supply. Heavy metal distribution in soils is related to both anthropogenic and natural sources. The main examples are volcanic eruptions, geological disintegration of parent rock, and other natural sources. Increased levels of inorganic pollutants in soils are a result of anthropogenic inputs such as the widespread use of agrochemicals (inorganic and organic) fertilizers, pesticides, waste water irrigation, sewage sludge supplementation, increased atmospheric depositions by industrial units, and the burning of fossil fuels.

1.4 Heavy Metals and the Impact on Human Health

Massive increases in human exposure to heavy metals have been brought about by the industrial activities of the previous century. The most frequent heavy metals to cause human poisonings have been mercury, lead, chromium, cadmium, and arsenic. Plant uptake of heavy metals from soils at

high concentrations may constitute a serious health concern to people due to the effects on the food chain.

An important source of human exposure to these toxins is through the cultivation of food crops polluted with heavy metals in heavy metal-affected soil. When heavy metals build up in soft tissues without being digested by the organism, they become poisonous [10,12]. Humans who consume hazardous metals at chronic levels experience negative effects, which don't become apparent for several years after exposure [10–11].

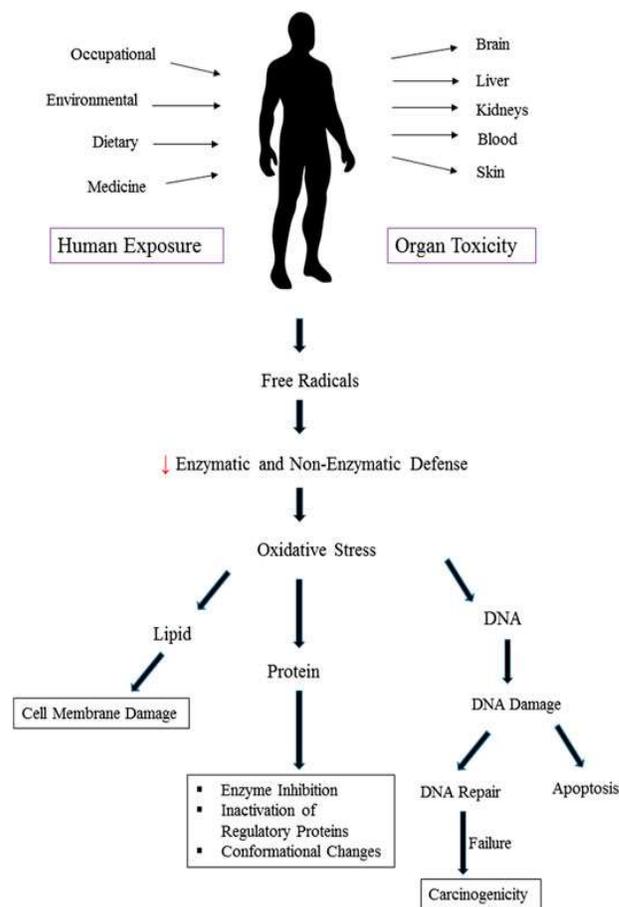


Figure 3: Heavy metal toxicity in humans

A well-known heavy metal toxin having a specific gravity 8.65 times higher than water is cadmium (Cd). The liver, placenta, kidneys, lungs, brain, and bones have been named as the organs most susceptible to Cd poisoning [10-12].

Particularly when given orally, zinc (zn) is thought to be largely non-toxic. Excessive amounts, however, might lead to system malfunctions that hinder growth and reproduction. Clinical symptoms of zinc toxicities have been described as anemia, liver failure, renal failure, icterus (yellow mucus membrane), vomiting, diarrhea, bloody urine, and icterus. In humans, excessive copper (Cu) consumption can result in severe mucosal irritation, gastro intestinal discomfort, capillary, hepatic, and renal damage, as well as irritation of the central nervous system, which can cause depression and possibly necrotic changes in the functions of the liver and kidneys.

Even in low concentrations, lead (Pb) poses a concern. The production of lead-acid batteries for automobiles accounts for more than three-quarters of all lead usage worldwide. Young children are more susceptible to the toxic effects of lead and can have severe, long-lasting health problems, especially on the brain and nervous system development [13].

