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Toxic microelements: bioavailability and mobilization in soil after 40 years of phosphorous fertilization

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Biodostupnost i mobilizacija toksičnih mikroelemenata u zemljištu nakon četrdesetogodišnje aplikacije fosfornih djubriva

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Biodostupnost i mobilizacija toksičnih mikroelemenata u zemljištu nakon četrdesetogodišnje aplikacije fosfornih djubriva

Abstrakt

U ovoj doktorskoj disertaciji proučavano je zemljište koje je 40 godina đubreno različitim količinama fosfornog đubriva monoamonijum-fosfata (MAP). Količine primenjenog mineralnog đubriva bile su 26, 39 i 52 kg po hektaru. Kao kontrolni uzorak korišćeno je neđubreno zemljište. Radi boljeg razumevanja tokova fosfora kroz zemljište i potencijalnih izvora fosfora uzorkovano je zemljište sa dve dubine: površinski sloj debljine do 30 cm i sloj od 30 do 60 cm. Uzorci zemljišta su tretirani različitim sekvencijalnim ekstrakcionim procedurama i to: modifikovanom Chang i Jackson metodom, modifikovanom Tesijerovom metodom i BCR metodom. Ehstrahovani su različiti oblici fosfora i metala. Takođe su, u cilju određivanja ukupnog sadržaja mikroelemenata, uzorci potpuno razarani mikrotalasnom digestijom. Istim metodama sekvencijalne ekstrakcije i totalne digestije tretirani su i sertifikovani referentni materijali CRM 684 (ekstratibilni fosfor rečnog sedimenta) i CRM 701 (ekstraktibilni elementi jezerskog sedimenta) u cilju potvrđivanja tačnosti instrumenta i primenjenih metoda.

Takođe je u ovom radu određivan sadržaj sledećih elemenata: Al, Cd, Co, Cr, Cu, Fe, Mn, Ni i Pb u uzorcima pšenice gajene na tom zemljištu. Uzorci pšenice su odvajani na stabljiku i zrno i potpuno razarani mikrotalasnom digestijom.

Koncentracije fosfora i metala u uzorcima zemlje i pšenice određivane su optičko emisionom spektrometrijom sa indukovano spregnutom plazmom (ICP-OES). Za bolje razumevanje i proučavanje prirode asocijacija tragova elemenata i fosfora korišćena je korelaciona analiza. Dalje, detaljno su ispitane promene u frakcijama organskog i neorganskog fosfora u zemljištu prouzrokovane višegodišnjom primenom različitih količina fosfornog đubriva. Poređeni su rezultati sekvencijalnih metoda fosfora sa sekvencijalnim ekstrakcijama metala kako bi se proniklo u asocijacije fosfornih vrsta sa substratima.

Rezultati ukazuju da se kontinualnim đubrenjem povećavaju svi oblici fosfora u zemljištu osim fosfora vezanog za kalcijum i organski fosfor. Primena većih količina fosfornog đubriva rezultira u dominaciji Al-P frakcije proučavanog zemljišta što ukazuje na to da je ta frakcija najodgovornija za migraciju fosfora kroz zemljišni profil. Izračunati su i bioakumulacioni faktori za sledeće elemente Al, Cd, Co, Cr, Cu, Fe, Mn, Ni i Pb za zrno i stabljiku pšenice.

Ključne reči:đubrenje zemljišta, sekvencijalna ekstrakcija, fosfor, metali u zemljištu, pšenica, korelaciona analiza, CRM 684, CRM 701, ICP-OES

Naučna oblast: Hemija, Uža naučna oblast: Analitička hemija

Toxic microelements: bioavailability and mobilization in soil after 40 years of phosphorous fertilization

Abstract:

The studied soil was of the Stagnosol type. Phosphate fertilizer was applied in 26, 39 and 52 kg P ha^{-1} amounts during a period of 40 years. Samples were subjected to sequential extraction according to a modified Chang and Jackson method, modified Tessier method, and the Community Bureau of Reference (BCR) sequential extraction procedure in order to extract different forms of phosphorus and metals, also Microwave Total digestion was applied to extract different forms of phosphorus and metals. The certified reference material CRM 684 (river sediment extractable phosphorus) and CRM 701 (Lake Sediment extractable Trace Elements) was used to confirm the accuracy of the instrument and of both employed methods.

Also the distribution of metals (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb) in wheat samples (*Triticumaestivum*) resulting from long-term fertilization (40 years) were investigated. All wheat samples were subjected to microwave digestion system, in order to extract metals and to determine them.

In the experimental plots wheat was harvested, grains were separated from stalk and then the total content of elements was determined.

The Concentrations of phosphorus and metals in soil and wheat samples were determined by ICP-OES. Correlation analysis was carried out in order to provide better understanding of the nature of associations of trace elements with phosphorus.

The changes in inorganic and organic phosphorus (P) fractions of soil resulting from long-term fertilization (40 years) were investigated. In order to improve understanding of the sink and sources of phosphorus, P-fractions were extracted from soil samples from depths of 0–30 and 30–60 cm with different amounts of monoammonium-phosphate (MAP) and then determined.

Furthermore, the association of phosphorus with substrates was provided by comparison of the results of sequential methods of phosphorus species with the sequential extraction of metals (modified Tessier method). The results of continuous fertilization during 40 years indicated increases of all forms phosphorus in the soil except of phosphorus bound to calcium and organic phosphorus. Application of higher amounts of P-fertilizer resulted in the dominance of the Al–P fraction in the studied soil, which indicated that this fraction was the most responsible for the migration of phosphorus along the soil profile.

Also the Bioaccumulation factor (BAF) of heavy metals in the Grain and Wheat were determined. Ten metals (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb) were studied.

Key words: Soil fertilization, sequential extractions, phosphorus, metals in soil, wheat yield, correlation analysis CRM 684, CRM 701, ICP-OES

Scientific field: Chemistry Field of Academic Expertise: Analytical Chemistry

Symbols and Abbreviations:

A E C	Alcohol ether carboxylate
BCR	Community Bureau of Reference
CDB	Citrate-Dithionite-Bicarbonate
CEC	Cation exchange capacity
CV	Coefficients of variation
CRM	Certified reference material
DCP	Dicalcium phosphate
DTPA	Diethylenetriaminepentaacetic acid
DCB	Dithionite-citrate-bicarbonate
DIW	Deionized water
D/W	Distilled water
EDTA	Ethylenediaminetetraacetic acid
EC	European Commission
E_0	Energy state
Eq	Energy excited state
GCS	Geological Society of Canada Procedure
HAP	Hydroxyapatite
ICP-OES	Inductively Couples Plasma Atomic Emission Spectroscopy
IP	Inorganic Phosphorus
IUPAC	International Union of Pure and Applied Chemistry
MAP ($NH_4H_2PO_4$)	Monoammonium phosphate
MCP	Monocalcium phosphate
OCP	Octocalciumphosphate
OP	Organic Phosphorus
O-P	Occluded phosphorus
PSB	Phosphorus Solubilizing Bacteria
PTEs	Potentially Toxic Elements
RSD	Relative standard deviation
R-P	Reducible Phosphorus
SMT	Standards Measurements and Testing
SOM	Soil Organic Matter
SEPs	Sequential Extraction Procedures
TP	Total Phosphorus

Contents

1. INTRODUCTION

1.1 SOIL

1.1.1 DefinitIons and Concepts

Preliminary Definitions of Soil

In the layman's mind, the soil is a very concrete thing, namely, the "dirt" on the surface of the earth. To the soil scientist, or pedologist, the word "soil" conveys a somewhat different meaning, but no generally accepted definition exists.

Hilgard [1] defined soil as "the more or less loose and friable material in which, by means of their roots, plants may or do find a foothold and nourishment, as well as other conditions of growth." This is one of the many definitions that consider soil primarily as a means of plant production.

Ramann [2, 3] writes: "The soil is the upper weathering layer of the solid earth crust." This definition is scientific in the sense that no reference is made to crop production or to any other utilitarian motive.

Joffe [4], a representative of the Russian school of soil science, objects to Ramann's formulation on the grounds that it does not distinguish between soil and loose rock material. According to Joffe, The soil is a natural body, differentiated into horizons of mineral and organic constituents, usually unconsolidated, of variable depth, which differs from the parent material below in morphology, physical properties and constitution, chemical properties and composition, and biological characteristics.

In the present-day concept the soil is considered a product of evolution and changes over time, with an own morphology and properties. The morphology of each soil, as expressed by a vertical section of different layers or horizons, is a direct reflection of the effects of the five genetic factors responsible for its development. This dynamic and evolutionary nature is embodied in the universal definition of soil as:

a natural body, located at the interface between the atmosphere, lithosphere and biosphere, consisting of layers of unconsolidated mineral and/or organic constituents of variable thickness which have been subjected to and influenced by genetic and environmental factors of: parent material, climate (including moistureand temperature effects), macro- and microorganisms, and topography, all acting over a period of time and producing a product-soil that differs from the material from which it is derived in many physical, chemical, and biological properties and characteristics.

1.1.2 The Soil Profile:

A soil profile, the unit of study in pedology1, is a vertical section between the soil surfaceat the top and weathered bedrock at the bottom. According to the natural pedogenicprocesses, a soil profile is divided into marked horizontal layers which are called“horizons” [5]. A typical soil profile is shown in (fig1.1)

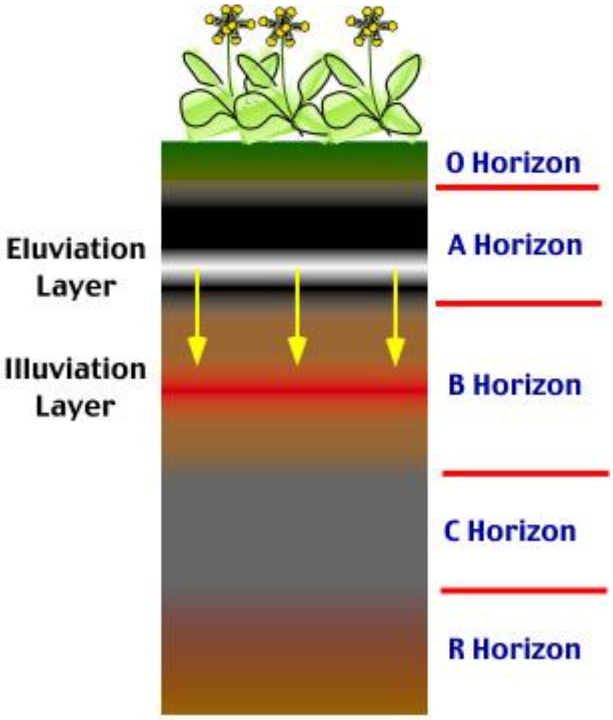

Figure 1.1 Different horizons in a podzol profile [5].

General distribution of metals in a soil profile (podzol) is as follows:

- Organic horizon (O horizon): Atmospheric deposits of metals and elements cycledthrough plants accumulate in this horizon.
- Organo-mineral horizon (A horizon): Metals accumulate in this horizon after litterhumification.
- Eluvial horizon (E horizon): This horizon contains some of the metals which havebeen adsorbed on clay particles and moved down the profile.
- Illuvial horizon (B horizon): The metals adsorbed on clay accumulate in thishorizon.

- Parent material (C horizon): This region comprises either the highly solublemetals or those which have fallen down the cracks in association with particles [5].

1.1.3 The Formation of Soil

Everywhere on the land surface there is either rock formation or soil exposed. When rocks thatwere formed deep in the earth are uplifted and exposed to the earth's atmosphere, the rocksadjust to the new environment. The chemical and physical changes that occur in the rock are called weathering. Weathering includes such physical processes as freezing, and thawing, andthe action of wind, moving water and glacial ice. Chemically, minerals in the rock decompose.The loose mantle produced by weathering is soil.The weathered soil is about one foot thick. Depending on the chemical composition and hardnessof the rock, the formation of a foot of soil could require as little as 100 years or as many as100, 000 years or more. We can also note that soil has a wide range in size of particles. Someparticles are large rock fragments. Some particles are sand sized, like those in sand dunes.

Particles of silt and clay size are usually so small they feel smooth and cannot be seen with thenaked eye. Further, we see that plants are growing on the soil. The plants produce organic matterin the soil. Plant growth and weathering usually occur together in soil formation. In soilformation the plants roots excrete carbon dioxide that dissolves in the soil water and formscarbonic acid. This acid speeds up chemical weathering. Chemical weathering, on the otherhand, causes elements that are essential to the growth of the plants to become soluble. In turn,chemical weathering contributes to the growth of the plants. Weathering and plant growthoperate as a “team” in soil formation.

1.2 PHOSPHORUS:

1.2.1 Soil Sample Collection for Phosphorus analysis:

The collection of a representative and reliable soil sample for phosphorus (P) analysis requires predetermination of sampling depth, position relative to nutrient application patterns, and sampling intensity within the field. The appropriate soil sampling depth is dependent upon the planned interpretation of the analytical data. If investigation of P distribution or concentration with depth is a specified research objective, three factors must be considered when determining the appropriate sampling depth:

1. Influence of changes in soil morphology with depth (i.e., horizonation).

2. Influence of surface soil management (e.g., tillage); and 3) necessity to maintain sample collection depth uniformity across numerous sites.

Sample collection depth based on observed morphological horizon depths is quite useful when attempting to associate soil P measurements with soil physical properties. This technique may generate very reliable data for a particular, well-defined location, but this laborious task is not very practical when a research project focuses on more than a few soils or when the data will be subjected to broader, perhaps watershed-scale, interpretation.

Depth of tillage will dramatically impact soil P distribution with depth. Tillage depth is seldom constant across a given field. Sampling depths should include soil collected from a depth confidently within the tillage zone and excluding soil from below the tillage zone. A second transitional depth should be collected that is expected to be variably affected by tillage and includes the lower tillage boundary. Deeper sampling depths should not be directly impacted by physical tillage activity [6].

Relating soil physical and chemical properties to the potential for P transport with surface runoff water requires a different approach to soil sample collection.Sharpley (1985) [7] studied five soils of varying physical and chemical properties and found that effective depth of interaction between surface soil and runoff ranged from 2 to 40 mm. The effective depth of interaction varied with soil type, surface slope, rainfall intensity, and crop residue. For most agricultural soils, samples collected to a depth of 20 mm would accurately define the effective depth of runoff interaction generated by moderate to high rainfall intensity (< 50 mm/h). For medium to coarse textured soils on steeper slopes (>12 %) that are subjected to high intensity rainfall (> 100 mm /h), soils should be sampled to a depth of 40 mm in order to more accurately relate the potential for P transport with surface runoff to soil physical and chemical properties.

Recommended soil sampling intensity is usually between 10 and 30 sub samples per composite sample [8-10]. A single composite sample may represent a single research plot or an entire production field, but generally not more than 10 ha. Discrete nutrient application patterns in a field can increase the complexity of appropriate soil sample collection procedures. In a review of positional P availability resulting from band application of fertilizer P, Sharply and Halvorson (1994) [11] stated that collection of 15 random samples (Ward and Leikam, 1986; Shapiro, 1988)[12, 13] to 30 random samples (Hooker, 1976) [14] were adequate to reflect crop P availability in conventionally tilled fields where previous P fertilizer bands exist. For no-till or minimum-till soils containing residual P fertilizer bands in which the location of the P bands is known, sampling to include one “in-the-band” soil sample for every 20 “between-the-band” samples for 76 cm band spacing, and one “in-the-band” sample for every 8 “between-the-band” samples for 30 cm band spacing, will accurately reflect the mean soil P status of the field [9].Twenty to 30 sub samples per composite are adequate. When the location of the P bands is not known, collection of 20 to 30 sub samples per composite is also adequate but paired sub samples should be collected where the location of the first sub sample of the pair is completely random and the second sub sample of the pair is located 50% of the band-spacing distance from the first, perpendicular to the band direction [9].

