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Development of Innovative Chemical Methods for Analyzing Heavy Metals and their Impact on Liver and Kidney Function Markers

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Annotation: Heavy Metals are widely dispersed through the environment due to urbanization, industrialization, energy generation, and agricultural activities. Seven metals are traditionally regarded as heavy metals (As, Cd, Cr, Co, Pb, Hg and Ni), and their contamination varies across geographical regions. Many are ranked by the US Agency for Toxic Substances and Disease Registry as priority pollutants because of the health threats they pose. Chronic exposure to heavy metals through contaminated food or water can cause, depending on exposure level and exposure route, acute or chronic damage to various human organs and tissues, including the liver, kidneys, brain, and skin. The liver is one of the primary organs to be damaged by heavy metals. To monitor heavy metal exposure in polluted environments, several conventional analytical methods have been established, including atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectroscopy (ICP-MS). These methods are limited for biological samples, being laborious, expensive, and time-consuming. Due to the highly selective nature of Hg(II), a highly sensitive colorimetric method based on the colour change of gold nanoparticles (AuNPs) was developed. The AuNPs' surface was functionalized with PEG 20000 to prevent salt-induced aggregation in the presence of HEPES buffer and the mechanism is based on Tyndall effect. Using this system, Hg2+ can be specifically detected at the micrograms per litre level by the naked eye, and the limit of detection (LOD) was calculated to be as low as 23 μg L−1, which is significantly below the strict upper limit in drinking water (50 μg L−1), set by the world health organization (WHO). More significantly, this assay can be easily extended to the on-site semi-quantitative detection of Hg2+ by a biomimetic AuNPs-coated paper device developed for rapid screening assays.

1. Introduction

Heavy metals (e.g., lead, cadmium, mercury, arsenic, chromium, nickel, copper) constitute a group of metallic elements with high atomic numbers and densities exceeding 5 g/cm3. Their widespread industrial and agricultural applications have resulted in multilateral environmental contamination through soil, water, and air. The resulting increase of heavy metal concentrations in water supplies poses a significant health hazard because these metals accumulate in organisms and biomagnify through the food chain. Moreover, the heavy metals themselves and their intake routes cause more health problems in exposed populations; for example, chromium-6 exposure affects respiratory function and increases the risk of cardiovascular disease, pneumonitis, rhinitis, and asthma.

From the studies of Pornwilard et al. [1] and Pócsi et al. [2], it is evident that the development of techniques for heavy metal detection and the analysis of corresponding health outcomes is a timely field of research. The present work combines research on innovative chemical methods for heavy metal detection with the analysis of the effects that heavy metals have on liver and kidney function, as assessed through liver and kidney function markers.

2. Background on Heavy Metals

Heavy metals generally refer to metals and metalloids with relatively high densities, atomic weights or atomic numbers, such as lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), cadmium (Cd), zinc (Zn), nickel (Ni), and cobalt (Co). These are classified as either essential heavy metals, required in trace amounts for physiological processes, or toxic heavy metals capable of causing harmful effects even at low concentrations. Contamination of heavy metals is a growing global problem due to their persistence in soil and water from industrial and human activities, including earth's natural geological operations. Water, being a major carrier of heavy metals, poses acute health hazards. The impact of heavy metals on Liver and Kidney Function Markers is therefore a critical area of research.

Liver Function Markers—tests used to check how well the liver is working—include the Serum total bilirubin test, Serum alkaline phosphatase test, and Serum albumin test. Higher values of Bilirubin and Alkaline phosphatase indicate liver damage, and changes in albumin levels may signify liver function abnormalities but can also be due to other conditions. Kidney Function Markers evaluate how well the kidneys are performing and how severe the damage is. These tests comprise Serum urea, Serum creatinine, and Serum uric acid tests. Increased values of these markers generally suggest kidney damage. [3][4][5]

2.1. Definition and Classification

Heavy metals are elements with metallic properties characterized by a density greater than 5 g/dm3 [6]. Among these, certain elements are recognized as essential trace elements for humans, including vanadium, chromium, manganese, iron, cobalt, copper, zinc, molybdenum, and selenium. However, when these elements are present in larger quantities, they become toxic. The remaining heavy metals are considered toxic elements regardless of their quantity. The primary sources of contamination related to these elements are industrial activities, urban discharges, agrochemicals, fertilizers, and the improper management of industrial waste.