2. LITERATURE SURVEYS

Mahdi Balali-Mood *et al.*, (2021) indicated that human exposure to heavy metals has significantly increased as a result of the century's industrial activity. The most frequent heavy metals to cause human poisonings have been mercury, lead, chromium, cadmium, and arsenic. Apoptosis, differentiation, growth, proliferation, and other biological functions are all affected by heavy metals. Mercury preferentially piles up in the kidneys and negatively affects them, particularly the proximal tubules. Lead inhibits ferro chelatase and ALAD, which reduces heme production and causes anemia.

Rahul S. Salunke *et al.*, (2021) revealed that heavy metal contamination of drinkable water is a severe environmental issue that directly impacts the sustainability of the entire planet. Heavy metals like arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg) are non-biodegradable and hence highly poisonous and detrimental to both human health and Electrochemical sensors made from nonmaterial and nanostructures have significantly improved the performance of sensing devices in terms of sensitivity.

Figure 4: shows various heavy metal types, their effects on human health, and the permitted limitations

Pollutents	Major source	Effect of human health	Permissible levels(mg/L)	References
Cadmium (Cd)	Welding, electroplating, welding, pesticides, fertilizer	Renal dysfunction, lung disease, lung cancer, bone defects, kidney damage, bone marrow	0.06	[13]
Zinc (Zn)	Refineries, brass manufacture, metal plating	Damage to nervous system, irritability	15	[14]
Copper (Cu)	Mining, pesticide production, chemical industry	Anemia, liver and kidney damage, stomach irritation	0.1	[15]
Lead (Pb)	Paint, pesticides, smoking, automobile emulsion, mining, burning of coal	Mental retardation in children, development delay, fatal infant encephalopathy, chronic damage to nervous system, liver, kidney damage	0.1	[16]

Kilaru Harsha V. et al., (2019) stated that the unplanned urbanization, unbridled population increase, human activities, rapid industrialization, and inadequate use of natural water resources have all had a negative impact on water quality. A set of metals and metalloids having an atomic density greater than 4000 kg/m³ are referred to as heavy metals. Even at very low concentrations, the heavy metals are poisonous by nature and can seriously harm both humans and animals. Aquifer systems are exposed to these heavy metals through industrial discharges and agricultural runoff. With varying degrees of success, there are various approaches available to remove heavy metals from the aquatic environment. The generation of secondary waste, high maintenance, operating costs etc. is all factors that are contributing to a significant portion of these treatment methods' deficiencies. To eliminate heavy metals from the aquatic environment and protect

the environment, it is crucial to create effective, eco-friendly friendly and commercially sustainable treatment technologies.

Paul B T. et al., (2012) Environmental contamination by these metals has recently been connected to increasing ecological and global public health concerns. Additionally, due to an exponential rise in their use in numerous industrial, agricultural, residential, and technical applications, human exposure has increased significantly. According to reports, geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources are all sources of heavy metals in the environment. The necessary heavy metals influence the biochemistry and physiology of plants and animals. They serve significant roles in several oxidation-reduction reactions and are significant components of several essential enzymes.

Bieby Voijant T et al., (2011) The ability of plants to absorb contaminants and then

release them into the atmosphere is termed as Phytovolatilization. This approach is utilized to manage with pollutants in soils, sediments, sludges, and groundwater that are complex organic compounds that decompose into simpler ones. Through the rhizofiltration (Latin for "root process"), which is the adsorption, precipitation, or absorption of pollutants into the roots, plant roots take up metal contaminants and/or excess nutrients from growing substrates.

Plant roots are able to solubilize and absorb micronutrients from extremely low levels in the soil, even from practically insoluble precipitates, with the help of plant-produced chelating agents, plant-induced pH changes, and redox processes. In order to transport and store micronutrients, plants have also developed extremely specialized systems

3. MATERIAL AND METHODS

Sample Collection: For this investigation, roadside plants were used (within 30 m of the roadside). In addition, locals consume certain species as food and use them medicinally. After the collection, it was placed in a distinct polyethylene bag for transit to the lab. Between April and May, during the dry season, samples were collected from root, stem, and leaf separation, the plants were rinsed with deionized water. It works well as a cleaning

agent because of its easiness in eliminating "gunk," [15].