1.2.2 Soil Sample Handling, Preparation, and Storage forPhosphorus analysis:

Air-drying should be satisfactory for investigations into relative changes in soil P concentrations in response to imposed treatments or for routine comparative P analyses. Soil samples should be air-dried (25 to 30^{o}C) and crushed to pass a 2 mm sieve. Air-dried and crushed soil samples are stable at room temperature. Air-drying may not be suitable for determination of the absolute quantity of the various P fractions in soils. Air-drying may artificially elevate the quantity of soluble reactive P above *in situ* conditions [6]. Bartlett and James (1980) [15] studied P solubility in the surface soil of a loamy fine sand and found water-soluble P concentrations to be five times higher in air-dried samples (~30 mg P L-1) than in samples stored at field moisture (~5 mg P/ L). The effect of air-drying was only partially reversed by rewetting and incubating the air-dried soil for one month (~20 mg P L-1). Water-soluble P in rewetted soil samples that had previously been air-dried was shown to decrease during three months of storage at 20^{o}C [15]. For quantitative characterization studies, soil and sediment samples should be stored at field moisture content under refrigeration, between 0 and 4^{o}C. Soil and sediment samples should not be stored frozen (<0^{o}C), because the water-soluble proportion of total P increases after freezing [16]. Mixing moist soil samples to achieve homogeneity is difficult, and careful attention should be paid to ensure thorough mixing prior to sub sampling. Moist soils are also difficult to sieve, but large particles (> 2mm) should be removed from the sample prior to analysis.

1.2.3 Phosphorus in soil:

Phosphates, the most abundant form of phosphorus in the environment, are available for assimilation, and for that reason, they have been traditionally used as fertilizers [17]. Various forms of phosphorus could be found in nature:

1. Mineral forms;
2. Organic forms;
3. Dissolved inorganic forms;
4. Gaseous forms and
5. Particulate or colloidal forms.

In soils, more than 90% of total phosphorous is present as insoluble and fixed forms including the primary phosphate minerals, humus P, insoluble phosphates of Ca, Fe, and Al and – phosphorus fixed by hydrous oxides and silicate minerals [18]. Duo to high fixation and immobilization of P in the soil, the agriculturists usually apply high amounts of P-fertilizer, what results in higher input of P into soil than plant uptake. The cumulative accumulation of available P in agricultural soils may partially saturate the capacity of soil for P sorption with resulting increase of P leaching into subsoil layers [19] or may sometimes reach depth more than 90 cm [20], suggesting that erosion, rather than leaching, would cause a threat to water bodies [21].

1.2.3.1 Available Phosphorus**:**

Soil P can be considered as non-available, potentially available and immediately available. Immediately available P is the inorganic form occurring in the soil solution and which is almost exclusively orthophosphate. Plants are unable to absorb P directly from solid compounds or from organic P compounds, even though the latter may be in soil solution. Hanaper and co-workers (1964) [22] considered that the large fraction of organic P in soil solution (Pierre and Parker, 1927 [23], had found 96% of the P in soil solution to be organic) must be colloidal as plant roots are unable to utilize it. On mixing sucrose with a soil, Hannapel found that organic P movement on leaching was increased, whereas treatment of the soil with formaldehyde decreased organic P movement. This, together with millipore (0.45 μm) filtration, showed that much of the organic P in soil solution is associated with microbial cells.

Plant does directly take up orthophosphate from solution and have been shown to do so quantitatively. Up to a point, plant growth increases with orthophosphate in the nutrient solution. As the concentration of water-soluble phosphate in soil solution is very small at any one time, it follows that it must be replenished during plant growth and the rate of this replenishment is more important than the concentration. The most important form of potentially available P is the organic; inorganic forms other than orthophosphate are largely unavailable. Relatively available inorganic P tends to accumulate in its most stable state under prevailing conditions; thus in calcareous soils the available inorganic P would be acid-soluble whereas in acid soils the adsorbed P would be more available. In flooded soils with low oxidation potentials, certain forms of P normally considered as unavailable, for example iron phosphate, can be regarded as available Gasser(1956) [24].

In most soils the main source of orthophosphate is the organic matter unless, of course, direct fertilization with soluble phosphate has been made. Friend and Birch (1960) [25] found that the organic P in a soil was of significant value in predicting P availability. Eid and co-workers (1951) [26] found that the significance of organic P content was of importance only in warm soils and did not apply in cold climates. Semb and Uhlen (1954) [27] more precisely stated that the significance of organic P content for predicting P availability was valid only if the pH exceeded 5.5 as more acid conditions adversely affected mineralization. It would seem, therefore, that knowledge of both the amount of organic P in a soil and of its rate of decomposition would be most useful for predicting availability. As will be discussed, Friend and Birch go further and include the reassimilation and fixation of the mineralized P. The measurement of total P in a soil is of no use in determining available P except that a very low total content will indicate a probable P deficiency.

The amount of P in a soil that is available to growing plants is determined by one of two methods, biological or chemical, but for the most reliable results both methods should be employed in conjunction. As in the determination of all available nutrients, laboratory methods are of use only as a routine with soils of know characteristics. For the intelligent and practical appraisal of a soil’s capacity to provide nutrient phosphate it is essential to first conduct field and greenhouse experiments embodying all probable variables such as crop, land management and so on. Subsequent chemical or biological experiments can then be correlated so as to obtain the best method for that particular soil under particular conditions.

1.2.4 Chemistry of Phosphorus in soil:

Phosphorus is essential to all forms of life on earth and is primarily conserved in soils and sediments. Understanding the chemical, biological, and physical processes in soils that affect the availability of P to terrestrial plants, and ultimately to animals and humans, is therefore of fundamental importance to the development of sustainable management practices for all agro ecosystems. Similarly, knowledge of processes controlling the transport of P from soils to waters, and the availability of P to aquatic biota is of considerable ecological significance because of the well-known role of P in eutrophication [28]. Soil phosphorus (P) has maintained its appeal to soil scientists for more than 150 years. During the first 100 of those years, research mainly focused on securing the P nutrition of cultivated plants. This was rational since few soils in the world can supply cultivated crops with sufficient amounts of P without fertilization or maturing. Due to the substantial response of yields to P fertilization and affordable products, the use of P fertilizers increased rapidly after the Second World War, and to date, nearly 600×106 Mg of fertilizer P has been applied on the Earth’s agricultural soils [29].

Considerably less P has been removed from soils through harvest, and the consequent positive P balance has created soils having an excessive P content in many areas of the developed world [30-32] .The statistics on intercontinental trade indicate that the P balance has been most positive in Europe, with more than double the net inputs of P per hectare than in any other region in the world [33]. Securing the P nutrition of crops with fertilizers has been one of the greatest successes in improving agricultural productivity, but long ago P fertilizers were discovered not to be a pure blessing. The first symptoms of the harmful effects of the increased P (and nitrogen) inputs in agriculture on the environment were noticed in the 1960’s in North America and Great Britain, where the nutrient concentrations of the surface waters were elevated from their natural state with numerous harmful consequences. Obviously, there also were and are other sources of P affecting the quality of surface waters, but extensive evidence from Finland, [34, 35], and elsewhere,[36-39] demonstrates that agriculture has an undisputable impact on the P loading of the surface waters wherever modern agriculture is being practiced.

The undesired effects of P fertilization shifted the focus of P research from agronomic issues to environmental ones. As a result, instead of studying ways to maximize the P supply for cultivated plants, an increasing number of soil scientists now work on the possibilities of reducing agricultural P inputs and balancing production with environmental values, ways of measuring and reducing the mobilization of the various forms of P in enriched soils, and ways of reducing the P Transport to the surface waters in drainage and runoff waters. Regardless of the related change in the norms and methodology applied in soil P research, the primary processes governing the behavior of P in soil are essentially the same as 150 years ago when Justus Liebig and John Bennet Lawes made their first experiments on the P nutrition of plants, [40]. A strong basis in understanding these processes is required both in agronomically and in environmentally oriented P research.

Within the past one and a half centuries of soil P research, a massive amount of literature has been produced, and the escalating knowledge has been reviewed in a number of articles, [41-50].

Today, the individual processes governing the behavior of P in various soils are well known. Then why is soil P still under active research? Most of the interest is, of course, explained by the widespread harmful effects of soil P on the surface waters and the increased public Concern about this issue, but the research is also driven by purely scientific incentives. In short, many aspects of soil P are not well known even today.

1.2.5 Phosphorus Cycle in soil:

Much modern soil chemistry and soil management now addresses this balance between sustainable agricultural practices and protection of the environment. Achieving production and environmental goals for soil P management begins with an understanding of how these goals are related to the soil cycle, the chemical and biochemical processes that control the forms, biological availability, and mobility of soil P (fig 1.2).

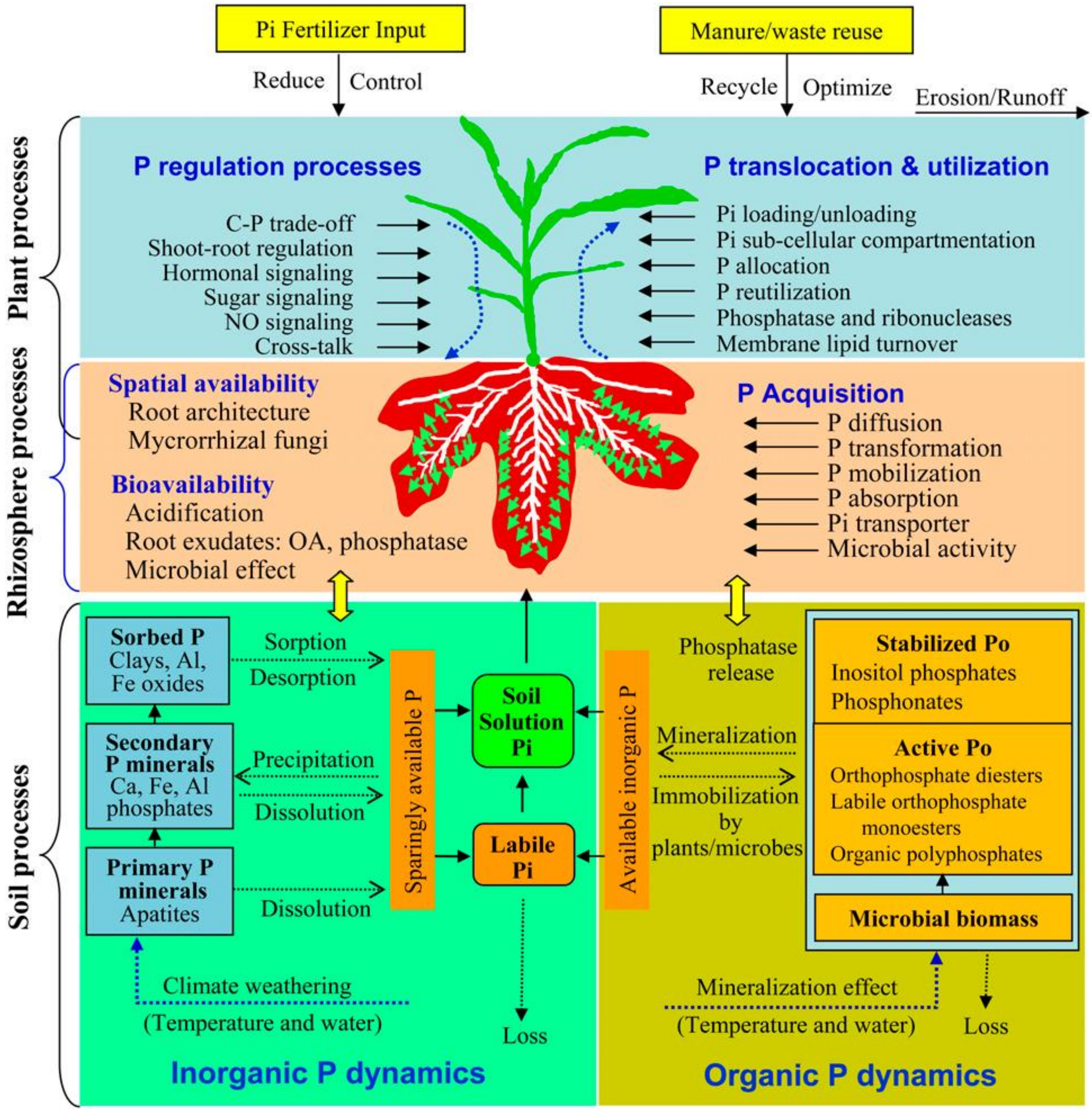

Figure1.2: P dynamics in the soil/rhizosphere- plant continuum. C-P, Carbon-P; NO, nitric oxide; OA, organic acids. [34]

Phosphorus is second only to nitrogen as a mineral nutrient required by both plants and microorganisms. Phosphorus in soilsis immobilized orbecomes less soluble either by absorption, chemical precipitation or both [51].

In fact, phosphorus can reach the soil by the organic residues decay or erosion. There is no phosphorus source through the rain or by microorganism fixation as in nitrogen cycle. The losses are different too: phosphorus leaves the soil by erosion and it’s not easily leached like NO^{3-}; although the organic forms can be mineralized and the mineral forms can be immobilized as it happened in the nitrogen cycle.

The basic difference between phosphorus cycle and the others mentioned before is that it doesn't have an atmospheric part and has some chemical processes more complex which play an important role in soil like phosphorus solubilization. In fact, plants are only able to uptake the phosphorus in the form of HPO_4^{2-} and $H_2PO_4^-$ and that's why the study of available phosphorus is so important for crops and agriculture. Some studies show that 85-88% of the phosphorus in soil is on the non reactive pool, which means that it is in the fixed or adsorbed form, consequently unavailable for soil transformations [52]. Besides that, the relative immobility of the ion in the soil may cause the phosphate supply to be the limiting factor in plant growths [51]. It's important to know which are the processes that contribute for this element availability in soil, observing the processes that contributes for the phosphorus solubilization, once plants and microorganisms just uptake soluble forms.

In the phosphorus cycle, there are organic forms which can represent 20-80% of the total phosphorus [53].

Mineralization may turn available some soluble forms, but these can be easily fixed in inorganic unavailable pools. That happens because in acid soils, phosphorus can precipitate in salts, combining with the aluminum and iron ions; in neutral or basic soils, the precipitate occurs with the calcium ions [54]. Some of this soluble phosphorus can also be adsorbed and become unavailable. In other way, some microorganisms may solubilize those inorganic forms [55]. This is particularly important because if these bacteria can live in the soil, they will act as bio fertilizers, [56],turning thephosphorus fertilizer usually fixed in inorganic forms more available. These bacteria, whose name is phosphorus solubilizing bacteria (PSB), can be identified and used for different soil types. At this point it can be understood how important are the soil conditions for the microbial development and their optimal conditions [55]. The PSB density can vary according to pH, moisture, organic matter or different crops [54].

Besides the microorganism metabolism, some plants have the ability to segregate some enzymes that solubilize phosphorus, making it available for them. That's why the soil-plant system has to be the most efficient for providing a sustainable solution [57].

Summarizing, the different forms of phosphorus in the soil can be a consequence of transport, immobilization, solubilization and mineralization [57]. Present organic phosphorus in soil is mineralized by the heterotrophic population which keeps some of it for their growth. The products of this decomposition are released in soluble phosphorus form available for plants. Nevertheless, this phosphorus mineral pool is easily fixed and adsorbed in soil and becomes unavailable both for plants and microorganisms. It’s important to determine how significant all these processes are in phosphorus cycle in order to adapt the agriculture managements and the fertilizers used to have the best solution. In Agrosystems, for example, plants residues are used to make the nutrients available [58].