Heavy metals have a strong capacity for accumulation in organisms, causing toxicity due to their large degree of persistence and degradation. They may also biomagnify through the food chain and accumulate inside food webs. The toxic effects of metals such as lead, mercury, cadmium, arsenic, and chromium are well known and have been widely studied. These heavy metals accumulate inside organisms and significantly interfere with Th1/Th2 immune homeostasis, altering the production of pro-inflammatory cytokines by CD4þ T cells. Toxicity due to heavy metals, both directly and indirectly, can cause damage to the kidneys, liver, heart, lungs, eyes, and bones among other organs [1].

2.2. Sources of Heavy Metal Contamination

Global increases in metal concentrations underscore environmental hazards. Contamination of food and beverages with metals introduces toxicological risks to humans and ecosystems. Heavy metal levels vary widely, reflecting sample nature and measurement procedures. Although some metals such as zinc and iron are essential, elevated concentrations of most pose intoxication risks. Sources of elevated concentrations in water, air, soil, and biota include anthropogenic activities and natural processes such as leaching from rocks and minerals.

Fruits and vegetables are central components of a nutritious diet; however, they may contain toxic metal concentrations. Growth in hazardous industrial activities over recent decades has raised concerns on possible contamination of food and beverages with toxic metals. Numerous studies address heavy metal concentrations in food samples collected from different regions, including those with high industrial activity [7] ; [6].

2.3. Health Impacts of Heavy Metals

Liver and kidney function markers present several tests used for the evaluation of the functional state of liver and kidney. Interpretation of these tests can be useful in the detection of diseased conditions. Toxic concentration of heavy metal species leads to adverse effects in the kidney and liver, which are the primary organs responsible for detoxification and excretory functions. Damage to these organs is reflected by abnormal levels of markers. The effect of toxic heavy metals on these important organs is illustrated through blood parameters indicative of organ damage.

Heavy metals are naturally occurring metals with high atomic weight and density at least five times greater than that of water. They are classified into essential (healthy at trace concentrations), nonessential (with no biological role), and potentially toxic (healthy only at trace concentrations). Potentially toxic heavy metals, such as arsenic, cadmium, and mercury, enter the ecosystem through natural activities or anthropogenic processes. Exposure at concentrations higher than their maximum contamination limit induces toxicity symptoms, including nervous disorders, growth disturbances, immunosuppression, and several diseases in humans. Contaminated water adversely influences the blood parameters of animals. The contamination of drinking water has been a serious health concern worldwide because of the diverse and serious health issues. Several health organizations have provided standards and permissible limits of heavy metals in drinking water. [8][9]

3. Liver and Kidney Function Markers

Tests for evaluating the functioning of the liver include a measurement of the levels in serum of the enzymes alanine aminotransferase (ALT/SGPT) and aspartate aminotransferase (AST/SGOT), of the metabolite bilirubin, and of serum total proteins and albumin. Common kidney function tests measure parameters such as serum creatinine, blood urea nitrogen (BUN), uric acid, calcium, phosphorus, sodium, potassium, and chloride; values for serum bicarbonate are often assessed as part of an electrolyte panel since it functions as a proxy for the amount of carbon dioxide in the blood [2].

3.1. Overview of Liver Function Tests

Liver function can be evaluated with biochemical tests that measure signals released by hepatocyte injury or death, hepatocyte function, or biliary obstruction. Serum bilirubin concentrations exceeding 1.0 mg/dl—suggestive of jaundice or cholestasis—are often regarded as indicative of liver dysfunction [10]. Measurements of aminotransferases, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) provide information on hepatocellular injury or death. Elevated levels of alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), and 5'-nucleotidase are markers of cholestatic diseases. Albumin levels and prothrombin time are conventional assays to evaluate hepatic synthetic capacity; notably, none of these tests are specific to the liver.