Drying: The following techniques were used to dry the leaves: Solar drying (leaves were dried into trays under direct sunlight at temperatures). Air drying in the shade at 25 °C is recommended. Drying for three hours at 60 °C in a hot air oven.

Size Reduction (Milling and Grinding): The process of mechanically breaking down materials into microscopic granules is termed as material grinding. Using an agate grinder/mortar and pestle and a homogenizer, the dry sample was first ground up. The process of homogenization involves reducing the particle sizes of pharmaceutical products while subjecting them to high pressures, shear, turbulence, acceleration, and impact.

Sample Digestion: In most cases, a microwave digesting method is employed. By dissolving a solid sample with reagents like potent acids, alkalis, or enzymes, digestion is the process of transforming the solid sample into a liquid state. Generally, the sample and reagent mixture is heated to boiling, considerably accelerating the dissolution process [15].

Wet acid digestion using nitric: Weigh the sample, which is then poured into a test tube with a volume of 20–30 mg. The test tube

was heated to 100 °C on a heating plate after adding 1 mL of concentrated HNO₃. The digestive process went conducted for an hour with a glass marble placed on top of each tube. The solution was added into a graduated polypropylene tube after cooling to room temperature, and deionized water was used to dilute it to 10 ml [16] *et al.*

Dry ash method: A porcelain crucible was loaded with a weighted 20 mg of sample material for dry digestion. The sample was heated to its highest altitude on a hot plate for 90 minutes. The bowls were then inserted into a furnace, where the temperature was gradually raised over the period of an hour to 500 °C. An ash residue that is white or light grey has been generated after 4 hours of ashing the sample. 1 mL of HNO₃ was used to dissolve the remainder. The solution is then poured into a polypropylene tube and diluted with deionized water to a volume of 10 mL [16].

Analysis Of Sample Using Spectroscopy

One of the most widely used analytical methods is ultraviolet-visible (UV-Vis) spectroscopy since it can identify almost all molecules and is very adaptable. By transmitting UV-Visible light through a sample, UV- Visible spectroscopy generates an absorbance spectrum that demonstrates a compound's absorbance at various

wavelengths. The chemical makeup of the molecule determines the quantity of absorbance at any wavelength [17].

By comparing the absorbance spectra of two compounds, UV-Vis can be used to confirm the identity of a compound or to identify functional groups in a qualitative way. As the analyte concentration and absorbance are connected by Beer's Law, it can also be employed quantitatively.

Water analysis, DNA or protein quantification, as well as a variety of chromatography detectors, all use UV-Visible spectroscopy. By taking numerous UV-Visible measurements across time, UV-Visible spectroscopy can also be used to study the kinetics of chemical reactions. Spectrophotometers are typically used to measure UV-Visible. Because it can identify a wide variety of substances, UV-Visible is also a very useful detector for other analytical procedures like chromatography.