1.2.6 Phosphorus Dynamics:

1.2.6.1 from Soil to Plant**:**

With increasing demand of agricultural production and as the peak in global production will occur in the next decades, phosphorus (P) is receiving more attention as a nonrenewable resource [59, 60]. One unique characteristic of P is its low availability due to slow diffusion and high fixation in soils. All of this means that P can be a major limiting factor for plant growth. Applications of chemical P Fertilizers and animal manure to agricultural land have improved soil P fertility and crop production, but caused environmental damage in the past decades. Maintaining a proper P-supplying level at the root zone can maximize the efficiency of plant roots to mobilize and acquire P from the rhizosphere by an integration of root morphological and physiological adaptive strategies. Furthermore, P uptake and utilizationby plants plays a vital role in the determination of final crop yield. A holistic understanding of P dynamics from soil to plant is necessary for optimizing P management and improving P-use efficiency, aiming at reducing consumption of chemical P fertilizer, maximizing exploitation of the biological potential of root/rhizosphere processes for efficient mobilization, and acquisition of soil P by plants as well as recycling P from manure and waste. Taken together, overall P dynamics in the soil plant system is a function of the integrative effects of P transformation, availability, and utilization caused by soil, rhizosphere, and plant processes. This Update focuses on the dynamic processes determining P availability in the soil and in the rhizosphere, Pmobilization, uptake, and utilization by plants. It highlights recent advances in the understanding of the P dynamics in the soil/rhizosphere-plant continuum [61].

1.2.7 Phosphorus Dynamics in Soil

1.2.7.1 Soil P Transformation

Soil P exists in various chemical forms including inorganic P (IP) and organic P (OP). These P forms differ in their behavior and fate in soils [62, 63]. IP usually accounts for 35% to 70% of total P in soil [64]. Primary P minerals including apatites, strengite, and variscite are very stable, and the release of available P from these minerals by weathering is generally too slow to meet the crop demand though direct application of phosphate rocks (i.e. apatites) has proved relatively efficient for crop growth in acidic soils. In contrast, secondary P minerals including calcium (Ca), iron (Fe), and aluminum (Al) phosphates vary in their dissolution rates, depending on size of mineral particles and soil pH [65, 66]. With increasing soil pH, solubility of Fe and Al phosphates increases but solubility of Ca phosphate decreases, except for pH values above 8 [67]. The P adsorbed on various clays and Al/Fe oxides can be released by desorption reactions.

All these P forms exist in complex equilibria with each other, representing from very stable, sparingly available, to plant-available P pools such as labile P and solution P (Figure: 1.1) in acidic soils, P can be dominantly adsorbed byAl/Fe oxides and hydroxides, such as gibbsite, hematite, and goethite [68]. P can be first adsorbed on the surface of clay minerals andFe/Al oxides by forming various complexes. The nonprotonated and protonated bidentate surface complexes may coexist at pH 4 to 9, while protonated bidentate innersphere complex is predominant under acidic soil conditions [69, 70]. Clay minerals and Fe/Al oxides have large specific surface areas, which provide large number of adsorption sites. The adsorption of soil P can be enhanced with increasing ionic strength. With further reactions, P may be occluded in nanopores that frequently occur in Fe/Al oxides, and thereby become unavailable to plants [70]. In neutral-to-calcareous soils, P retention is dominated by precipitation reactions [71], although P can also be adsorbed on the surface of Ca carbonate [42] and clay minerals [72]. Phosphate can precipitate with Ca, generating dicalcium phosphate (DCP) that is available to plants. Ultimately, DCP can be transformed into more stable forms such as octocalcium phosphate and hydroxyapatite (HAP), which are less available to plants at alkaline pH [70]. HAP accounts for more than 50% of total Pi in calcareous soils from long-term fertilizer experiments (H. Li, personal communication). HAP dissolution increases with decrease of soil pH [73], suggesting that rhizosphere acidification may be an efficient strategy to mobilize soil P from calcareous soil. Po generally accounts for 30% to 65% of the total P in soils [64].

Soil Po mainly exists in stabilized forms as inositol phosphates and propionates, and active forms as orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates [74, 75]. The Po can be released through mineralization processes mediated by soil organisms and plant roots in association with phosphates secretion. These processes are highly influenced by soil moisture, temperature, surface physicalchemical properties, and soil pH and Eh (for redox potential). Po transformation has a great influence on the overall bioavailability of P in soil [63]. Therefore, the availability of soil P is extremely complex and needs to be systemically evaluated because it is highly associated with P dynamics and transformation among various P pools (Figure 1.2)

1.2.8 Chemical Fertilizer P in Soil:

The modern terrestrial P cycle is dominated by agriculture and human activities [66]. The concentration of available soil Pi seldom exceeds 10 μm [76], which is much lower than that in plant tissues where the concentration is approximately 5 to 20 mM Pi [77]. Because of the low concentration and poor mobility of plant-available P in soils, applications of chemical P fertilizers are needed to improve crop growth and yield. The major forms of phosphate fertilizers include monocalcium phosphate (MCP) and monopotassium phosphate.

Application of MCP can significantly affect soil physicochemical properties. After application to soil, MCP undergoes a wetting process, generates large amounts of protons, phosphate, and DCP, and eventually forms a P-saturated patch [78]. This Pi-saturated patch forms three different reaction zones including direct reaction, precipitation reaction, and adsorption reaction zones. The direct reaction zone is very acidic (pH = 1.0–1.6), resulting in enhanced mobilization of soilmetal ions. These metal ions can also react with high concentrations of Pi in the zone thus causing further precipitation of Pi.

The amorphous Fe-P and Al-P that thereby form can be partly available to plants. In calcareous soil, new complexes of MCP and DCP can be formed and with time DCP is gradually transformed into more stable forms of Ca phosphates (octocalcium phosphate or apatite). Because the Pi concentration is relatively low, P adsorption by soilminerals is dominant in the outer zone [79]. In contrast, the application of monopotassium phosphate has little influence on soil physical and chemical properties [80]. Therefore, matching P fertilizer types with soil physical and chemical properties may be an efficient strategy for rational use of chemical fertilizer P.

1.2.9 Manure P in Soil:

Manure can be applied to soil to increase P fertility. The total P content in manure is very variable and nearly 70% of total P in manure is labile. In manure, Pi accounts for 50% to 90 % [81]. Manure also contains large amounts of Po, such as phospholipids and nucleic acids [82], which can be released to increase, soil Pi concentrations by mineralization. Furthermore, small molecular organic acids from mineralization of humic substances in manure can dissolve Ca phosphate, and especially for citrate, it can efficiently weaken the Nan particle stability of HAP, by controlling the free Ca availability and thereby the nucleation rate [83]. P adsorption to soil particles can be greatly reduced through applying organic substances. The humic acids contain large numbers of negative charges, carboxyl and hydroxyl groups, which strongly compete for the adsorption sites with Pi. Manure can also change soil pH and thus alter soil P availability. However, mechanisms of manure induced P transformation processes between Pi and Po in soil still need further investigation.

1.3 METHODS OF PHOSPHORUS ANALYSIS FOR SOILS

1.3.1 Background and Theory

1.3.1.1Total phosphorus:

The total quantity of P in most soils is relatively small. For example, Lipman and Conybeare (1936) [84] found an average of 0.06%in the top 15 cm of cropland soils throughout the United States, and Metson (1961) [85] states that the total P in soils rarely exceeds 0.2%. Phosphorus occurs in soil in inorganic and organic forms, the relative proportions of which vary with organic matter content but usually the organic forms predominate. The element tends to accumulate in the finer fractions of soil and thus increases as the clay content increases.

Three basic methods have been employed to bring the total P in a soil into solution: digestion with strong acids, fusion with alkalis and ignition followed by acid extraction. The acids recommended for the digestion are perchloric or hydrofluoric and, of the two, perchloric acid is to be perferred as silica is removed by filtration and methods exist for the determination of the resultant soluble phosphate in which perchloric acid does not interfere; Furthermore, the use of expensive platinum waer is avoided.

Metson considers that the digestion method is not always quantitative and prefers a method of prolonged fusion or else a double acid digestion. In his final recommendation, Metson advocates the destruction of organic matter with nitric acid before igniting the soil and to follow the ignition with an acid digestion. The acid used is a mixture of nitric and hydrofluoric and the P is finally measured by the gravimetric procedure of Lorenz (1901) [86]. For fusion method of bringing P into solution, sodium carbonate is the usual reagent and typical experimental details are given by Jackson (1958) [87] who, however, points out that, whereas the method is suitable for siliceous soils, it is not satisfactory for soils in which the silica content is less than the calcium plus fluoride, for example calcareous soils. Jackson also describes a modified fusion method in which the melt is taken up in sulphuric acid and iron (III) eliminated by passing the solution through a Jones reductor. Fusion with magnesium nitrate is recommended by the Association of Official Agricultural Chemists (1950) [88] but according to Jackson this does not extract all the P. it may be that if the magnesium nitrate was carefully purified a more satisfactory extraction would result.

Stewart (1910) [89] introduced an ignition method whereby the soil is ignited and then extracted with cold 12% hydrochloric acid. In later modifications the soil was ignited at 550°C for 1 hour and then extracted for 16 hours with 1 M sulphuric acid. Beckwith and little (1963) [90],after a similar ignition, extracted the soil for 4 hours with dilute (2:1) hydrochloric acid at 100°C. Beckwith and Little considered that one result of the ignition is to render titanium oxide less soluble, thus reducing the absorption of P.

For the simple determination of total P in a soil, the perchloric acid digestion is here recommended but if it is also desired to make a broad distinction between inorganic and organic P, then the ignition procedure is more suitable.

1.3.1.2 Inorganic phosphorus compounds

1.3.1.2.1 Total inorganic phosphorus:

An approximation of the total inorganic P in a soil can be obtained by extraction with strong acid, for example 1 M sulphuric acid.

Parton (1963) [91] suggested that as the solubility of inorganic P compounds is largely dependent upon pH, the total amount can be brought into solution provided that the pH is made sufficiently low. At pH 1.0 all the inorganic P, Parton considered, will be mobilized, but not necessarily in solution as some re-sorption of P will occur. To overcome this difficulty Parton added radioactive P, ^{32}P, in known amount to the soil and any re-sorption of P was reflected in a decrease of radioactivity in solution. Parton's procedure was thus to mobilize the inorganic P using a hydrochloric acid solution at pH 1.0 and containing potassium dihydrogen orthophosphate labelled with ^{32}P. When equilibrium was obtained the inorganic P in solution was determined and the radioactivity of the solution compared with that of the original solution. The inorganic P mobilized will then be given by the expression,

$$\left[\frac{\textbf{Specific activity of applied P}}{\textbf{Specific activity of P in solution}} - 1\right] \times \text{Quantity of added.}$$

1.3.1.2.2 Fractionation of inorganic phosphorus compounds:

The individual forms of inorganic P in soil are not, as yet, completely identified. Chang and Jackson (1957) [92] postulated three division of soil inorganic P:

i. Discrete phosphates precipitated upon surfaces and which are the most readily available. These include calcium phosphate precipitated upon calcium carbonate, aluminium phosphate precipitated upon aluminosilicates and iron phosphate precipitated upon iron (III) oxides.

ii. Discrete phosphate particles which are slightly available and which include calcium phosphate such as apatite and dicalcium phosphate, aluminium phosphate such as variscite, iron phosphate such as strengite and aluminium-iron phosphate such as barrandite.

iii. Occluded phosphate which are but little available and consist of calcium phosphate occluded in calcium carbonate, aluminium phosphate occluded in iron(III) oxides and reductant –soluble iron phosphate also occluded in iron(III) oxides.

Two main methods of identifying the inorganic forms of P have been used, chemical fractionation, depending upon the solubility of different forms in different solvents, and a more indirect method based upon the concept of solubility products. If in a system we have a sparingly soluble solid-phase crystalline compound in equilibrium with its ions, the activities of those ions are controlled by the nature of the compound. For example if crystals of $CaHPO_4$ are in equilibrium with Ca^{2+} and HPO_4^{2-} ions in solution, then the product of the activities of those ions is constant and characteristic. Aslyng (1954) [93] attempted to use this method to find whether dibasic calcium phosphate ($CaHPO_4$. $2H_2O$) or hydroxyapatite ($Ca_5OH(PO_4)_3$) was present in a soil.

Characteristic and constant solubility products, however, are theoretically obtained only with a well crystallized solid phase which is in true equilibrium with its ions and as this is probably seldom the case in soil suspensions, the method is limited in application.

Fraps (1906) [94] was among the first to chemically fractionate inorganic soil phosphate. Fisher and Thomas (1935) [95] extracted soil with a mixture of acetic acid and sodium acetate at pH 5 for different periods of time and with dilute sulphuric acid at PH 3. This procedure resulted in three main groups of P compounds: calcium, magnesium and manganese phosphates; Iron and aluminium phosphates; And adsorbed phosphates and apatites. Holman (1936) [96] plotted pH-solubility curves; Williams (1937) [97] extracted with sodium hydroxide obtaining organic, exchangeable and soluble P leaving an alkali-insoluble fraction, and Dean (1937) [98] followed the alkali extraction with dilute sulphuric acid extraction to separate calcium phosphate. Ghani (1943a) [99] modified Dean's procedure by extraction first with acetic acid to remove mono-, di- and tri-calcium phosphates, then with sodium hydroxide to remove organic, aluminium and iron phosphates and finally with dilute sulphuric acid to dissolve apatites. The disadvantage of this procedure was that the acetic acid soluble phosphorus was re-adsorbed by the soil and appeared in the alkali fraction. This led to a modification (Ghani, 1943b) [100] in which 8-hydroquinoline was added to the acetic acid.

8- hydroxyquinoline forms compounds in which the hydrogen of the hydroxy group is replaced to form substances such as $Al(C_9H_6NO)_3$ which are precipitated in dilute acetic acid. Williams (1950) [101] further modified Ghani’s method showing that successive single extractions with 2.5% acetic acid-8- hydroxyquinoline and 0.1M sodium hydroxide give a better soil P fractionation. Williams separated the soil fractions by centrifuging instead of using a filter candle, as recommended by Ghani, and he investigated the relative efficiency of 8- hydroxyquinoline and cupferron as reagents for blocking the re-adsorption of phosphate by iron and aluminium. He found that cupferron precipitated aluminium more completely than did8- hydroxyquinoline at the pH employed, but is less stable and may possibly attack organic P compounds. As 8- hydroxyquinoline extract are coloured it is necessary to evaporate them to dryness with a little calcium acetate and measure the P content of the ash after ignition. Cooke (1951) [102] found that addition of selenious acid to 0.5% was more efficient in preventing re-adsorption than 8- hydroxyquinoline, whereupon Ghani and Islam (1957) [103] reported that a combination of selenious acid with 8- hydroxyquinoline was even better. Wiklander (1950) [104] fractionated soil P as that present in the free soil solution, that present in micellar soil solution, that fixed in the interphase between micellar solution and the solid phase, and precipitated, insoluble forms. Talibudeen (1957;1958) [105, 106] suggested four main groups of soil P based upon the speed with which phosphate groups are exchanged with ortho-phosphate in the soil solution. A major advance in the field of P fractionation was made by Chang and Jackson (1957) [92] who pointed out that in nearly all of the early fractionation methods iron and aluminium phosphate were determined together.

Turner and Rice (1954) [107] had reported that neutral ammonium fluoride solution can dissolve aluminium phosphate but not iron phosphate and this was utilized by Chang and Jackson to separate the two phosphate. Acid extractions made in the early stages of fractionation, as in the procedure of Ghani and Williams for example, dissolve not only calcium phosphate but some aluminium and iron phosphate as well. Chang and Jackson arranged the order of the various extractants so as to more discretely separate the fractions; They used synthetics of known composition as test sample. In this method phosphate are first removed with dilute ammonium chloride solution and then the aluminium phosphate is removed with neutral ammonium fluoride solution. Iron phosphate is extracted next with sodium hydroxide and finally, calcium phosphate is dissolved in dilute acid. The phosphate not extracted by these four reagents is completely dissolved by a dithionite-citrate treatment and was called ‘reductant-soluble’ iron phosphate.