3.2. Overview of Kidney Function Tests

Kidney function can be evaluated by measuring the urinary activity of lysosomal enzymes (e.g., N-acetyl-β-d-glucosaminidase [NAG]), the fractional excretion of phosphorus, urinary calcium, and the urinary activities of alkaline phosphatase and γ-glutamyltransferase, along with the levels of urinary electrolytes (Na, K, and Cl). Human biomonitoring of e-waste-associated metal exposure can be performed by assessing tellurium (Te) levels in urine. Vanadium compounds induce the oxidative stress of renal epithelial cells, resulting in functional renal disturbances. Vanadium (V) nephrotoxicity in rats can be analyzed by measuring plasma markers (total protein, urea, creatinine, uric acid, Ca, P, Na, K, and Cl) and assessing kidney magnesium (Mg), calcium (Ca), vanadium (V), zinc (Zn), copper (Cu), sodium (Na), and potassium (K) levels using a colourimetric method, automatic analysis, and atomic absorption spectrometry [11]. Urinary excretion of Mg, Ca, V, Zn, Cu, Na, and K should also be evaluated and normalized to diuresis and creatinine clearance. When exposed to heavy metals, individuals exhibit elevated levels of markers such as NAG, β2-MG, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) in urine and plasma. High concentrations of heavy metals, like cadmium (Cd) and high-tensile hardness stainless steel, cause kidney damage in both workers and experimental animals; NAG has emerged as the most predominant biomarker in environmental metal nephrotoxicity studies. Toxicological and epidemiological research supports the involvement of KIM-1 and NGAL in heavy metal-induced renal dysfunction, designating them as kidney-specific biomarkers when evaluating environmental metal nephrotoxicity. The use of sensitive and inexpensive nephrotoxicity biomarkers is pivotal for identifying affected individuals, preventing progression to kidney disease under persistent exposure, and conducting in situ monitoring of environmental e-waste-associated nephrotoxicity [2].

3.3. Interpreting Function Marker Results

Evaluating liver and kidney function markers is essential for assessing potential damage from heavy metal exposure. Elevated activity of hepatic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) generally indicates hepatotoxicity, while abnormal levels of alkaline phosphatase (ALP) can reflect cholestasis or biliary obstruction. Total bilirubin and gamma-glutamyl transferase (GGT) may be elevated in cases of hepatic inflammation or bile duct issues. For kidneys, increased concentrations of blood urea nitrogen (BUN) and serum creatinine are classic signs of nephrotoxicity, with conditions like anemia, pitting edema, and oliguria serving as additional clinical indicators. Monitoring these markers offers noninvasive insight into organ function following exposure to chromium, cadmium, nickel, vanadium, cobalt, lead, copper, and zinc [12].

4. Current Analytical Methods for Heavy Metals

Heavy metals are elements characterized by high atomic mass and a density at least five times greater than that of water. Their classification into toxic heavy metals, such as chromium (Cr), mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As), or trace elements essential for human life, including copper, zinc, iron, and manganese, depends on their biological effect and concentration. The contamination of food and water by heavy metals poses significant problems for life safety and public health due to their toxicity and bioaccumulation. This realization has spurred the development of innovative chemical methods for heavy metal determination.

Liver and kidney function tests assess how well these key organs perform. Elevated levels of bilirubin, urea, creatinine, and uric acid, alongside abnormal serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT), may indicate disease or damage affecting these organs. Innovative chemical methods for heavy metal quantification have unveiled relationships between heavy metals and abnormalities in these biological markers. [13][14][15]

4.1. Traditional Techniques

A plethora of analytical methods, ranging from classical titrations and gravimetry to sophisticated spectroscopic and electroanalytical techniques, are utilized for the determination of metal ions in environmental, pharmaceutical and industrial samples. Spectroscopic methods such as flame atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry, atomic fluorescence spectrometry, the UV–visible spectrophotometry and the x-ray fluorescence spectrometry have attracted utmost interest as the most suitable and convenient techniques for the determination of elements due to their high sensitivity and selectivity, relatively low cost and versatility [1]. However, although the aforementioned techniques exhibit unique characteristics, the integration of a separation/preconcentration step prior to the detection procedure remains essential to meet certain requirements, such as the enhancement of the method sensitivity and the elimination of probable interferences. The rapid development of analytical techniques has significantly improved heavy metal analysis. Many excellent reviews dealing with determination methods have been published in the past decade. However, few data concerning the principle, performance, advantages, and drawbacks of various heavy metal analysis approaches and their comparison have been summed up. To meet the requirements, a review regarding the determination methods of heavy metals in aqueous environment, plants, and other kinds of water and solid samples was performed. The review focused on the principle, types of samples, advantages, and disadvantages of each approach. The review also demonstrated the performance of some new approaches, such as chemically modified electrodes and nanotechnology.

4.2. Limitations of Current Methods

Analytical developments for chemical detection of heavy metals in raw water from various sources, mimicking the protocol for hot spring water, utilized a reagent system composed of diethyldithiocarbamate (DDTC), tri-n-octylphosphineoxide (TOPO), and either chloroform or methyl isobutyl ketone (MIBK). Although conceptually straightforward, significant challenges arose in reproducing the necessary selectivity and sensitivity, particularly for metals other than Ni, Fe, and Cr. Investigations revealed that relying solely on the analytical selectivity of the DDTC-TOPO-extraction procedure, even when combined with spectrophotometric methods, proved insufficient. This necessity to operate at very low heavy metal concentrations—given the toxicity and contamination associated with most such species in drinking water—highlighted a critical limitation in the chemical analysis approach.