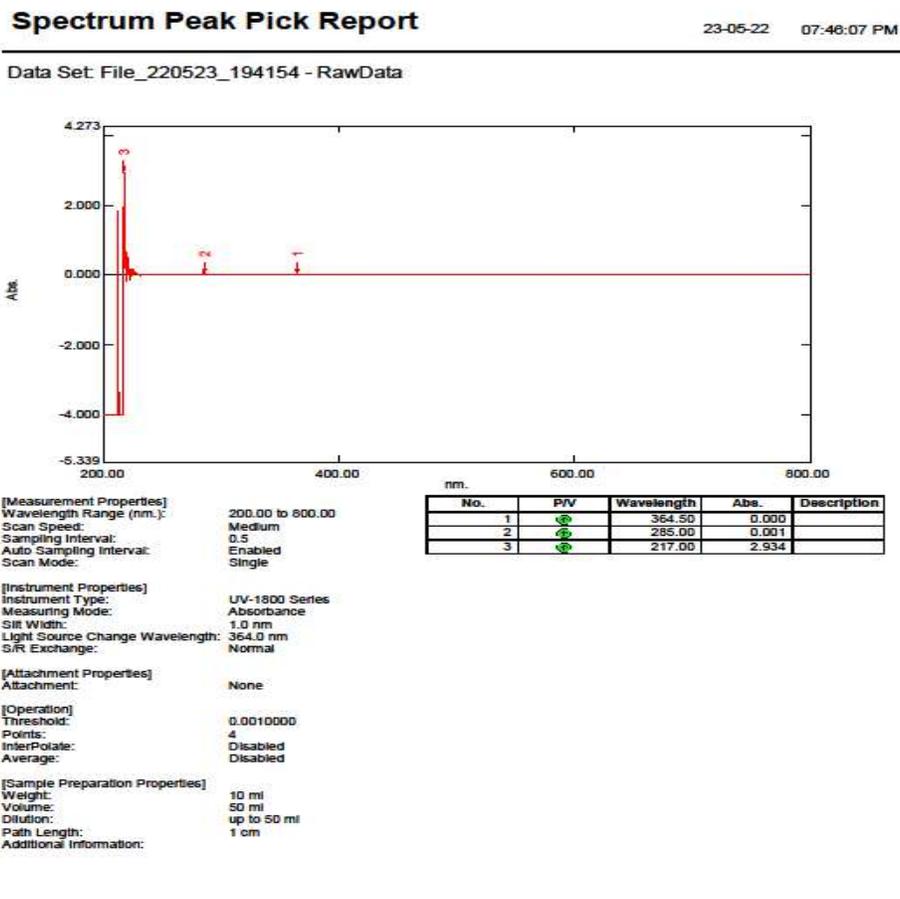
Other spectroscopic approaches, including fluorescence, possess higher sensitivity but are less often used since few molecules are fluorescent. The sensitivity of UV-Visible is comparable to that of other absorbance measurements [17].

4. RESULT AND DISCUSSION

Various procedural approaches and their substitute methodologies were learned to

carry out detection, identification, and UV-visible spectrophotometer housed in the institute's instrumentation room was utilized to illustrate the spectrophotometer's operation, applications, and features. It was learned how heavy metals affect human health and disease, as well as how their toxicity affects the environment and how it changes over time, and the steps that may be taken to prevent it. The UV-spectrophotometer was used to analyze the heavy metals. By executing successive

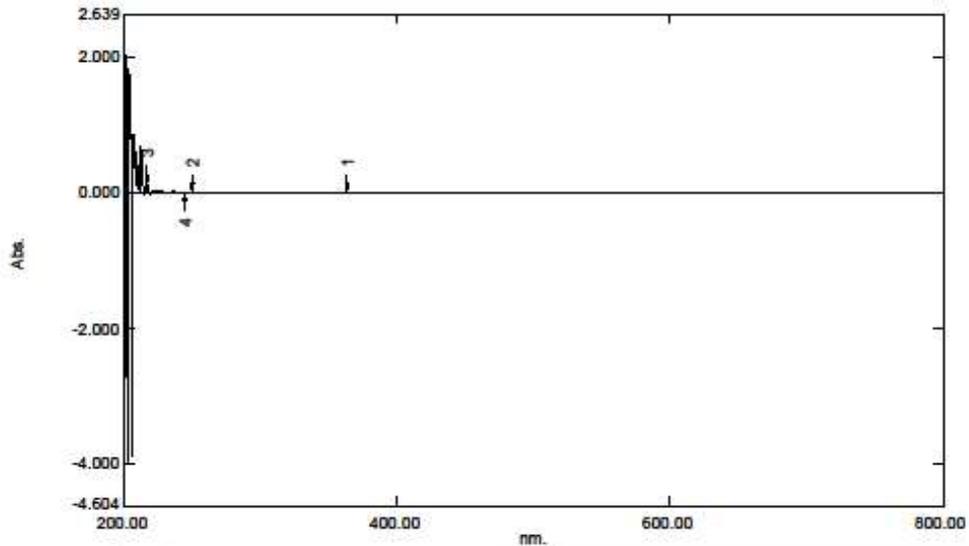
identification tests for heavy metals. The dilutions of the solution obtained after performing digestion on the sample being examined, the data below was demonstrated. Although detection and identification were learned, they could not be applied since they require for highly developed IR spectroscopy. The spectral peak pick reports for serial dilutions of 1ug/ml, 2ug/ml, 3ug/ml, and 4ug/ml are each recorded using a spectrophotometer.