Chang and Jackson explained the reductant-soluble phosphate as being due to an iron oxide precipitate formed on the surface of iron and aluminium phosphate particles during weathering by hydrolysis of iron (III) salts. Some soils rich in iron oxides contain significant amounts of iron and aluminium phosphate occluded within the oxide and thus not removed by the dithionite treatment; The occluded phosphate, however, can be extracted after the dithionite treatment with fluoride or alkali. Chang and Jackson’s procedure for extracting aluminium phosphate was criticized (e.g. Yuan et al., 1960) [108] as not adequately separating aluminium from iron phosphate due to the pH of the ammonium fluoride solution. Fife (1959; 1962) [109, 110] carefully evaluated ammonium fluoride as a selective extractant for aluminium-bound phosphorus, Using three different soils he investigated the effects of extraction time, soil-liquid ratio and pH of extractant (0.5 M ammonium fluoride solution). Fife found that the efficiency of extraction increased with dilution and more markedly with neutral than with alkaline (pH 8.5) conditions. With alkaline extraction, adsorption of P by free iron oxide was not influenced by time of extraction between 24 and 72 hours but a linear increase in P release occurred.

This P release was ascribed to slow hydrolysis of iron phosphate and not to continued attack upon aluminium phosphate. Fife concluded that the presence of high amounts of allophane or of amorphous alumina is unlikely to give problems when determining aluminium phosphate with alkaline ammonium fluoride solution. Difficulties would arise, however, if much gibbsite is present, when aluminium phosphate appears to be released continuously with time. One suggested explanation for this was that the aluminium phosphate may be protected by coatings of iron (III) oxide, but Fife thought it more likely to be due to slow attack of gibbsite by the fluoride ion, and he recommended a direct alkaline extraction at high dilution, the initial alkalinity of the solution being such as to give a final pH of soil suspension of 8.5. Askinazi et al. (1963) [111] also found that for selective extraction of aluminium phosphate a 0.5 M solution of ammonium fluoride at pH 8.5 was best and that under these conditions removal of iron and calcium phosphate was minimal. Similarly Pratt and Garber (1964) [112] modified the original Chang and Jackson ammonium fluoride extractant by making it to pH 8.5 but reported that whereas the procedure is satisfactory for virgin soils, there is uncertainty about the separation of aluminium phosphate from recently fertilized soils.

Chang and Liaw (1962) [113] examined Fife's procedure for extracting aluminium phosphate and considered that the phosphate was more discretely separated from iron phosphate by extracting with neutral ammonium fluoride solution as in the original Chang and Jackson method, for one hour rather than with the alkaline reagent for longer periods. However, as re-precipitation of phosphate from aluminium by iron occurs, they modified the procedure as being a measurement of total aluminium phosphate, thus dispensing with the need for correcting the result for dissolved iron phosphate. Smith (1965a) [114] stated that for the accurate distinction between aluminium and iron phosphates a separate correction factor has to be calculated for each level of standard phosphate added to the ammonium fluoride solution. The pre-existing distribution of phosphate between the soil and fluoride solution can be calculated by plotting recoveries from a series of additions against concentration of added phosphate. Chang and Jackson subsequently modified their extraction procedure by making the ammonium fluoride extractant to pH 8.5 and by extracting reductant and occluded form of P before extracting calcium phosphates.

The procedure was further studied by Bromfield (1967) [115] who concluded that ammonium fluoride is not a reliable extractant for aluminium phosphate unless the soil calcium and iron phosphates have solubilities similar to those of the controls used by Chang and Jackson and unless reliable corrections are made for re-sorption of P on sesquioxides. Bromfield found that appreciable amount of dicalcium phosphate are dissolved in the ammonium fluoride reagent, particularly in soils fertilized with superphosphate, and that some calcium phosphate is dissolved during the preliminary extraction with ammonium chloride. Part of this dissolved calcium phosphate is then resorbed on to sesquioxides, leading to under-estimation of calcium phosphate and over-estimation of iron phosphate.

Before determining the P extracted by dithionite the excess dithionite and citrate present must be destroyed. This was originally done by oxidation with hydrogen peroxide which is not an easy operation and can lead to errors. Chang et al. (1966) [116] thus suggested a procedure whereby the dithionite is oxidized with iron (III) chloride in alkaline solution, citrate being precipitated. After filtration, aliquots are taken for analysis. Petersen and Corey (1966) [117] used potassium permanganate for oxidizing the dithionite. Avnimelech and Hagin (1965) [118] fractionated soil phosphorus by means of a gradient elution technique in which samples were leached with a linearly increasing acidity. Thus soil P was extracted according to its solubility in acid. The method involves a non-equilibrium extraction as opposed to conventional methods which employ equilibrium extraction.

1.3.1.3 Organic Phosphorus compounds

1.3.1.3.1 Total organic phosphorus

Hopkins and Pettit (1908) [119] estimated organic P in soils by a calculation procedure. Soils were chosen having the same amounts of potassium in the subsoil as in the topsoil and thus assumed to have a uniform mineral composition. P present in the subsoil was then subtracted from that in the topsoil and the difference taken as organic P. The N/P ratio was then calculated and organic P determined in other kinds of soil by multiplying the nitrogen content by this ratio. The method was later taken up by Stewart (1910) [89] but was much criticized by Fraps (1911) [120]. The practical determination of total organic P in soils has been attempted by two main methods, direct chemical extraction and indirectly by the difference between total and inorganic P. In most cases even the so-called direct method depends eventually upon a distinction between total and inorganic material.

According to Russell (1961) [121] neither procedure is acceptable but at present it would be difficult to suggest an alternative. For the mere determination of total organic P the indirect method by difference is preferable to extraction procedure and is certainly more simple and expedient. The principle of the method is to determine the total inorganic P in the soil before and after oxidation of the organic matter, the difference being taken as a measure of organically bound P. Sources of error lie in the quantitative oxidation of organic matter and, perhaps more importantly, in the extraction of the inorganic P.

Schmoeger (1897) [122] suggested hydrolyzing soil under pressure at 140-160°C and then extracting with cold 12% hydrochloric acid; the difference in P between the extracts of hydrolyzed and non-hydrolyzed soil samples was taken as organic P. Potter and Benton (1916) [123] based a method on the solubility of organic matter in ammonium hydroxide and their procedure was improved by Schollenberger (1918) [124] although it remained tedious. Destruction of organic matter by ignition was used from early times (e.g. Stewart, 1910) [89] and several different temperatures and times of heating have been advocated. The procedure most commonly followed is that of Legg and Black (1955) [125] who ignited the soil for 1 hour at 240°C and made the acid extractions with concentrated hydrochloric acid. Saunders and Williams (1955) [126] used 1 M sulphuric acid for the extractions and ignited the soil at 550°C for 1 hour. The chief error in destroying the organic matter by ignition is that the solubility of the inorganic P already present in the soil is affected, thus rendering the two acid extractions non-comparable. During the initial acid extraction of the non-ignited soil its possible also that some organic P will be mineralized by acid hydrolysis [127] which will cause low results for organic P.

Dahnke and co-workers (1964) [128] first extracted soil with 0.1 M hydrochloric acid and 0.06 M ammonium fluoride solution for 5 minutes to remove readily extractable P. The soil was then ignited at 500°C for 1 hour and re-extracted; The P in the second extract was assumed to be organic. Dickman and De Turk (1938) [129] suggested a method which was later slightly modified by Bray and Kurtz (1945) [130] in which P, extracted as acid-soluble plus adsorbed, was measured before and after oxidation with hydrogen peroxide. 1 g of soil in a tube graduated at 50 cm^3 was treated with 15 cm^3 of 30% hydrogen peroxide and 10 cm^3 of water. The suspension was warmed in a steam bath for 30 minutes, diluted acidified and shaken with ammonium fluoride solution; after filtering, the P was determined in the filtrate. Jackson gives a similar procedure.

The objection to destroying the organic matter with hydrogen peroxide is the difficulty of obtaining a phosphorus-free reagent. The commercial product must be mixed with a little calcium hydroxide and distilled under reduced pressure below 60°C; The distillate will be less concentrated than the original peroxide (20% instead 30%) due to decomposition. Most chemical extraction procedures for estimating organic P differ from those just described only in that oxidation of organic matter is carried out after extracting the organic P compounds from the soil and not on the soil as a whole. Wrenshall and Dyer (1939) [131] extracted organic P from soil with 4 M hydrochloric acid followed by 2.9 M ammonium hydroxide solution, and Pearson (1940) [132] recommended an eighteen-hour extraction with 0.5 M ammonium hydroxide at 90°C. Bower (1945) [133] suggested hot 0.5 M sodium hydroxide solution as a more efficient extractant than ammonium hydroxide and his view was confirmed by Black et al . (1948) [134] who added, however, that organic P may be lost by hydrolysis, Saunders and Williams (1955) [126] compared Pearson's ammonium hydroxide extraction with that in which the soil is pre-treated with 0.1 M hydrochloric acid and then extracted with cold 0.1 M sodium hydroxide. Saunders and Williams found that the results of Pearson method were influenced by the initial acid extraction and that although the extraction with 0.1 M sodium hydroxide gave better results, it was still affected by hydrolysis losses.

Mehta (1951) [135] investigated the methods of Wrenshall and Dyer, Pearson and Bower and found none of them satisfactory. Mehta et al. (1954) [136] later recommended a pre-extraction of soil with concentrated hydrochloric acid followed by two extractions with 0.5 M sodium hydroxide, one cold and the other at 90°C. Mehta's procedure is given by Jackson (1958) [87] who present it as a flow-sheet. Kaila (1962) [137] discovered that the results given by Mehta's method were the same as those obtained by the much simpler procedure of extracting first with 2 M sulphuric acid and then once with 0.5 M sodium hydroxide at room temperature. Kaila and Virtanen (1955) [138] had attempted an extraction involving organic solvents but difficultly was experienced in choosing a solvent which did not interfere with the subsequent determination of P. Dormaar and Webster (1963) [139] found that acetone and 0.2 M acetyl acetone did not interfere with the molybdenum blue reaction and modified the Kaila-Virtanen procedure accordingly.

Anderson (1962) [140] found the procedure of Saunders and Williams to be superior to that of Mehta, and Hance and Anderson (1962) [141] developed a modification of Mehta's method in which the soil is extracted with alkali before acid treatment as well as after and which reduces loss by hydrolysis. Martin (1964) [142] found that 0.3 M potassium hydroxide extracted 83% of the organic P from soils previously treated with acid. Martin fractionated the extracts by passing through an anion exchange resin and obtaining humic-associated P, inorganic P and acid-soluble organic P. Boswell and De Long (1959) [143] considered the acid and alkali treatments too harsh and liable to cause hydrolysis and they presented a modified oxine extraction procedure. Unless an acid treatment is given, the soils are first converted to the ammonium form and the pH of their water suspension adjusted to 9.2. A 2.5% solution of oxine in benzene is added and the suspension shaken, with periodic pH adjustment. The benzene phase is separated and washed with water and the aqueous phase is analyzed after centrifuging. A pre-treatment with 0.5 M hydrochloric acid followed by an 18-hour extraction increased the yield of organic P.

The benzene modification permits isolation of the oxine which otherwise interferes with the subsequent P determination. Pre-extraction of a soil with acid removes bases, which precipitate dissolved organic P, and all polyvalent ions which interfere with the subsequent treatments and may disintegrate clay complexes, thus releasing adsorbed organic P.

Saxena (1964) [144] investigated the strength of acid used for pre-treatment and found concentrated hydrochloric acid most effective. However, found concentrated hydrochloric acid causes hydrolysis of organic P and Saxena finally recommended making very short time (2 minutes) extractions with the acid and to neutralize the extracts immediately with ammonium hydroxide. Saxena pointed out that ammonium hydroxide will not dephosphorylate organic P compounds, whereas sodium hydroxide will, and he found that one extraction with ammonium hydroxide at 75°C removes all the organic P which would be hydrolyzed by sodium hydroxide. To compensate for the poor extracting power of ammonium compared to that of sodium hydroxide, the ammonia extraction is followed by another using 0.5 M sodium hydroxide at 90°C. Saxena’s final procedure for extracting organic P is as follows:

10 g of soil are shaken for 2 minutes with 50 cm^3 of concentrated hydrochloric acid, diluted, centrifuged and washed. The extract is neutralized with ammonium hydroxide. The soil residue is then extracted with 100 cm^3 of 1.5 M ammonium hydroxide at 75°C for 2 hours and the extract added to the first, neutralized extract. The soil is finally extracted with 100 cm^3 of 0.5 M sodium hydroxide solution at 90°C for 6 hours, the extract neutralized with acid and mixed with the previous extracts.

Organic P is then determined in the usual manner as the difference between total and inorganic P in the extracts. Saxena claimed that hydrolysis is almost completely eliminated by this procedure.

Many workers have compared the ignition and chemical extraction procedures for estimating organic P in soil and a wide range of soils has been examined. On the whole, the ignition method has been found to give the highest organic P recovery [126, 141, 145, 146], using seventeen soils, and found little significant difference between the methods. Dormaar (1964) [147] compared several techniques and came to on conclusion other than that organic P cannot yet be determined quantitatively. Kaila (1962) [137] found that the ignition procedure gave higher values than extraction procedure and that the results were not related to pH, carbon, clay or iron (III) ; He concluded, however, that for the most accurate assessment of organic P the average result of the two methods should be taken.

Anderson and Black (1965) [148] developed a procedure for measuring small amounts of organic P in the presence of large amounts of inorganic P, this being part of a fertilization investigation and in which the difference methods were unreliable. They found that organic P was retained almost quantitatively by the carbon and that inorganic P was quantitatively removed; No competition between inorganic and organic P for sorption on the carbon was noted. Some of the organic P is retained on the carbon chromatographically, some is precipitated on acidification and retained mechanically and a little, less than 5% may be washed out during leaching of the inorganic P.

As compared with other methods of determining organic P, the method of Anderson and Black has the advantage of requiring only a small correction for inorganic P even when the quantities of organic P are of the order of 10 μg accompanied by as much as 10^4 μg of inorganic P. The column method of separating the two forms of P is better than shaking the extracts with carbon as suggested by Goring (1955) [149]. In the recent years and in order to achieve comparable measurements in the determination of phosphate forms in freshwater sediments, the Standards, Measurements and Testing Programme (formerly BCR) of the European Commission has launched a project the first step of which was to select and evaluate collaboratively existing extraction procedures. This was carried out through expert consultations and a literature survey, and was followed by the organization of a first interlaboratory study for the evaluation of four sequential extraction schemes, one of which was selected as the most promising method for achieving comparability.

This scheme, a modified version of the Williams protocol, was further tested in a second interlaboratory study. The so-called SMT extraction scheme seems promising and will provide, in the near future, a valuable tool for water managers in the field of lake restoration. It will be particularly helpful in estimating the stock of P potentially available, hence the risk of eutrophication due to internal P release. Eutrophication (*i.e.*, the proliferation of algae due to an excess of nutrients) has become one of the major water pollution problems throughout Europe, with strong economic consequences (*e.g.*, the preparation of drinking water becomes more difficult because of filter clogging by algae). Phosphorus (P) is regarded as a key factor responsible for the eutrophication of freshwater [150, 151].

Its concentration in lakes and rivers results from both external inputs and internal loading from the sediment, which can contribute phosphate to the overlying water at levels comparable to the external source;[152] its release depends on the form of phosphate in the sediment. Most of sediment phosphorus is in the particulate form, dissolved P being only a few per cent of total P. Not all the forms of phosphorus are bioavailable and, therefore, likely to increase eutrophication.