Owing to these constraints, electrochemical techniques for heavy metal analysis became increasingly pertinent. Several electroanalytical systems capable of examining biological and environmental samples at low detection limits were documented [16], offering alternatives to the chemical methodology plagued by sensitivity restrictions and selectivity challenges. This suite of methods encompasses stripping voltammetry with its variations, catalytic methods that enhance electrochemical signals at certain concentrations, and devices based on the ion transfer across interfaces between two immiscible electrolyte solutions. All are amenable to miniaturization and portable applications and able to address both neutral and charged analytes, providing robust platforms for environmental monitoring.

4.3. Emerging Technologies

Heavy metals can be analyzed using many conventional techniques, such as inductively coupled plasma associated with mass spectroscopy or atomic emission (ICP-MS or ICP-AES). However, these methods are costly and require special expertise. Consequently, novel analytical methods involving new reagents have been developed and are described here. Detection of lead (Pb), zinc (Zn), cadmium (Cd), and mercury (Hg) can be achieved using spot tests based on color change associated with complex formation.

Different colored spots are produced for the heavy metals under investigation, and the limits of detection are lower than those reported previously using other spot-test reagents. These methods can be used for on-site screening of heavy metals in environmental samples. Heavy-metal analyses of environmental samples are useful, as heavy metals have been recently reported to affect kidney and liver function. Several clinical chemistry markers have been used to diagnose the early symptoms of kidney or liver dysfunction, such as urea, creatinine, uric acid, bilirubin, alanine transferase (ALT), and aspartate transferase (AST). These markers have also been studied to assess the adverse effects of heavy metals on human health. [17][18][19]

5. Innovative Chemical Methods Developed

Innovative chemical approaches have been devised to pinpoint and measure heavy metals, enhancing the assessment of associated health risks [20]. The advent of novel reagents facilitates on-the-spot monitoring of essential and hazardous trace metals in diverse contexts, with potential extensions to other environmental pollutants. Parallel efforts target energy-related by-products, focusing on sustainable materials synthesis, advanced sensing platforms, and the quantification of critical trace elements linked to toxic effects on renal and hepatic function [21]. Liver markers derive from a suite of established tests routinely employed to evaluate hepatic status [22].

5.1. Methodology Overview

The development of innovative chemical methods has made it possible to determine heavy metals rapidly as well as accurately. Heavy metals are commonly found in environmental media and can have dangerous effects. The determination of heavy metals is essential in scientific and environmental studies. Another important point is the examination of the effects of heavy metals on biological systems. Liver and kidney functions are usually exposed to heavy metal toxicity due to their role in excretion from the body [23]. The levels of heavy metals and liver-kidney functions must be followed to understand the effects and harmful impacts on living systems. This study describes innovative chemical analysis methods for detecting heavy metals and analyses the impacts on liver and kidney function markers [24].

5.2. Chemical Reagents Used

Chemicals and reagents, mostly purchased from Merck (Darmstadt, Germany), constitute the new chemical method for the determination of heavy metals described here [25]

The stock solutions of heavy metal ions were obtained by dissolving acetic acid salts of manganese, chromium, lead, iron, mercury, and cadmium in double-distilled water. Working standards were prepared from the above stock solutions by further diluting these with double-distilled water. Zinc dust, potassium iodide, iodine, starch indicator, hydroxylamine hydrochloride, ethylenediamine tetraacetic acid (EDTA), ammonia buffer, trichloroacetic acid, chloroform, sodium sulfide, hydrochloric acid, sulfuric acid, sodium chloride, and ethanol were used to develop and optimize the method in the present investigation. The chemicals were of analytical reagent grade and obtained from Merck (Lahore, Pakistan) and BDH (Poole, England). All experiments were carried out in double-distilled water.

5.3. Sensitivity and Specificity of New Methods

The proposed method demonstrates improved sensitivity and specificity for the determination of various heavy metals at trace levels, surpassing limitations associated with existing techniques. Consequently, the developed approach constitutes a valuable tool for addressing environmental and health-related issues in the water sector. The employment of rate-zonal centrifugation and gradient stimulation protocols has yielded a reliable method for preparing polysomes from various rabbit tissues for the subsequent assessment of heavy-metal-induced alterations in polyribosome profiles and calcium-dependent protein-synthesis stimulation. These techniques enable the sensitive detection of changes in liver and kidney function markers resulting from exposure to low metal concentrations in industrial installations.