Spectrum Peak Pick Report

23-05-22 07:39:15 PM

Data Set: File_220523_193423 - RawData



[Measurement Properties]
 Wavelength Range (nm.): 200.00 to 600.00
 Scan Speed: Medium
 Sampling Interval: 0.5
 Auto Sampling Interval: Enabled
 Scan Mode: Single

No.	P/V	Wavelength	Abs.	Description
1	⊕	363.00	0.001	
2	⊕	250.00	0.000	
3	⊕	217.00	0.124	
4	⊖	244.00	-0.002	

[Instrument Properties]
 Instrument Type: UV-1800 Series
 Measuring Mode: Absorbance
 Slit Width: 1.0 nm
 Light Source Change Wavelength: 364.0 nm
 S/R Exchange: Normal

[Attachment Properties]
 Attachment: None

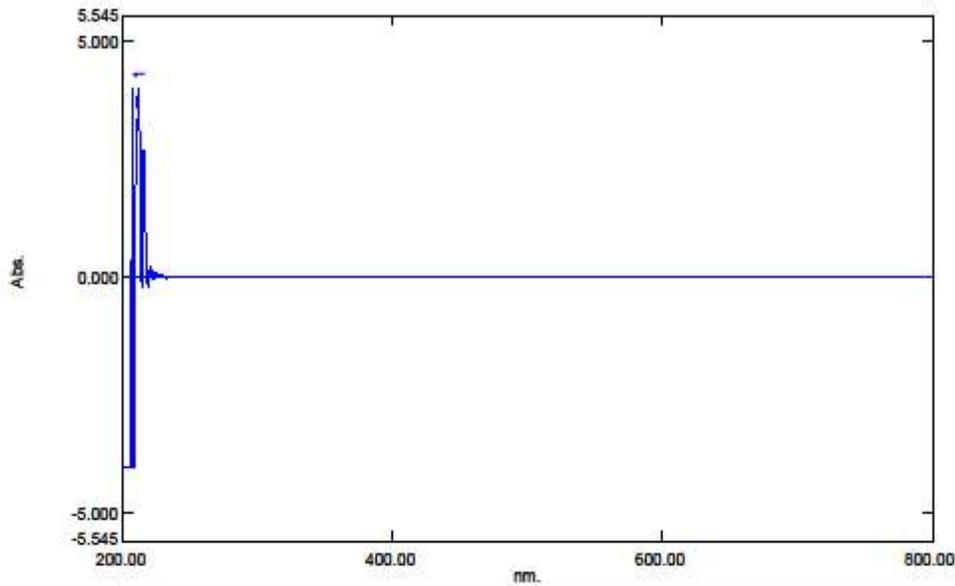
[Operation]
 Threshold: 0.0010000
 Points: 4
 Interpolate: Disabled
 Average: Disabled

[Sample Preparation Properties]
 Weight: 10 ml
 Volume: 50 ml
 Dilution: up to 50 ml
 Path Length: 1 cm
 Additional Information:

Spectrum Peak Pick Report

23-05-22 07:54:29 PM

Data Set: File_220523_194948 - RawData



[Measurement Properties]
 Wavelength Range (nm.): 200.00 to 800.00
 Scan Speed: Medium
 Sampling Interval: 0.5
 Auto Sampling Interval: Enabled
 Scan Mode: Single

No.	P/V	Wavelength	Abs.	Description
1		211.50	3.613	

[Instrument Properties]
 Instrument Type: UV-1800 Series
 Measuring Mode: Absorbance
 Slit Width: 1.0 nm
 Light Source Change Wavelength: 364.0 nm
 S/R Exchange: Normal