Although standardised methods to quantify total phosphorus in waste- and freshwater exist, such a method is not yet available for the determination of different forms of phosphate; these forms can be determined using sequential extraction procedures which are operationally defined methods related to specific reagents and procedures, *i.e.*, results are interpreted to be related to a specific phase of sediment (although, *sensus stricto*, it is related solely to a chemical procedure). In the absence of a standardised method(s), it is not possible to compare data from one laboratory with another or to extrapolate results from one lake to another. At this stage, consequently, it becomes urgent to identify and standardise a harmonised method which would enable comparability of data to be achieved among monitoring laboratories and hence would be an essential tool for water managers. In order to improve this situation, the European Commission through the Standards, Measurements and Testing (SMT) Programme has launched a collaborative project which aimed to (1) design a harmonised sequential extraction scheme, (2) test the selected scheme in interlaboratory studies involving expert European laboratories and (3) certify the extractable trace element contents of a sediment reference material. The project started in 1996 with a literature search which identified four methods, among the most widely recognised, for the determination of phosphorus compounds in lake sediment; these were taken as a basis for comparison and for working out a harmonised procedure.

Sequential extraction schemes were first developed for soils and then extended to sediments [92]. Many operationally defined schemes are available, most of them addressing inorganic forms of phosphate. The main inorganic forms of P are

i. the fraction adsorbed by exchange sites and referred to as loosely bound, labile or exchangeable P; [153-155] this fraction is easily releasable and becomes available for algal growth.

ii. the fraction associated with Al, Fe and Mn oxides and hydroxides; phosphorus and iron are often bound in sediments, P is then adsorbed on iron complexes by ligand exchange [156] and the amount of FeOOH is one of the factors controlling P releasefrom the sediment; and (iii) the fraction in Ca-bound compounds generally referred to as apatite-P [157, 158] or Ca-bound [159, 160]. Adsorption of P on calcium carbonate is one of the mechanisms for the formation of calcium-P in sediments.

However, contrary to the formation of iron-P, Ca-bound P can also be formed by precipitation. Organic-P is a complex fraction the exact nature of which is not precisely known. De Groot and Golterman [161] have shown that organic-P is partly composed of phytates. Among the four sequential extraction schemes selected for evaluation in the frame of this project, threerely on ‘strong’ reagents, namely the procedures developed by Williams *et al.*, [157]Hieltjes and Lijklema [153] and Ruttenberg; [154] whereas chelators are used in the methoddeveloped by Golterman; [159] these procedures are summarisedin (Table 1.1).

The Williams and Hieltjes–Lijklema methods use NaOH to solubilise Fe and HCl to dissolve Ca; in the case of the Hieltjes–Lijklema method, the NaOH extraction is preceded by NH_4Cl extraction. The shortcomings of these methods have already been outlined [162, 163]. The Williams method leads to resorption by carbonates in calcareous sediments whereas dissolution of small amounts of Fe-P and Al-P by NH_4Cl is possible in the Hieltjes–Lijklema scheme. The Citrate-Dithionite-Bicarbonate Extractable (CDB) extraction, which was part of the earlier versions of the Williams scheme, was later omitted [164]. NaOH–HCl is replaced by EDTA in the Golterman method; this is supported by the suspicion that strongly acidic and alkaline solutions are too aggressive and should be avoided inextraction schemes. In this method, chelating agents such EDTA would extract Fe-bound and Ca-bound phosphorus without disturbing clay-bound or organic phosphorus. The Golterman method is not without pitfalls and the determination of phosphate can be perturbed by interference generated by EDTA.

The Ruttenberg method, initially developed for marine sediments, is a five-step procedure which allows the separationof the following sedimentary P pools: loosely sorbed or exchangeable; ferric Fe-bound P; authigenic carbonate fluoroapatite+biogenic apatite+$CaCO_3$ associated P; detrital apatite; and organic P. It is the first method designed to separate chemically authigenic apatite from detrital apatite. Furthermore, the problem of analytical artefacts resulting from redistribution of P on residual solid surfaces during extraction has been resolved.

The advantages and disadvantages of each method are listed in (Table 1.2).

Table 1.1: Reagent used and corresponding P fractions in the four sequential extraction procedures

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5
Williams [157, 164]	1 mol L^{-1}NaoH Iron-bound P bioavailable	1+3.5 mol L^{-1}HCl Ca-bound P nonavailable	HCl 3.5 mol L^{-1} + calcination Total P	HCl 1 mol L^{-1} +calcination Organic P partly available	
Hieltjes–Lijklema [153]	1 mol $L^{-1}$$NaH_4Cl$ Labile P bioavailable	0.1+2 mol L^{-1}NaOH Iron-boundP bioavailable	0.5 molL^{-1} HCl Ca-P non-available		
Golterman [159]	H_2O Labile P bioavailable	0.05 mol L^{-1} Ca-EDTA dithionite Iron-P bioavailable	0.1 molL^{-1} Na_2-EDTA Ca-P non available	0.25 mol L^{-1} H_2SO_4 Acid soluble organic P biavailable	2 mol L^{-1} NaOH reductant Organic P nonavailable
Ruttenberg [154]	1 mol $L^{-1}$$MgCl_2$ Loosely sorbed P bioavailable	0.3 mol L^{-1} Na_3-citrate 1 mol L^{-1} $NaHCO_3$ Fe-bound P bioavailable	1 mol L^{-1} Na-acetate Authigenic apatite Ca-bound P biogenic apatite non available	1 mol L^{-1} HCl Detrital apatite non available	1 mol L^{-1} HCl + calcination Organic P partly available

Table 1.2: The advantages and disadvantages of each method

Method	Advantages	Disadvantages
Williams	Simple, practical	Partial resorption of P extracted by NaOH on $CaCO_3$
Hieltjes–Lijklema	Simple, practical	Dissolution of small amounts of Fe-P and AL-P by NaOH, hydrolysis of organic P, no relation with bioavailability
Golterman	Extracts specific compounds permits extraction of organic P fractions, provides information on bioavailable fractions	Not practical, NTA and EDTA interfere with P determination, complicated solution preparation, in some sediments, extraction must be repeated
Ruttenberg	Distinction between different apatite forms; no redistribution of P on to residual solid surfaces during extraction	Very long, not practical, the butanol extraction is very difficult to achieve

The discussion of the results showed that the Williams method does not pose any particular problem and gives good analytical results; it is simple to use compared with theRuttenberg and Golterman methods. In both cases the average value for total P was very close to that determined by ICP. However, there are some pitfalls, *e.g.*, OP could be slightly underestimated owing to losses during calcination. Several laboratories complained about the imprecision and duration of Golterman's method. Furthermore, one can note thatfor all the laboratories, the sum of the different fractions was always less than TP determined by ICP. Although Hieltjes–Lijklema's method seemed simple, involving the same reagents as in the Williams method.

The results of the trial were not very good. Similarly to the Golterman method, Ruttenberg's method is time consuming and the many steps are a source of cumulative error in spite of the detailed protocol [165].

Reasons for adopting the Williams extraction scheme:

The benefit of undertaking the trial was twofold: (i) the trial showed the large difference in the results obtained for the same sediment using different extraction schemes and, therefore, confirmed the necessity of developing a harmonised procedure and (ii) it also showed that the modified Williams protocol seemed be the most promising method for achieving comparability of results. Following this first trial and by common consent from boththe EC Commission and the laboratories, it was decided toreject the Ruttenberg and Golterman protocols. Although these methods might present a few advantages, *e.g.*, extraction of different organic P fractions, information on bioavailable fractions (Golterman) and distinction between different apatite forms (Ruttenberg), they are not reproducible and they aretedious to carry out.

Consequently, they were not consideredto be suitable for adoption as a harmonised procedure, whichshould be both reliable and precise. A discussion started ofthe Williams and Hieltjes–Lijklema methods. Although thesame reagents are used in both protocols, their concentrations differ; they are higher in the Williams method, which seems better. The Williams method yields OP, which is not the case with the Hieltjes–Lijklema method (OP can be calculated as the difference between TP and IP). The Hieltjes–Lijklema method yields soluble P, whereas this phase is not determined in the Williams scheme. Besides, the Hieltjes–Lijklema method is a sequential extraction scheme, whereas in the Williams protocol the steps are independent, not sequential. Also, the Williams method gave better results in the trials and the participants agreed to take this protocol as a working basis for the harmonised procedure.

The modified Williams scheme was then tested on three sediments with different characteristics, *i.e.*, organic, siliceous and calcareous the results were generally good (RSD <10% in most cases) and only a few laboratorieshad problems, which were solved. A good quality control procedure was highly recommended. No difference due to the nature of the sediment could be seen, and consequently the method should be suitable for all sediments. The digestion step, which is not part of the Williams protocol, was carried out to test its advantages and disadvantages as regards TP measured by the Williams method. This method is more difficult to handle than the Williams method because of the use of HF, which is not a routineprocedure in many laboratories.

Moreover, the results (accepted data) were very similar and it was decided not to use the digestion step and to adopt the Williams protocol.

One laboratory used ICP as the determination method; theresults were systematically higher for NAIP, and consequentlythe spectrophotometric determination was recommended. It should be noted that the SMT protocol might not be the bestwith regard to the evaluation of the bioavailability of the different P fractions but is it certainly the most suitable one to allow laboratories to achieve reproducible results. Moreover, the SMT method is simple to handle and could provide a useful tool for water managers on a routine basis in the field of lake restoration. Especially the method could help in calculating the releasable P stock in lake sediment, and hence provide information on the lake recovery delay. Following the discussion of the results, slight modifications were suggested in order to improve the protocol and make it as clear as possible, even for laboratories which are not familiar with sequential extraction schemes. The good results allowed the modified protocol to be adopted for the final step of the project, *i.e.*, the certification campaign.

Follow-up of the project:

The modified Williams protocol has been reviewed by all participants and accepted as a common procedure for the certification of a lake sediment reference material which is planned within the first half of 1999. Provided that the results are in good agreement, this reference material certified on the basis of the modified Williams protocol could be made available by the end of 1999 [165].

1.4 HEAVY METALS

1.4.1 Definition:

1.4.1.1 Terms commonly used to specify groups of metals**:**

Terms that have been commonly used in specifying groups of metals in biological and in environmental studies are listed with comments in (Table 1.3).The limitations of these terms are clear. They are arbitrary and imprecise. Several categories overlap, making them inexact. The term “heavy metal”, because it is often used with connotations of pollution and toxicity, is probably the least satisfactory of all the terms quoted as it leads to the greatest confusion. “Heavy” in conventional usage implies high density. “Metal” in conventional usage refers to the pure element or an alloy of metallic elements. Knowledge of density contributes little to prediction of biological effects of metals, especially since the elemental metals or their alloys are, in most cases, not the reactive species with which living organisms have to deal.

Table 1.3: Terms often used to classify metals in biological and environmental studies (after [166]).

Term	Comments
Metal	Metals may be defined by the physical properties of the elemental state as elements with metallic luster, the capacity to lose electrons to form positive ions and the ability to conduct heat and electricity, but they are better identified by consideration of their chemical properties. The term is used indiscriminately by nonchemists to refer to both the element and compounds.
Metalloid	See “semimetal”.
Semimetal	An element that has the physical appearance and properties of a metal but behaves chemically like a nonmetal [167].
Light metal	A very imprecise term used loosely to refer to both the element and its compounds. It has rarely been defined, but the originator of the term, Bjerrum [168], applied it to metals of density less than 4 g/cm^{-3}.
Heavy metal	A very imprecise term (see Table 1.4) for definitions), used loosely to refer to both the element and its compounds. It is based on categorization by density, which is rarely a biologically significant property.
Essential metal	Broadly, one which is required for the complete life cycle of an organism, whose absence produces specific deficiency symptoms relieved only by that metal, and whose effect should be referred to a dose–response curve. The term is often used misleadingly since it should be accompanied by a statement of which organisms show a requirement for the element. Again, it is used loosely to refer to both the element and its compounds.

Term	Comments
Beneficial metal	An old term, now largely disused which implied that a nonessential metal could improve health. Another term that has been used loosely to refer to both the element and its compounds.
Toxic metal	An imprecise term. The fundamental rule of toxicology (Paracelsus, 1493–1541) is that all substances, including carbon and all other elements and their derivatives, are toxic given a high enough dose. The degree of toxicity of metals varies greatly from metal to metal and from organism to organism. Pure metals are rarely, if ever, very toxic (except as very fine powders, which may be harmful to the lungs from whatever substance they may originate). Toxicity, like essentiality, should be defined by reference to a dose–response curve for the species under consideration. This is another term that has been used loosely to refer to both the element and its compounds.
Abundant metal	Usually refers to the proportion of the element in the earth’s crust, though it may be defined in terms of other regions, e.g., oceans, “fresh water”, etc.
Available metal	One that is found in a form which is easily assimilated by living organisms (or by a specified organism).
Trace metal	A metal found in low concentration, in mass fractions of ppm or less, in some specified source, e.g., soil, plant, tissue, ground water, etc. Sometimes this term has confusing overtones of low nutritional requirement (by a specified organism).
Micronutrient	More recent term to describe more accurately the second of the meanings of trace metal, above.

The term "heavy metal" has been queried over many years, for example by Heuman [169], by Phipps [166], and by VanLoon and Duffy [170], but efforts to replace it by chemically sound terminology [171] have so far failed. As will be shown below, the term "heavy metals", however defined, always covers an extremely disparate group of elements, and an even more disparate group of compounds of the elements. Thus, any assumption of underlying functional similarity in biological or toxicological properties is bound to be wrong.

1.4.1.2 Current usage of the term "heavy metal**:**

(Table 1.4) lists all the current definitions of the term "heavy metal" that the author has been able to trace in scientific dictionaries or in other relevant literature. It must be noted that frequently the term has been used without an associated definition, presumably by authors who thought that there was agreement about the meaning of the term. The table shows how wrong this is and explains some of the confusion in the literature and in related policy and regulations. It should also be noted before going further that the term "heavy metal" has even been applied to semimetals (metalloids) such as arsenic, presumably because of the hidden assumption that "heaviness" and "toxicity" are in some way identical.

This illustrates further the confusion that surrounds the term. Before 1936, the term was used with the meanings "guns or shot of large size" or "great ability" [172,173]. The oldest scientific use of the term to be found in the English literature, according to the *Oxford English Dictionary*, is in Bjerrum's *Inorganic Chemistry*, 3rd Danish edition, as translated by Bell incollaboration with Bjerrum, published in London in 1936 [168]. It is worth noting that no comparableinorganic chemistry textbook published since seems to have used Bjerrum's classification, and it has notbeen included in the IUPAC *Compendium of Chemical Terminology* [174], which is the gold standard interminology for chemists. Bjerrum's definition of "heavy metals" is based upon the density of the elemental form of the metal, and he classifies "heavy metals" as those metals with elemental densities above 7 g/cm^3.

Over the years, this definition has been modified by various authors, and there is no consistency. In 1964, the editors of Van Nostrand's *International Encyclopaedia of Chemical Science* [175] and in 1987, the editors of Grant and Hackh's *Chemical Dictionary* [176] included metals with a density greater than 4 g/cm^3. A little later, in

1989, 1991, and 1992, Parker [177], Lozet and Mathieu [178], and Morris [179] chose a defining density "greater than 5 g/cm^3". However, Streit [180] used a density of 4.5 g/cm^3 as his reference point, and Thornton [181] chose 6 g/cm^3. The *Roemp Chemical Dictionary* [182] gives 3.5 g/cm^3 as a possible defining density. At some point in the history of the term, it has been realized that density is not of great significance in relation to the reactivity of a metal. Accordingly, definitions have been formulated in terms of atomic weight or mass, which brings us a step closer to the periodic table, traditionally the most sound and scientifically informative chemical classification of the elements. However, the mass criterion is still unclear. Bennet [183] and Lewis [184] opt for atomic weights greater than that of sodium (i.e., greater than 23), thus starting with magnesium, while Rand et al. [185] prefer metals of atomic weights greater than 40, thus starting with scandium. Lewis [184] suggested that forming soaps with fatty acids is an important criterion of "heaviness". This, together with the absurdity of classifying magnesium as a "heavy metal", when there has developed a conventional association of "heaviness" with toxicity, makes the Bennet and Lewis definition untenable. As for starting with scandium, it has a density of just under 3 and so would not be a "heavy metal" under any of the definitions based on density. Thus, again we have no consistent basis for defining the term. Another group of definitions is based on atomic number. Here there is more internal consistency since three of the definitions cite "heavy metals" as having atomic numbers above 20, that of sodium. Interestingly, one of them comes from the chapter by Lyman in Rand (1995) [186] and contradicts the definition favoured by Rand himself cited in the previous paragraph.