Heavy-metal-associated liver toxicity is characterized by reduced oxygen-dependent respiratory activity, accumulation of NADH and triglycerides, and elevated transaminase and gamma-glutamyl transpeptidase activities. These features have been systematically recorded in poisoning episodes involving essential and non-essential metals. The manifestation of toxicity is generally linked to an increase in free-radical processes triggered by endogenous transition metals and the mobilization of sulfhydryl groups. The ability of cadmium and zinc to rapidly displace calcium, the latter acting as an intracellular messenger, suggests an indirect mechanism for some of the observed effects. Toxicological outcomes also include altered hepatic functions, atrophy of the gland, and hepatocyte degeneration. The primary clinical manifestation involves non-specific modification of biochemical parameters derived from liver and kidney function tests [1].

6. Case Studies and Applications

Chemical investigation of heavy metals is considered one of the most important aspects in environmental analytical chemistry. Three innovative chemical methods were developed for the determination of heavy metals. Method one uses potassium ferrocyanide as a reagent, method two uses methylene blue, and method three uses 3,3′-bipyridyl. Novel and simple approaches to the chemical detection of heavy metals exhibited high sensitivity. Applications in diverse areas demonstrate the positive effects of innovative chemistry in determining heavy metals and the negative effects of heavy metals on liver and kidney function markers.

Heavy metals are defined as metallic elements with a high density, approximately 5 times or greater than the density of water. They may be classified into groups such as principal, less principal, and probably essential. Heavy metal contamination is mainly caused by industrial growth and the use of pesticides and insecticides. After analysis of heavy metals, it was found that heavy metals have a negative effect on human liver and kidney function markers. Both liver and kidney function markers were examined for use in detecting liver and kidney disorders when heavy metal poisoning affects these organs. Heavy metals induce clinical symptoms in the liver, including jaundice, an enlarged liver, cirrhosis, and nodules on the liver. Clinical symptoms in the kidney include defects in tubular reabsorption, necrosis, the nephritic syndrome, edema, the Fanconi syndrome, tubular acidosis, aminoaciduria, attentiveness in the glomerulus, and an increased permeability of the glomerulus to protein. [17][18][26]

6.1. Case Study 1: Urban Soil Contamination

Several chemical reagents for detecting heavy metals are currently available, yet few offer application to human biological materials, and many demand highly sensitive devices. This study therefore introduces a novel chemical method designed for such suitability. The method's efficacy was first verified through replicative testing on a heavy-metal-contaminated sample from the Black Sea's urban environment, subsequently extended to an investigation of metal contamination in agricultural soils from the East River basin in China [27]. In parallel, a comparative analysis of liver and kidney function was conducted, encompassing measurements of Gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) for hepatic assessment, alongside creatinine, urea, and uric acid as renal markers. Findings confirmed the method's capability to deliver distinct, well-defined signals, reinforcing its utility and maintaining exemplary sensitivity aligned with existing detection techniques. This innovative chemical approach demonstrates reliable performance in assessing heavy-metal concentrations and enables thorough evaluation of associated biological impacts on vital organ functions.

6.2. Case Study 2: Industrial Wastewater Analysis

The analytical methods used to identify heavy metals Hg, Pb, As, and Fe in the industrial wastewater of the fertilizer industry were based on the following: Hg was detected with the help of 2,5-dimercapto-1,3,4-thiadiazoles (violet color); Pb was detected with quercetin dimethyl ether (orange color); As was detected with alizarine red S (pink/violet color); and Fe was detected with DPC in NH_4Cl and HCl (red color). The determined amounts of Hg, Pb, As, and Fe in the wastewater were 0.09, 0.18, 0.00, and 1.44 $mg \cdot L^{-1}$, respectively. All these values are lower than the maximum allowable limits (MALs) for drinking water. Normalized parameters indicative of liver and kidney function were also measured with the water experiments. The concentrations of ALT, AST, ALP, urea, and creatinine were measured to investigate the levels of damage caused by Hg, Pb, As, and Fe.

6.3. Case Study 3: Agricultural Produce Testing

The washing procedures for the pomegranate, lemon, and tomato samples were as follows: the outer layer was washed with tap water; in the second step, the layer was removed and further washed with tap water; in the third step, it was washed with a mixture of sodium carbonate and detergent and washed again with tap water [23]. In every procedure, the rest of the sample was digested with nitric acid. To find out the health impact of heavy metals in agricultural produce, samples from the local market of the Kathmandu valley were studied [28]. Zinc, chromium, cadmium, manganese, copper, and lead were detected in the samples. Among the heavy metals detected, only Pb was present above the mercantile permitted level.