[Attachment Properties]
 Attachment: None

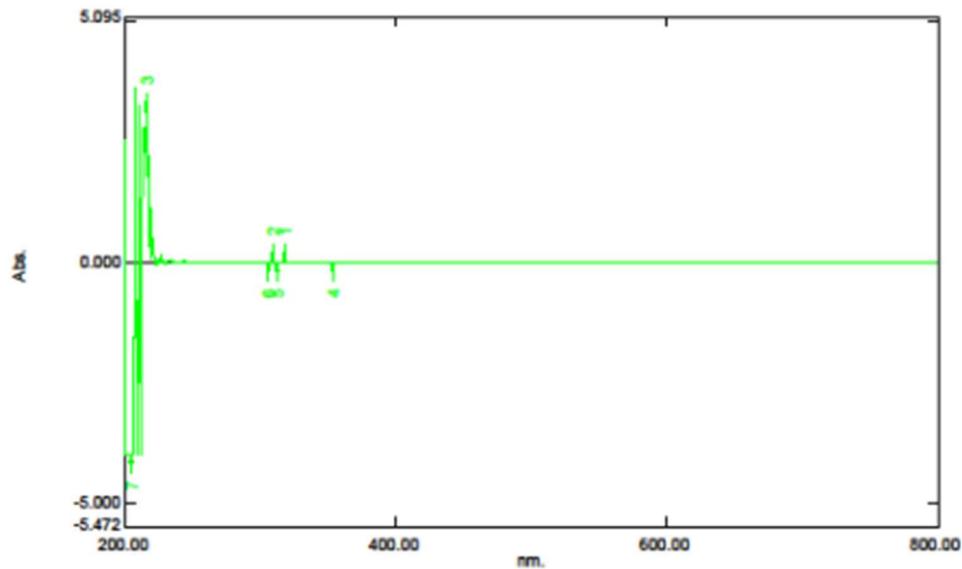
[Operation]
 Threshold: 0.0010000
 Points: 4
 InterPolate: Disabled
 Average: Disabled

[Sample Preparation Properties]
 Weight: 10 ml
 Volume: 50 ml
 Dilution: up to 50 ml
 Path Length: 1 cm
 Additional Information:

Spectrum Peak Pick Report

23-05-22 08:00:35 PM

Data Set: File_220523_195634 - RawData



[Measurement Properties]
 Wavelength Range (nm.): 200.00 to 800.00
 Scan Speed: Medium
 Sampling Interval: 0.5
 Auto Sampling Interval: Enabled
 Scan Mode: Single

[Instrument Properties]
 Instrument Type: UV-1800 Series
 Measuring Mode: Absorbance
 Slit Width: 1.0 nm
 Light Source Change Wavelength: 364.0 nm
 S/R Exchange: Normal

[Attachment Properties]
 Attachment: None

[Operation]
 Threshold: 0.0010000
 Points: 4
 Interpolate: Disabled
 Average: Disabled

[Sample Preparation Properties]
 Weight: 10 ml
 Volume: 50 ml
 Dilution: up to 50 ml
 Path Length: 1 cm
 Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	●	318.00	0.001	
2	●	308.50	0.001	
3	●	216.00	3.136	
4	●	353.50	-0.001	
5	●	312.00	-0.001	
6	●	305.50	-0.001	
7	●	204.50	-4.000	

5. CONCLUSION

We have examined at the practical features of using a UV spectrophotometer to regulate, measure, and process data for the purpose of identifying heavy metals in a sample of roadside plants. The development of commercially available equipment for precise on-site analysis still faces obstacles (sensor replacement, regular multisensory array recalibration, drift correction) notwithstanding the successful deployment of new methods in the simultaneous detection of contaminants. It is recommended to conduct more research in the direction of creating processes and procedures that are more dependable, robust, and compact. An expanded sensing network might be established with further Internet of Things integration, delivering continuous, real-time data on the environmental health of resources throughout the world. Additionally, the application of computational analysis has produced improved results in sample quantification and classification.

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ABBREVIATIONS

API-Activepharmaceuticalingredient

R&D-Researchanddevelopment

QC-Qualitycontrol

ADL-Analyticaldevelopmentlaboratory

IVIVC-Invivo- invitrocorrelation

Pb-Lead

Cr-Chromium

As-Arsenic

Zn-Zinc

Cd-Cadmium

Hg-mercury

NaOH-Sodiumhydroxide

H₂O₂-Hydrogenperoxide

UV-Ultraviolet

LC-MS/MS-Liquidchromatography-tandemmassspectrometry

DL-Detectionlimit

QL-Quantitationlimit

RSD-Relativestandarddeviation

HPTLC-

Highperformancethinlayerchromatography

GC-Gaschromatography

LC-Liquidchromatography

CAN-Acetonitrile

µm-Micrometer

µg-Microgram

HNO₃-Nitric acid

DNA-Deoxyribonucleic acid

NO₂-Nitrogen Dioxide

AQI-AirQuality Index

Kg/m³-kilogram per cubic meter

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