The problem with citing metals of atomic number greater than sodium as being "heavy" is that it includes essential metals such as magnesium and potassium and flatly opposes the historic basis of definition based on density, since it includes elements of density lower than any that has been used as a defining property by other authors. Burrell's definition [187] even includes the semimetals, arsenic and tellurium and the nonmetal selenium. A fourth group of definitions is based on other chemical properties, with little in common, density for radiation screening, density of crystals, and reaction with dithizone. This brings us to the definitions based vaguely on toxicity. One of these definitions [188] even refers to "heavy metals" as an "outdated term". The same authors also point out, as we have already noted in (Table 1.3), that the term has been applied to compounds of the so-called “heavy metals”, including organic derivatives where the biological and toxic properties may reflect more on the organic moiety than on the metal itself, thus making the term even more misleading than usual in the literature.

With the above in mind, it is not surprising that the most widely used textbook in toxicology, *Casarett and Doull’s Toxicology* [189] never uses the term “heavy metal”, although it does include both arsenic and arsine as “Major Toxic Metals”! It is not surprising either that Phipps, one of the authors whose definitions are cited in the table, calls the term “hopelessly imprecise and thoroughly objectionable” [166] or that recently VanLoon and Duffy conclude that “there is no chemical basis for deciding which metals should be included in this category (heavy metals)” [170]. What is surprising is the persistence of the term and its continuing use in literature, policy, and regulations, with widely varying definitions leading to confusion of thought, failure in communication, and considerable waste of time and money in fruitless debate.

Table 1.4: Definitions of “heavy metal”: A survey of current usage (April 2001).

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1. Definitions in terms of density (specific gravity):

- Metals fall naturally into two groups—the light metals with densities below 4 and the heavy metals with densities above 7 [168].
- Metal having a density greater than 4 [175].
- Metal of high density, especially a metal having a density of 5.0 or over [190].
- Metal with a density greater than 5 [191].
- Metal with a density greater than 6 g/cm^3[192].
- Metal of density greater than 4 [176].
- Metal with a density of 5.0 or greater [193].
- Metal whose density is approximately 5.0 or higher [177].
- Metal with a density greater than 5 [178].
- (In metallurgy) any metal or alloy of high density, especially one that has a density greater than 5 g/cm^3[179].
- Metal with a density higher than 4.5 g/cm^3[180].
- Metal with a density above 3.5–5 g/cm^3[182].
- Element with a density exceeding 6 g/cm^3[181].

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2. Definitions in terms of atomic weight (relative atomic mass):

- Metal with a high atomic weight [194].
- Metal of atomic weight greater than sodium [183].
- Metal of atomic weight greater than sodium (23) that forms soaps on reaction with fatty acids [184].
- Metallic element with high atomic weight; (e.g., mercury, chromium, cadmium, arsenic, and lead); can damage living things at low concentrations and tends to accumulate in the food chain [195].
- Metallic element with an atomic weight greater than 40 (JHD note—starting with scandium Atomic Number 21). Excluded are alkaline earth metals, alkali metals, lanthanides and actinides [185].
- Metal with a high atomic mass [196].
- “Heavy metals” is a collective term for metals of high atomic mass, particularly those transition metals that are toxic and cannot be processed by living organisms, such as lead, mercury, and cadmium [197].
- Metal such as mercury, lead, tin, and cadmium that has a relatively high atomic weight [198].
- Rather vague term for any metal (in whatever chemical form) with a fairly high relative atomic mass, especially those that are significantly toxic (e.g., lead, cadmium, mercury). They persist in the environment and can accumulate in plant and animal tissues. Mining and industrial wastes and sewage sludge are potential sources of heavy metal pollution [199].
- A metal such as cadmium, mercury, and lead that has a relatively high relative atomic mass. The term does not have a precise chemical meaning [200].
- Metal with a high relative atomic mass. The term is usually applied to common transition metals such as copper, lead or zinc [201].

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3. Definitions in terms of atomic number:

- In electron microscopy, metal of high atomic number used to introduce electron density into a biological specimen by staining, negative staining, or shadowing [202].

- In plant nutrition, a metal of moderate to high atomic number e.g., Cu, Zn, Ni, Pb, present in soils due to an outcrop or mine spoil, preventing growth except for a few tolerant species and ecotypes [202].
- The rectangular block of elements in the periodic table flanked by titanium, hafnium, arsenic, and bismuth at its corners but including also selenium and tellurium. The densities range from 4.5 to 22.5 g/cm^3[187].
- Any metal with an atomic number beyond calcium [203].
- Any element with an atomic number greater than 20 [204].
- Metal with an atomic number between 21 (scandium) and 92 (uranium) [186].
- Term now often used to mean any metal with atomic number >20, but there is no general concurrence [166].

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4. Definitions based on other chemical properties:

- "Heavy metals" is the name of a range of very dense alloys used for radiation screening or balancing purpose. Densities range from 14.5 g/cm^3 for 76 % W, 20 % Cu, 4 % Ni to 16.6 g/cm^3 for 90 % W, 7 % Ni, 3 % Cu [205].
- Intermetallic compound of iron and tin ($FeSn_2$) formed in tinning pots which have become badly contaminated with iron. The compound tends to settle to the bottom of the pot as solid crystals and can be removed with a perforated ladle [206].
- Lead, zinc, and alkaline earth metals that react with fatty acids to form soaps. "Heavy metal soaps" are used in lubricating greases, paint dryers, and fungicides [207].
- Any of the metals that react readily with dithizone (C_6H_5N), e.g., zinc, copper, lead, etc. [208].
- Metallic elements of relatively high molecular weight [209].

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5. Definitions without a clear basis other than toxicity:

- Element commonly used in industry and generically toxic to animals and to aerobic and anaerobic processes, but not everyone is neither dense nor entirely metallic. Includes As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Zn [210].

- Outdated generic term referring to lead, cadmium, mercury, and some other elements which generally are relatively toxic in nature; recently, the term toxic elements has been used. The term also sometimes refers to compounds containing these elements [188].

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6. Nonchemical definitions used before 1936:

- Guns or shot of large size [172].
- Great ability [173].

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1.4.2 Trace metal release to the environment:

In recent decades, there has been growing concern about trace metal contamination of the environment. Trace metals are released to the environment from natural as well as anthropogenic sources. Natural inputs come from wind-blown dust, volcanic activities, forest fires, sea-salt emissions and biogenic sources [211].

Trace metals are released from a wide range of anthropogenic sources, including fossil fuel combustion, agricultural chemicals such as fungicides and fertilizers, waste disposal such as farm manure, sewage sludge and mine wastes, chemicals, electronical and metallurgical industries, metalliferous mining and smelting and incidental accumulation because of warfare and military trainings. Different sources can contribute toenvironmental contamination in several forms; for example, trace metal content of coal or petroleum can be emitted as part of air-borne particles or accumulate in residue ash which may cause water and soil contamination. Trace metals from metallurgical industries can also be emitted to the atmosphere as aerosols or dust or accumulate in liquid effluents or solid wastes [212].

Anthropogenic emissions of trace metals may have significant temporal cycles; e.g. trace metal emissions from heat production sources are highest during the winter and emissions from electrical power production and road transport are lowest during the night [213].

1.4.3Trace metals in terrestrial ecosystems:

The elemental composition of soil generally reflects the local geology and geomorphology, but surface soils may be considerably influenced by atmospheric metal deposition. Hence, the soil may be both a source and a sink of trace metals; the concentration of trace metals inherited from soil parent materials is modified by natural as well as anthropogenic inputs [214]. Forest soils are normally not exposed to direct discharges of pollutants and the main pollution pathway is via atmospheric dry- and wet deposition [215]. Trace metals from the atmospheric input typically react with the functional groups on the surface of soil particles. They may form surface complexes and accumulate in the soil [216].

In general, the mobility and bioavailability of a large number of trace metals increase under acidic conditions. Particularly the more mobile trace metals such as Cd and Zn can be easily taken up by plants, microbial biomass and soil fauna; this may also lead to increasing surface water and ground water pollution [217]. Less mobile trace metals such as Cu and Pb are more strongly complexed with organic matter and may accumulate in top soil [218]. Some of the trace metals, such as Co, Cu, Mn and Zn are essential in low concentrations for the normal healthy growth of animals and plants, but excess amounts of all trace elements are toxic to living organisms [219]. In plants, various biochemical and physiological symptoms of trace metal toxicity have been observed, including reduced growth of roots and shoots, decreased enzymatic activity and declined nutrient concentrations in foliar tissues [220].

High content of trace metals can also damage microorganisms and disturb the microbial soil processes such as litter decomposition and soil respiration [221]. Agricultural soils may be contaminated from irrigation and use of fertilizers. This may lead to ecotoxicological and possibly human toxicological impacts that occur as a result of crop consumption or ground water use for drinking. Blood disorder and effects on liver, kidneys and nervous system are some of the trace metal effects on human health [222]. High concentration of trace metals in agricultural soils affects the diversity and abundance of soil fauna such as nematodes and earth worms or damages the essential organs of terrestrial fauna such as birds and mammals [223].

1.4.4 Range of metals in uncontaminated soils:

(Table 1.5) lists the range of metals in uncontaminated soils, but, depending on the local geology, the concentration of metals in a soil may exceed the ranges listed here. “Only by direct analysis of uncontaminated soils can background levels of metals be determined” [224]

Table 1.5 Content of Elements in Soils

Metal	Selected Average for Soils (mg/kg)	Common Range for Soils (mg/kg)
Fe	38,000	7,000-550,000
Cu	30	2-100
Zn	50	10-300
Cr	100	1-1,1000
Cd	0.06	0.01-0.70
Ni	40	5-500
Pb	10	2-200

Source: Lindsay, 1979, in McLean and Bledsoe, 1992

1.4.5 Physical- chemical forms of trace metals in the atmosphere:

Trace metals such as As, Cd, Cr, Ni and Pb and their compounds are usually present in the atmosphere associated with particles. Atmospheric particle sizes can be in the range 5 nm-20 μm, although a majority of atmospheric particles are often 0.1-10 μm in diameter and have an average residence time in the atmosphere of 10-30 days [215]. As, mostly trivalent, is emitted both as gaseous species and associated with articulates.

Elemental Cd and its oxides are the predominant forms of this element in different emissions [211]. Aerosols containing Cr (III) or Cr (VI) are generated from specific industries. Ni species present in the atmospheric deposition probably include sulphates and oxides [225]. Vehicles using leaded gasoline emit lead halides primarily in gaseous form which condense to $PbCl_2$, $PbBr_2$ and PbClBr particles. It has been shown that lead is usually emitted from smelters in the form of Pb and PbO. Tetraalkyl lead has also been observed in the gaseous phase in the atmosphere [211].

1.4.6 Forms of Heavy Metals in Soils:

The loading of ecosystems with heavy metals can be due to excessive fertilizer and pesticide use, irrigation, atmospheric deposition, and pollution by waste materials. In natural ecosystems, and especially in wetlands, watershed management plays an important role, determining not only the degree of plant uptake and soil retention of the heavy metals but also the extent to which they are leached into aquifers. A precise knowledge of heavy metals concentrations, the forms in which they are found, their dependence on soil physicochemical properties provide a basis for careful soil management which will limit, as far as possible, the negative impact of heavy metals on the ecosystem. Knowledge of present heavy metal pollution levels within the watershed would be a starting point in estimating the consequences of poor watershed management regimes which may mobilize previously unavailable forms of the heavy metals and lead to their incorporation into the food chain. Heavy metals in soil may be found in one or more of the following forms:

a) Dissolved (in soil solution),

b) Exchangeable (in organic and inorganic components),

c) As structural components of the lattices of soil minerals,

d) As insoluble precipitates with other soil components.

The first two forms are available to the plants while the other two are potentially available in the longer term. Understanding the mechanisms by which a heavy metal element changes from one form to another and the speed at which it does so, is imperfect but improving. In general, the concentration of an element in the soil solution is believed to depend on the equilibrium between the soil solution and solid phase, with pH playing the decisive role [226]. Heavy metals tend to form complexes with organic matter in the soil (humic and fulvic acids), which are different for each metal [227]. Organic matter plays an important role not only in forming complexes, but also in retaining heavy metals in an exchangeable form. These two properties affect each heavy metal differently. For example, Cu is bound and rendered unavailable chiefly through the formation of complexes [228], while Cd is retained in an exchangeable form and is more readily available [229].

1.4.7 Behavior of trace metals in soils:

The behavior of heavy metals in soil depends on many factors such as pH of the soil solution, organic matter content, cation exchange capacity (CEC), Eh and microbiological condition. Of prime importance, however, is whether or not the heavy metals have been incorporated into the soil as inorganic salts or in forms bonded to organic matter [230]. The general risks of environmental pollution is that a heavy metal could enter into food chains depend mainly on: (i) the mount of metal present in the soil solution, (ii) the forms that may be solubilized (e.g. complexes and chelates between heavy metals and humic substances), and (iii) the heavy metals that can be held in exchangeable form on the surface of soil particles [231]. Total contents of heavy metals in soil have been determined, but the results are generally of uncertain interpretation because there is no correspondence between the total content in the soil, absorption by plants and leaching to ground waters [230, 232]. In order to predict the availability to plants, many extractant solutions have been used to assess potential solubility or mobility in the soil of both metals in general and heavy metals in particular[233, 234].

In compost production, determination of total heavy metals represents an important parameter to assess quality, but it is inadequate to predict whether a heavy metal may be absorbed by plants, leached to ground waters or insolubilized in the soil [235]. Chaney [236], for instance, has suggested both the Cd/Zn x 100 ratio and the load of 'zinc equivalent' as toxicity indexes of sludge and town wastes for utilization in agriculture. In addition, the organic matter, and especially the humic substances, form complexes with heavy metals that may both increase or decrease their solubility in soil and consequently their absorption by plants. Both inorganic (e.g. F^{-1}, Cl^{-1}, SO_4^{-2}, and OH^{-1}) and organic matter (e.g. single aliphatic acids, amino acids, sugar acids, and humic substances) constitute ligands present in the soil solution [237]. In this respect, humic substances play the most predominant role in the behavior of heavy metals in soil. Copper forms stronger complexes with humic substances [238], and the relative affinity of heavy metals towards humic substances is generally as follows: $Cu^{+2} > Pb^{+2} > Ni^{+2} > Zn^{+2} > Cd^{+2}$ [239].

1.4.8 Heavy Metals and Soil PH:

In general, adsorption of cationic metals increases with increased pH. The pH of the soil affects “several mechanisms of metal retention by soils” [224]. Soil pH increases the absorption of all cationic metals. However, a study by Harter (1983) of Pb, Ni, Zn, and Cu concluded that the “retention of metals did not significantly increase until the pH was greater than 7” (cited in [224]).

[240] describes soil chemistry and acid-forming features: “soils become more acid when excess hydrogen (H) and aluminum (Al) ions replace basic cations such as Ca, Mg, K, and Na on the surface of clays and soil humans. Cations are positively charged ions and anions are negative charged ions. The basic cations are often leached below the root zone, leaving H and Al behind because they are more strongly attached to the negative charges on the soil surface.” As far as increasing base-forming features, or alkalinity, “any process that will encourage high levels of the exchangeable base-forming cations (Ca, Mg, K, Na) will contribute toward an increase in alkalinity,” including liming,

weathering, content of water, and recycling of cations by deep-rooted plants, which can bring cations to the surface “and incorporate them in the topsoil.” When the soil pH is too high, “deficiencies of iron, manganese, and other micronutrients occur” [240].