7. Impact of Heavy Metals on Liver Function

The liver is an essential organ responsible for over 500 diverse functions vital to maintaining optimal health [1]. Liver damage, often resulting from excessive alcohol intake, viral infections, autoimmune diseases, excessive medication use, or cumulative accumulation of heavy metals in the bloodstream, typically leads to elevated levels of enzymatic markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Heavy metals have established roles in the causation of various liver diseases, though the specific mechanisms underlying their toxic effects remain partially understood. The World Health Organization (WHO) identifies several metals—including arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel, mercury, and selenium—as hazardous due to their cumulative toxicological impact on the liver and kidneys. Clinical symptoms of hepatic retention of these metals span a range from fever, loss of appetite, and malaise to jaundice, hepatomegaly, abdominal pain, distension, nocturnal bone pain, and skin changes.

7.1. Mechanisms of Toxicity

The potential of some heavy metals to harm human organs is well documented. Several are toxic to humans via occupational exposure, environmental contamination, water, and food. Most accumulate in the body over time, preferentially in certain organs, and may cause early organ failure. The mechanisms for organ targeting and distinctive pathological signs remain unclear. Thiol-binding metals exert toxicity mainly through interaction and inhibition of essential thiol groups in enzymes and proteins, yet their systemic effects differ. Different metals elicit distinct health and behavioral outcomes: manganese causes parkinsonism or epilepsy, cadmium leads to loss of olfaction, and mercury and lead produce specific neurobehavioral syndromes [29].

Heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), zinc (Zn), copper (Cu), iron (Fe), arsenic (As), and cobalt (Co) are widely used in industry, agriculture, and domestic activities. These metals create significant environmental pollution with severe impacts on human health [30]. Global regulations set maximum allowable concentrations, often based on coastal and marine recommendations, but they continue to permit high levels of contamination in various ecosystems. Therefore, efficient, cost-effective, and in-field methods are essential for monitoring and controlling heavy metal concentrations.

7.2. Symptoms and Clinical Findings

Penetrating heavy metals such as iron, copper, zinc, cadmium, chromium, and lead affect various physiological and regulatory processes in the human body by altering the biological metabolism of critical substances. The heavy metal concentration of the matrix can be determined with atomic absorption spectrometry. However, wood and water samples require careful pretreatment to convert the analytes to an aqueous form since most samples do not satisfy the conditions for atomic absorption spectrometer direct analysis. Accordingly, the development of a chemical system for sorption–spectrophotometric determination is imperative because the approach saves time and reduces the amount of chemical waste. To address this gap, an innovative chemical method for the determination of heavy metals is proposed, based on the synthesis and application of a chemical reagent called dimethylglyoxime-2-(4-nitrophenylazo)-1.8-dihydroxy-3,6-naphthalene disulfonic acid (DMG-NA). After extraction with an organic solvent, dimethylglyoxime (DMG) reagent is immediately added to establish the presence of heavy metals. This result provides a preliminary indication of the presence and type of heavy metal contaminants in a water or timber sample. Then, 2-(4-nitrophenylazo)-1.8-dihydroxy-3,6-naphthalene disulfonic acid (NA) reagent is introduced and the absorbance of the complex is measured at 515–520 nm. After colour development, simultaneous determination of nickel, cobalt, and copper is accomplished by measuring the absorbance of the complexes formed with heavy metals at 465 nm. On the basis of the available concentration of metals, the concentrations of liver and kidney function markers are determined specifically [1].

8. Impact of Heavy Metals on Kidney Function

Heavy metals can be highly toxic to the kidneys, leading to damage of proximal tubular cells. Chronic intoxication may disrupt cellular functions and renal architecture, culminating in proteinuria and hematuria. Although toxins vary in chemical composition, they all contribute to renal insufficiency and increased rates of hypertension, stroke, cardiovascular disease, and chronic kidney disease (CKD). Both acute and chronic exposures to heavy metals are capable of inducing various types of kidney damage, including acute renal failure, proximal tubular dysfunction, nephrotic syndrome, distal tubulopathy, and chronic kidney failure.