According to [224] the “pH dependence of absorption reactions of cationic metals is due, in part, to the preferential adsorption of the hydrolyzed metal species in comparison to the free metal ion. The proportion of hydrolyzed metal species increases with pH”. For the specific metals this report is concerned with, [224] summarize the data (additional sources are noted):

Copper: Copper adsorption by soils shows a stronger pH dependence than Cd. Cu is absorbed to a greater extent by soils and soil constituents than the other metals studied, except Pb. Copper, however, has a high affinity for soluble organic ligands, and the formation of these complexes may greatly increase Cu mobility in soils. Cu hydrolysis at pH 6 increases its retention by soil.

Cu, however, has behaved differently from the other cationic metals in studies, actually decreasing with increased pH, when other factors are present: “The adsorption of Cu to montmorillonite, in the presence of water soluble ligands extracted from sludges and various other organic materials, decreased with increasing pH. This behavior is the opposite of the typical relationship between metal adsorption and pH” [224]. The explanation is that “at low pH, H^+ competes with the Cu for complexation with the organic matter. As the pH increases, more of the Cu can be complexed with the organic matter and less is therefore adsorbed by the clay” [224].

Cadmium: Adsorption mechanisms may be the primary source of Cadmium removal from soils. As with all cationic metals, the chemistry of Cd in the soil environment is, to a great extent, controlled by pH. Under acidic conditions, Cd solubility increases and very little adsorption of Cd by soil colloids, hydrous oxides, and organic matter takes place. At pH values greater than 6, cadmium is absorbed by the soil solid phase or is precipitated, and the solution concentrations of cadmium are greatly reduced. Cd does not hydrolyze until pH 8.

Iron: Fe is considered pH dependent. Fe and manganese (Mn) play a principal role in the retention of metals by soil.

Zinc: Retention of Zn is increased with increasing pH. When the pH was increased above 7.5, however, the solution concentration of Zn increased. The greatest percent of the total Zn in polluted soils and sediments was associated with Fe and Mn oxides in studies. Zinc hydrolyzes at pH greater than 7.7, and these hydrolyzed species are strongly adsorbed to soil surfaces. Zinc forms complexes with inorganic and organic ligands that will affect its adsorption reactions with the soil surface. The normal zinc soil content, says Etherington, is between 10 and 300 μg g^{-1} [241].

Lead: At pH values above 6, Pb is either adsorbed on clay surfaces or forms lead carbonate. Of all the trace elements studied, Pb is retained by soils and soil constituents to the greatest extent under the conditions of these studies. Some have found decreased sorption of Pb in the presence of complexing ligands and competing cations. Lead has a strong affinity for organic ligands, and the formation of such complexes may greatly increase the mobility of Pb in soil.

Nickel: Ni does not form insoluble precipitates in unpolluted soils, and retention for Ni is, therefore, exclusively through adsorption mechanisms. Nickel will adsorb to clays, iron and manganese oxides, and organic matter and is thus removed from the soil solution. The formation of complexes of Ni with both inorganic and organic ligands will increase Ni mobility in soils.

Chromium: Chromium exists in two possible oxidation states in soils: the trivalent chromium, Cr (III) and the hexavalent chromium, Cr (VI). Trivalent Cr is readily absorbed by soils. In a study of the relative mobility of metals in soils at pH 5, Cr (III) was found to be the least mobile. Hydrox species of Cr (III) precipitate at pH 4.5 and complete precipitation of the hydroxy species occurs at pH 5.5. Haxavalent Cr was found to be the only metal studied that was highly mobile in alkaline soils. The parameters that correlated with Cr(VI) immobilization in the soils were free iron oxides, total manganese, and soil pH, whereas the soil properties, cation exchange capacity, surface area, and percent clay had no significant influence on Cr(VI) mobility.

Molybdenum: Molybdenum occurs in natural soils as “the sulphid or the molybdate, usually entering soil solution as the molybdate anion” [241]. “The usual soil content is 1-10 μg g^{-1}, but most of this is insoluble” [241]. “A few calcareous, P-rich soils show excessive Mo availability”[241].

1.4.9 Cation Exchange Capacity (CEC):

The cation exchange capacity is the total amount of exchangeable cations that a soil can absorb. It is sometimes called "total exchange capacity" "base exchange capacity," or "cation adsorption capacity." It is expressed in milliequivalents per 100 grams of soil or of other adsorbing materials such as clay.

Any element with a positive charge is called a cation. The amount of these positively charged cations a soil can hold is described as the CEC and is expressed in milliequivalents per 100 grams (meq/100g) of soil. The larger this number, the more cations the soil can hold. A clay soil will have a larger CEC than a sandy soil.

According to [240], "the nutrient holding capacity of the soil is largely determined by the cation exchange capacity (CEC). Soil colloids with negative charges attract and hold cations (e.g., Ca, Mg, K, Na, Al, NH, H). Humus can hold cations in much the same way as clay minerals because of the negative charges on the humus particles. The clay and humus account for most of the CEC, but finer fractions of silt can have a limited number of exchange sites. The CEC is expressed as milliequivalents (meq) of positive charge that can be held (absorbed) by 100 grams of soil (oven-dry basis) or as centimoles of positive charge per kilogram of soil (cmol/kg); 1 meq/100 g=1 cmol/kg. Sandy soils have a lower CEC than clay soils because the coursetextured soils are commonly lower in clay and humus content. The texture and organic matter of a soil influence the CEC. The CEC of a loam of silt loam soil could range from 10-25 meq/100 g." In general, the CEC of a soil depends upon its organic matter content and clay type and content, the higher the CEC the greater the ability to retain heavy metals. The type and quantity of clay determines the CEC, which increases with clay content, particularly when it contains a high proportion of 2:1 lattice type minerals (e.g., montmorillonite). The specific soil surface is also closely related to clay content and type.

1.4.10 Heavy Metals Mobility:

The soil's ability to immobilize heavy metals increases with rising pH and peaks under mildly alkaline conditions. Heavy metal mobility is related to their immobilization in the solid phase. Fuller [242], in discussing the relatively high mobility of heavy metals with regard to pH, considered that in acid soils (pH 4.2-6.6) the elements Cd, Ni, and Zn are highly mobile, Cr ismoderately mobile, and Cu and Pb practically immobile, and in neutral to alkaline (pH 6.7-7.8), Cr is highly mobile, Cd and Zn are moderately mobile and Ni is immobile. Apart from pH, othersoil properties, such as cation exchange capacity (CEC), organic matter content, quantity and type of clay minerals, the content of the oxides of iron (Fe), aluminum (Al), and manganese (Mn), and the redox potential determine the soil’s ability to retain and immobilize heavy metals. When this ability is exceeded, the quantities of heavy metals available to plants increase, resulting in the appearance of toxicity phenomena.

Generally, metals added to soil will stay at the soil surface. Movement to groundwater, surface water, or the atmosphere is minimal “as long as the retention capacity of the soil is not exceeded” [224]. Metals movement in soil is “related to the solution and surface chemistry of the soil and to the specific properties of the metal and associated waste matrix” [224]. Metals in soil are “subject to mass transfer out of the system by leaching to groundwater, plant uptake, or volatilization,” [224]. “At the same time, metals participate in chemical reactions with the soil solid phase. The concentration of metals in the soil solution, at any given time, is governed by a number of interrelated processes, including inorganic and organic complexation, oxidationreduction reactions, precipitation/dissolution reactions, and adsorption/desorption reactions.”

Whether or not metals will move from the soil to the groundwater depends much on the type of soil. Generally, metals do not travel downward from the soil surface to any great extent. [224] explain that are metals movement in soil is directly related to the surface chemistry of the soil matrix and soil solution. When metals are introduced at the soil surface, “downward transportation does not occur to any great extent unless the metal retention capacity of the soil is overloaded, or metal interaction with the associated waste matrix enhances mobility.

Changes in soil environmental conditions over time, such as the degradation of the organic waste matrix, changes in pH, redox potential (Eh), or soil solution composition, due to various remediation schemes or to natural weathering processes, also may enhance metal mobility.” Metals associated “with the aqueous phase of soils are subject to movement with soil water, and may be transported through the vadose zone to groundwater” [224].“Metals, unlike the hazardous organics, cannot be degraded.” However, some metals, such as Cr, can be transformed to other oxidation states in soil, reducing their mobility and toxicity [224].

1.4.11 Soil Organic Matter and Mobility:

According to [223] Soil organic matter consists of the following:

- **Living organisms:** Soil organic matter can be the main source of soil cation exchange capacity, contributing more than 200 meq/100 g of organic matter in surface mineral soils. Organic matter content, however, decreases with depth, so that the mineral constituents of soil will become a more important surface for sorption as the organic matter content of the soil diminishes.

- **Soluble biochemicals :** (amino acids, proteins, carbohydrates, organic acids, polysaccarides, lignin, etc). The biochemicals form water-soluble complexes with metals, increasing metal mobility.
- **Insoluble humic substances:** The humic substances consist of insoluble polymers of aliphatic and aromatic substances produced through microbial action. Binding of metals to organic matter involves a continuum of reactive sites, ranging from weak forces of attraction to formation of strong chemical bonds. The Fe and Mn oxides are the principal soil surface that controls the mobility of metals in soils and natural water [224].

1.4.12 Soil Properties Affecting Adsorption:

“Adsorption of metal cations has been correlated with such soil properties as pH, oxidation-reduction (redox) potential, clay, soil organic matter (SOM), Fe and Mn oxides, and calcium carbonate content” [224]. Adsorption processes are also affected by the form of the metal added to the soil and by the solvent introduced along with the metal [224]. These interactions can either increase or decrease the movement of metals in soil water. Soils with heavier textures and higher pHs are effective in attenuating metals, “while sandy soils and/or soils with low pH did not retain the metals effectively” [224]. The lead and copper are the least of the cationic metals [224]. Chromium is considered quite mobile.

1.4.13 Potential contaminants by heavy metals:

Most metals and metalloids are present in trace amounts in soil and water. These trace elements occur naturally as a result of the weathering of rocks. They can be leached into surface water or groundwater, taken up by plants, released as gases into the atmosphere, or bound semi-perma nently by soil components such as clay or organic matter. Metals appear in the municipal solid waste stream from a variety of sources. Batteries, consumer electronics, ceramics, light bulbs, house dust and paint chips, lead foils, plastics and some inks and glass can all introduce metal contaminants into the solid waste streams. Similarly, sludge from tannery, textile and food industries can potentially contain much of the polluted chemical evidence of that environment. Thus, composts made from recycled organic material will inevitably contain Potentially Toxic Elements (PTEs). In small amounts, many of these elements may be essential for plant growth; however, in higher concentrations they are likely to have a detrimental effect upon plant growth. For instance, cadmium (Cd) is not essential for plants or animals and although it is phytotoxic when added to acidic soils, it has not been found to be toxic to plants under natural conditions [243]. Cd up-take differs among plant species and among cultivators within species, and accumulation varies in the different plant organs as follows: leaves > storage roots > fruits and grain [243]. Chromium (Cr) is also a naturally occurring elements found widely in rocks, soils, and plants. Due to its low solubility, only a little (Cr) is bioavailable, which means that even when crops are grown in soils treated with sludge relatively high in Cr, phytotoxicity is rarely observed [244].

Copper (Cu) is essential for plants and animals. The natural concentration of copper found in plants usually ranges from 5-25 mg/kg (dry weight). A concentration below this level will render the plant deficient in Cu, while a concentration above 25 mg/kg will promote phytotoxicity. However, when biosolids and compost with normal Cu concentration have been land applied, even at very high cumulative loading rates, no evidence of Cu phytotoxicity was observed [245]. Only when biosolids with very high Cu concentration (2000mg/kg) were applied to strongly acidic soils did Cu phytotoxicity occur in sensitive crops [245].

Lead (Pb) is not essential for plants or animals, and it can be toxic to both. Plant tolerance to it is very high because Pb is strongly adsorbed by soils. When the soil fertility is appropriate for crop production, plants tend to take-up little soil Pb; because phosphate inhibits Pb transport [245].The potential risk from Pb in compost is not through plant uptake of compost-applied Pb, but rather through direct soil ingestion of Pb by children or livestock, or the result of deposition of edible plant portions [245]. Zinc (Zn) is essential for a wide variety of plants and animals. A Zn tissue concentration of less than 15 mg/kg (dry weight) leaves the plant deficient, while concentration over 400 mg/kg (dry weight) is potentially phytotoxic [245]. Zinc tolerance in animals is affected by a variety of elements, including Cu, Mn, Fe, Pb and Cd. Zinc toxicity in plants occurs before reaching concentration in tissues that could be harmful to humans. Over toxicosis of Zn is seldom observed until levels in excess of 1000 mg/kg are reached [245]. As such, this study aimed to make a preliminary investigation into the fate of Cr, Cd, Cu, Pb, and Zn as potentially toxic elements, throughout the process of composting of tannery sludge.

1.5 SAMPLING OF SOIL FOR HEAVY METALS ANALYSIS:

Spatial [246, 247] and seasonal variability [248-250] are known to influence significantly the results of sequential extraction schemes in soils. Wenzel et al [251] showed that no general trend exists that would predict mobile metal fractions to have more pronounced partial variability than less mobile ones. Despite limitations in comparability of data, this may be explained by the influence of variation in total metal concentrations. The opposite effects of the spatial variation are in factors governing metal solubility (e.g., pH and organic matter contents). Accordingly, the spatial variability of mobile metal fractions may either be increased or decreased by these factors. The coefficients of variation for metals extractable by neutral salt solutions or complexing agents are usually high, often exceeding 50 %, limiting the potential use of these extractants for monitoring temporal changes of metal mobility for environmental or soil management purposes. For total Pb, this problem was addressed by Schweikle [252] given coefficients of variation (CV) of extractable metals are up to 340 %. This problem has to be faced when soil tests for bioavailability or ecotoxic relevant metal fractions are designed, i.e., for legislative purposes.

Soil management practices (fertilizing, liming, sludge application) may cause significant seasonal changes in mobile fractions, but also natural seasonal variation of extractable metals in extensively used forest and range soils or undisturbed ecosystems may occur as well [253-256]. Seasonal variation of extractable metals is an inherent process that is at least as significant as spatial variability [257-259]. Due to the variation in weather conditions, seasonal patterns of extractable fractions are not necessarily predictable from a few years observation and may differ from site to site. Accordingly, there is a clear possibility of obtaining biased results when sampling only once. Distinction should also be made between sampling of (1) natural, agricultural, grassland, forest, or moorland soil where to some extent element distribution and speciation can be regarded as homogeneous and (2) industrially contaminated soils will usually have an element distribution and speciation that is heterogeneous not only over the surface area but also with depth. In the first case, representative samples of the area topsoils may be required.

In the second case, statistical sampling may be desirable but will often be uneconomic, and the so-called judgmental sampling using selected pit sampling of soil profiles may be required. Soil properties may vary considerably on a micro scale of about 1 to 100 mm. Thus, metal solubility and extractability may be affected either directly by micro inhomogeneity of the total metal contents or by simultaneous variation in soil properties (pH, CEC, organic matter, mineral composition, and soil texture). Differences in the fraction of outer- and inner-sphere aggregates may be caused either by natural processes of soil formation or by anthropogenic inputs. It was found that moderately acidic soils with high silt and clay contents had significantly higher CEC and exchangeable Mg (0.1 mol/l $BaCl_2$), but lower amounts of exchangeable Ca and K in the outer sphere aggregates [256, 260].

As indicated by higher levels of exchangeable Al and lower amounts of basic cations, aggregate surfaces are frequently more acidified than homogenized bulk soil, particularly in well-aggregated soils low in basic cations [261]. This is also reflected by higher concentrations of Al^{3+}, Fe^{2+}, and H^+ ions in the saturation phase of acidic soils [261-265]. Wilcke et al [266] revealed that the sorption capacity of the outer-sphere aggregates in acidic soils is lower than that of the inner sphere. Total and mobile Pb fractions were usually enriched on aggregate surfaces, probably due to widespread Pb deposition [266]. It has been concluded that the mobility of metals may frequently be underestimated when assessed by chemical extraction of disturbed, homogenized, and sieved soil samples of well aggregated, acidic soils, particularly when anthropogenically polluted, and probably overestimated in soils with organic fillings and linings in macropores. These chemical effects are obviously confused with transport nonequilibria in aggregated soils [267-269]. That should commonly lead to lower metal concentrations in the real soil solution than predicted by structure destroying equilibrium methods, i.e., the saturation phase.