Adverse health effects associated with kidney malfunction often appear only after significant damage has occurred. Therefore, the assessment of liver and kidney functions is a vital component of clinical diagnosis, especially when monitoring the pathogenic effects of heavy metals on human health. Evaluation typically involves serum biological parameters such as aspartate aminotransferase (S.G.O.T), alanine aminotransferase (S.G.P.T), blood urea, and creatinine. Alterations in liver and kidney functions may provide early indications of various diseases and serve as markers of organ damage during poisoning. [3][31]

8.1. Mechanisms of Toxicity

Heavy metal toxicity involves a multifaceted process encompassing different events such as metal challenge, several signaling pathways activated by cell receptors responding to the challenge, and alternative stress-related genes triggered by different levels of reactive oxygen/nitrogen species (ROS/RNS) induced by the stress. The toxicity of heavy metals is primarily connected with perturbations of various metabolic processes, in the first instance by reacting with the sulfhydryl groups of key enzymes engaged in critical cellular functions such as respiration, membrane transport, maintenance of the cellular antioxidant status, and DNA transcription.

These metals also exert an indirect effect on cell function by inducing the production of reactive oxygen and nitrogen species (ROS and RNS), known collectively as ROS. A substantial elevation in the intracellular ROS concentration, it is assumed, causes the oxidation of lipid membranes, DNA, and proteins responsible for the functioning and structure of cells, thereby resulting in mutagenesis, carcinogenesis, and ultimately the induction of degenerative processes. These events are also closely associated with the potential of heavy metals to affect the content of essential nutrients in cells and/or tissues, resulting in the substitution of essential metal ions in enzymes and related proteins. [32][33][34]

8.2. Symptoms and Clinical Findings

Heavy metal intoxication can induce prolonged or acute liver and kidney pathology. Clinical findings depend on exposure, time lapse, heavy metal type and concentrations. Some shared features are: jaundice, a dark discoloration of the mucous membranes, an extensive hemorrhagic syndrome and an increase in the dimensions of the abdomen. Changes of the hepatic, digestive, neurological and psychiatric systems accompany the intoxications [1] [2].

9. Regulatory and Safety Considerations

Regulatory guidelines for maximum limits of toxic metal concentrations are implemented worldwide to ensure consumer safety. An accurate risk assessment, either by biological or non-biological monitoring, represents the first step in understanding exposure and evaluating risk to public health. The development of an analytical method for the determination of zinc, cadmium, lead, copper, and iron in milk, water, fruit juice, and soil samples plays a fundamental role in environmental health, as heavy metals contribute to many illnesses and disorders worldwide due to high exposure. The proposed procedure involves generating complex species at room temperature, acidifying the solution, extracting the complexes into an organic phase, and back-extracting with a new reagent to prepare a stock solution for advanced analysis [2].

9.1. Global Standards and Guidelines

The presence of heavy metals in environmental and biological systems is a worldwide concern to human health. In this context, specific chemical protocols are developed for the determination of cadmium, copper, lead and zinc in soil and in liquid samples (water, juices and oils). In the first case, samples are digested with a HNO3/HCl mixture earlier of the analysis by flame atomic absorption spectrometry (FAAS), and in the second case, a liquid-liquid extraction method was used to isolate the analytes before the determination by FAAS. [35][36]

9.2. Risk Assessment Protocols

Risk assessment is a process that estimates the nature and probability of adverse health effects in humans who may be exposed to chemicals, directly or indirectly, in contaminated environmental areas. This process includes a risk characterization of any significant health effects that could occur in exposed populations. Large amounts of heavy metals have been observed and were reported in fresh waters throughout the world, possibly resulting from waste discharges from industrial and domestic activities. In such cases, a definitive assessment of the health risks posed by the elevated levels of heavy metals is crucial.

An approach for the terrestrial environment has been developed that links chemical contamination to ecotoxicological effects. The vulnerability of sediments bathing water and soil is evaluated from the toxicological perspective. Furthermore, exposure analysis provides an estimate of the potential contact of humans with metals. The Human Health Risk Assessment involved consideration of the general public in non-high-exposure situations, with identification of potential health problems associated with hazardous elements in the environment. To mitigate health threats to the environment and public, regulatory standards for heavy metals and their compounds in various media have been promulgated and periodically revised by the U.S. Environmental Protection Agency (U.S. EPA) and/or corresponding authorities of some countries. [37][38][39]

10. Future Directions in Research

Innovative approaches have been proposed to quantify heavy metals, some of which have been implemented and validated through analysis of contaminated samples. Novel methods have been introduced to investigate the effects of heavy metals on liver- and kidney-function markers.

The global accumulation of heavy metals, particularly through anthropogenic activities, constitutes a serious environmental threat. Such pollutants reside predominantly in soil and water and consequently enter the food chain, leading to biomagnification. Elevated levels induce toxicity in various organisms, including humans. Among numerous heavy metals analysed, particular attention is devoted to lead (Pb), cadmium (Cd), and chromium (Cr) because of their widespread application in industry and commerce, their high toxicity, and their potential to accumulate in biological organs, triggering deleterious effects on human health. The liver and kidneys are principal targets for heavy-metal accumulation, where even minor exposure can lead to significant adverse effects and increased cancer risk. The assessment of liver and renal functional markers represents a key strategy for identifying hidden health risks in contaminated areas. [1] [2]

10.1. Innovations in Analytical Chemistry

In analytic chemistry, oxidative coupling of phenols has attracted considerable attention in both academia and industry due to the importance of dimeric biphenols in natural products and their potential applications in catalyst design, new materials, and biologically active frameworks. A novel phenol oxidative coupling system is described, comprising a catalytic system utilizing a Cr-salen complex that contains a redox-active ligand and inorganic secondary oxidants, such as bromine, in the presence of LiClO4 [16]. The protocol gives biphenols from phenols in good to excellent yields under mild conditions and can be applied on a multi-gram scale [24]. Heavy metal pollution is an immense danger for the health of living organisms and plants; by polluting natural waters, it enters into the food chain and accumulates in different parts of the body of any individual after entrance through lungs, skin, or by digestion. A new strategy for the detection of heavy metals by using a heteronuclear ring complex was developed. This green approach for the determination of heavy metals shows great advantage over traditional methods and is applied in natural waters, vegetables, medicines, milk, and other edible materials [22]. In the liver and kidney function tests, the values observed in heavy metals and lead intoxication reveal the presence of hepatotoxicity and nephrotoxicity. A novel chemical method utilizing a certain heteronuclear ring complex was developed to detect heavy metals in various environmental samples. Concentrations of heavy metals, including Pb, Cu, Hg, Ni, Mn, Cd, Cr, Fe, and Zn, were determined in natural waters, vegetables, medicines, milk, and other edible materials. The observed levels of heavy metals indicate direct linkage to increased levels of serum bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, and blood urea nitrogen (BUN), markers indicative of liver and kidney dysfunction.

10.2. Potential for New Therapeutics

There is some potential for the use of heavy metals in the development of new therapeutics. Major chelating agents effective against heavy metal toxicity include BAL, DMPS, DMSA, MiADMSA, MmDMSA, MchDMSA, CaNa2EDTA, D-penicillamine, TETA, NTA, DFO, and deferiprone. BAL, the first reported antidote with sulfhydryl and hydroxyl groups, forms stable complexes for excretion. DMPS, a water-soluble analog of dimercaprol, is used mainly for arsenic and mercury poisoning. DMSA, an oral, non-toxic, water-soluble chelator, has been used since the 1950s. CaNa2EDTA is employed primarily for lead toxicity. Penicillamine chelates copper, while TETA and NTA target copper as well. DFO binds tightly to trivalent ions to treat aluminum toxicity, and deferiprone chelates iron. Oxidative stress constitutes a key mechanism in metal toxicity, justifying antioxidant therapy. Vitamins E and C function as chain-terminating antioxidants and electron donors, with vitamin C activating biological antioxidant defenses. Astaxanthin prevents oxidative stress induced by cadmium, copper, and cobalt, alleviating cell damage caused by these metals [40].

11. Conclusion

A new and innovative chemical method for the determination of trace level heavy metals has been successfully developed, effectively applied for the detection of selective heavy metals not only in water and air but also in various biological samples. This newly developed method has also been utilized to monitor the presence of toxic trace metals found in cigarette smoke. Specifically, traces of cadmium (Cd), mercury (Hg), nickel (Ni), manganese (Mn), lead (Pb), and vanadium (V) in a selected area of the lake within the Bangkok region were thoroughly analyzed and documented. The remarkable ability of this method to determine the concentration of heavy metals in both water and air samples across a wide range of concentrations, while simultaneously testing for several metals at any given time, has proved to be immensely helpful for ongoing monitoring of overall environmental pollution levels. The heavy toxicity associated with these metals is linked to a variety of serious toxic effects, which may lead to impairments in several crucial organs, as well as hepatic injuries and renal failures. Moreover, the development of these innovative chemical methods can serve as vital tools for the determination of trace level heavy metals, which are directly related to liver and kidney function markers, thereby enhancing understanding and monitoring of public health concerns regarding these contaminants.

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