1.6 STORAGE AND PREPARATION OF SOIL SAMPLES FOR HEAVY METALS ANALYSIS:

Sample preparation generally involves the following steps: (1) drying or rewetting, (2) homogenizing and sieving, (3) storage, and, occasionally, (4) grinding. Usually, soil samples are air-dried prior to extraction. Although changes in the extractability of some elements (i.e., of Mn) have been reported earlier [270], this problem only recently received more attention [271-276]. Air-drying prior to extraction is a standard procedure, but leads to an increased extractability of Fe and Mn, whereas other metals are more or less unaffected [271-276]. As the effect of air-drying depends on soil properties and the initial moisture conditions, no general regression equations are available for prediction of metal levels in the field moist soils from analysis of air-dried samples.

Since extraction of field moist samples cannot be recommended for routine analysis, individual relations on a local or regional scale should be obtained to avoid errors in the determination of mobile pools of Mn and other metals in soil. Several authors identified possible mechanisms of these changes in metal extractability upon air-drying. The observed decrease in easily reducible (oxidic) Mn-fraction was related to (i) dehydration of Mn oxides [270], (ii) reduction of Mn-oxides by organic matter [277], and (iii) alterations of soil functional groups that were forming unavailable Mn complexes [278]. In summary, drying of samples prior to the determination of mobile metal fractions usually results in unrealistically large amounts of extractable Mn, Fe, Cu, and Zn, and underestimation of Ca, Mg, K, and probably Co, Ni, and V. The changes in extractability upon air-drying are related to soil properties (i.e., pH and organic matter content) and to the initial soil moisture conditions. Prediction of changes in metal extractability upon air-drying seems to be possible for most metals when individually based on selected soils of a data set.

Although homogenizing and sieving are essential steps in performing representative and repeatable soil analysis, these procedures suffer from some serious drawbacks. Firstly, the effects of structure disturbing soil sampling are obviously reinforced, thus creating new surfaces for reactions with metals in the solute phase, giving raise to adverse readsorption or desorption processes during metal extraction [279]. Secondly, homogenization of soil material from different horizons may result in erroneous changes in pH and carbonate content of the fine earth. In soils with high variability on a microscale, sieving and homogenization may cause erroneous results (i.e., by the destruction of weathered rock fragments or carbonate nodules).

Navo et al. [280] reported frequent nitrification during storage of air-dried samples to nonmicrobial changes in the physical structure (i.e., to an increase in the surface area of the organic fraction). Based on these results, Wenzel et al. [256] concluded that Mn was continually mobilized through the reduction of Mn-oxides by electron transfer from newly created organic surfaces. Accordingly, air-drying may reduce microbial activity in soils effectively, but physical changes of the organic fraction may affect the extractability of Mn and probably of other metals sensitive to changes in the redox potential.

As a conclusion, sample storage seems to be generally less critical to the analysis of extractable metal fractions than air-drying, but it is likely to enhance the effects of air-drying in the case of redoxsensitive elements. Occasionally, soil samples are ground prior to extraction. This procedure causes physical breakdown of soil microaggregates, thus potentially altering the extractability of metals from soil samples [271]. The exposure of fresh surfaces may, depending on soil properties, increase the extractability of some metals, but potentially may also cause readsorption of metals during the batch process [271].

1.7 HEAVY METAL AND TRACE METAL ANALYSIS IN SOIL:

1.7.1 Sequential Extraction Techniques:

Sequential extractions have been applied using extractants with progressively increasing extraction capacity, and several schemes have been developed to determine species of the soil solid phase. Although initially thought to distinguish some well-defined chemical forms of trace metals [281, 282], they rather address operationally defined fractions [279, 283]. The selectivity of many extractants is weak or not sufficiently understood, and it is questionable as to whether specific trace metal compounds actually exist and can be selectively removed from multicomponent systems [284]. Due to varying extraction conditions, similar procedures may extract a significantly different amount of metals. Concentration, operational pH, solution/solid ratio, and duration of the extraction affect considerably the selectivity of extractants. The conventional approach of equilibration during a single extraction step is the shaking or stirring of the solid-phase/extractant mixture. Recently, an accelerated extraction has been presented using an ultrasonic probe [285]. The resolution sought in the chemical fractionation depends on the purpose of the study, as does the choice of the single extractant in each step in a sequential scheme. The selectivity of the procedure can be considerably improved by incorporation of the various nonselective single extraction steps into a carefully designed sequential extraction scheme.

There is no general agreement on the solutions preferred for the extraction of various components in sediment or soils, due mostly to the matrix effects involved in heterogeneous chemical processes [286]. The aim of the study, the type of the solid materials and the elements of interest determine the most appropriate extractants. Partial dissolution techniques should include reagents that were sensitive to only one of the various components significant in trace metal binding. In sequential multiple extraction techniques, chemical extractants of various types are applied successively to the sample, each follow-up treatment being more drastic in chemical action or different in nature from the previous one. Selectivity for a specific phase or binding form cannot be expected for most of these procedures. In practice, some major factors may influence the success in selective leaching of components, such as:

- The chemical properties of the extractant chosen,
- Experimental parameter,
- The sequence of the individual steps,
- Specific matrix effects such as cross-contamination and readsorption, and
- Heterogeneity, as well as physical associations (e.g., coatings) of the various solid fractions.

All these factors have to be critically considered when an extractant for specific investigation is Chosen. Fractions of sequential extraction schemes include the following:

- Exchangeable fractions: Most of the recommended protocols seek to first displace the exchangeable portion of metals as a separate entity using $MgCl_2$ or NH_4Ac (pH = 7) treatments.
- Bound to carbonates: Removal of carbonates using HAc, with or without buffering by NaAc (pH 5).

- Easily reducible fractions: $NH_2OH*HCl$ at pH 2 is generally used, but procedures differ in minor operational details such as solid/solution ratios, treatment time, and interstep washing procedure.
- Oxidizable oxides and sulfides fractions: H_2O_2/NH_4Ac is used most frequently.
- Residual minerals: Strong acid mixtures are applied ($HF/HClO_4/HNO_3$) to leach all remaining metals.

The fractions of a sequential extraction procedure can be divided into the following steps:

- MOBILE FRACTION: this fraction includes the water-soluble and easily exchangeable (nonspecifically adsorbed) metals and easily soluble metallo-organic complexes. Chemicals used for this fraction fall commonly in one of the following groups [279, 287]:

1. Water or highly diluted salt solutions (ionic strength < 0.01 mol/l).

2. Neutral salt solutions without pH buffer capacity (e.g., $CaCl_2$, $NaNO_3$).

3. Salt solutions with pronounced pH buffer capacity (e.g., NH_4Ac).

4. Organic complexing agents (e.g., DTPA, EDTA-compounds).

- EASILY MOBILIZABLE FRACTION: This fraction contains the specifically bound, surface occluded species (sometimes also $CaCO_3$ bound species and metallo-organic complexes with low bonding forces).
- CARBONATE-BOUND FRACTION: To dissolve trace elements bound on carbonates, commonly buffer solutions (e.g., HAc/NaAc; pH = 4.75) are used. Zeien et al. [288] proposed to dissolve carbonates by adding equivalent amounts of diluted HCl to 1 mol/l NH_4Ac/HAc-buffer, addressing specifically adsorbed and surface-occluded trace element fractions of soil with 5 % m/m carbonates.
- ORGANICALLY BOUND FRACTION: Various approaches for the dissolution of organic bound elements are known: (i) release by oxidation, (ii) release by dissolution, and (iii) addition of competing ligands. Different methods extract the organically bound fraction before the oxide fraction, before the carbonate-bound fraction or directly after the carbonate-bound fraction or after the oxide-bound fraction. The organically bound fraction itself can again be divided into up to three separate fractions [283].
- Mn-OXIDE BOUND FRACTION: This fraction is sensitive to drying procedures prior to extraction. They are most susceptible to changes in pE and pH. Trace metals

bond to Mn-oxide may be readily mobilized upon changed environmental conditions. This fraction is to be separated prior to Fe- or Al-oxides.

- Fe- and Al-OXIDE BOUND FRACTION: In this fraction, the Fe-bound fraction can also be distinguished in AMORPHOUS Fe-BOUND FRACTION and CRYSTALLINE Fe-BOUND FRACTION.
- RESIDUAL FRACTION: This fraction mainly contains crystalline-bound trace metals and is most commonly dissolved with high concentrated acids and special digestion procedures.

1.7.1.1 Main parameters for a sequential extraction schemes**:**

A wide range of extraction procedures is readily available for different metals and variations of the extraction conditions are utilized due to varying sediment and soil composition. The following points have to be considered when designing an adequate extraction procedure:

- *Extractants*: Chemical and physical interferences both in extraction and analysis steps, respectively.
- *Extraction steps*: Selectivity, readsorption processes, and redistribution processes. If the single extractants for the different steps are chosen with respect to their ion-exchange capacity or reduction/ oxidation capacity, each step has to be designed individually following special considerations [256].
- *Concentration of the chemicals*: The efficiency of an extractant to dissolve or desorb trace metals from sediment and soils will usually be increased with increasing concentration or ionic strength. Thermodynamic laws predict the efficiency of an extractant to dissolve or desorb trace metals from solid samples [289-293].
- *Extraction pH*: Extractants with a large buffering capacity or extractants without buffer capacity can be used [289, 293-297].
- *Solution/solid ratio and extraction capacity*: The relative amount of extractant added to the sediment and soil has various implications on the results. Essentially, Wenzel et al. (256) distinguished four cases, e.g., (1) pure dissolution of metal compounds according to the solubility product, (2) pure ion exchange by 0.1–1 mol/l neutral salt solutions, or (3) by water or highly diluted neutral salt solutions (<<0.1 mol/l), and (4) combinations of (1) with either (2) or (3). If, over a sufficiently wide solution/solid ratio, the capacity of the extractant to dissolve a metal fraction exceeds its total amount

present in the solid sample, then the metal concentration in the extract (mg/l extract) will decrease with an increase in solution/solid ratio.

However, the total amount (mg/kg) extracted will be constant with increasing solution/solid ratio. Nevertheless, as sediment and soils are multiphase/multicomponent systems, dissolution of other compounds due to the nonselectivity of the extractant may confuse this behavior [289, 290,298-302] Wenzel et al. [256] concluded that the efficiency of mild reagents for extraction of abundant metal cations (e.g., Ca, Al, Mg, K) usually increased by increasing the solution/soil ratio, although often the concentrations in the extract concurrently decreased. With stronger reagents, this should also be valid for the more abundant metal cations as long the capacity of the extractant to dissolve a particular compound exceeded the amount present in the soil.

- *Extraction time and batch processes*: The effect of extraction time is related to the kinetics of the reactions between solid sample and extractant. Extractions may be predominantly based either on desorption or dissolution reactions. For desorption of metal cations from heterogeneous soil systems, Sparks [303] identified four rate-determining steps, e.g., (i) diffusion of the cations in the (free) bulk solution, (ii) film diffusion, (iii) particle diffusion, and (iv) the desorption reaction. Accordingly, the rates of most ion-exchange reactions are film- and/or particle diffusion-controlled. Vigorous mixing, stirring, or shaking significantly influences these processes. Film diffusion usually predominates with small particles, while particle diffusion is usually rate-limiting for large particles.

Dilute solutions usually favor film-diffusion-controlled processes. The time to reach equilibrium for ion exchange on soils varies between a few seconds and days and is affected by soil properties [304]. For mineral dissolution, essentially three rate controlling steps have been identified, e.g., (i) transport of solute away from the dissolved crystal (transport-controlled kinetics), (ii) surface reaction-controlled kinetics where ions are detached from the surface of crystals, and (iii) a combination of both [304]. Batch processes (e.g., stirring or shaking) increase the rate of transport-controlled reactions, while they do not affect surface-controlled reactions. Shaking and other batch processes may enhance the dissolution of readily soluble salts effectively, but are unlikely to affect the dissolution rate of less soluble minerals. Experiments reported by several authors generally revealed an increase of the extractable amounts of metals with time of extraction as expected from the theory of reaction kinetics [289, 291, 293, 305-308].

• *Extraction temperature:* Within the normal range of extraction temperatures (20–25 °C or roomTemperature), the effect of temperature on metal extractability is usually small, but has to be considered for interpretation of small differences [293, 306]. Finally, the whole procedure has to be optimized with regard to selectivity, simplicity, and reproducibility.

1.7.2 Standardization and standardized sequential extraction procedure as proposed by BCR:

Sequential extraction schemes have been developed during the past 20 years for the determination of binding forms of trace metals in sediment. The lack of uniformity of these schemes, however, did not allow the results so far to be compared worldwide or the procedures to be validated. Indeed, the results obtained by sequential extraction are operationally defined (i.e., the "forms" of metals are defined by the procedure used for their determination). Therefore, the significance of the analytical results is related to the extraction scheme used. Another problem, which hampered a good comparability of data, was the lack of suitable reference materials that precluded control of the quality of the measurements. Thus, standardization of leaching and extraction schemes is required, which goes hand in hand with the preparation of sediment and soil reference materials that are certified for their contents of extractable trace element, following standardized single and sequential extraction procedures [309]. Owing to thisLack of comparability and quality control, the Community Bureau of Reference (BCR, now Standards, Measurements and Testing Program) has launched a program of which one of the aims was to harmonize sequential extraction schemes for the determination of extractable trace metals in sediment [310]. This program involved the comparison of existing procedures tested in two interlaboratory exercises, and it developed into a certification campaign of extractable trace element contents in a sediment reference material, following a three-step sequential extraction procedure duly tested and adopted by a group of 18 EUR laboratories.

The significance of the analytical results depends on the "operationally defined characters" of the used extraction schemes, which requires the use of standardized protocols. Moreover, those schemes have to be validated and require the preparation of certified reference materials with certified contents of leachable elements if analyzed following standardized single and sequential extraction procedures [309]. BCR has proposed a standardized 3-stage extraction procedure (BCR EUR 14763 EN), which was originally developed for the analysis of heavy metals in sediments [311]. This procedure is currently used and evaluated also as extraction method for soils [312, 313]. So far, the BCR procedure has been successfully applied to a variety of sediment [314-318], sludge [318], and soil samples [312, 319].

The BCR scheme was recently used to certify the extractable trace element contents of a certified reference material (CRM 601, IRMM). Although this procedure offers a tool for obtaining comparable data, poor reproducibility and problems with lack of selectivity were still reported [312, 320-323]. Various research groups used this technique and found partially discrepancies when applying the scheme. The same extraction scheme was used for the determination of extractable elements in soils, as well [314-324]. Sahuquillo et al. [325] investigated potential sources of irreproducibility when applying the BCR three-stage procedure to the lake sediment CRM 601. Factors such as the type of acid used for pH adjustment, temperature, and duration of extraction did not affect the precision. The most critical factor was the pH of step 2 (NH_2OH*HCl extraction).

Improved precision could be obtained when the NH_2OH*HCl concentration was increased from 0.1 to 0.5 mol/l and the centrifugation speed was doubled [320]. The use of filtration did not affect the reproducibility, but it was not recommended since it promoted the dissolution of nontargeted phases. Neither ammonium hydrogen oxalate nor oxalic acid proved suitable alternatives in step 2 owing to precipitation of insoluble lead salts, particularly in the presence of calcium. A modified BCR procedure incorporating these changes has been applied to a sludge-amended soil (CRM 483) and provides indicative values for Cd, Cr, Cu, Ni, Pb, and Zn. It also recommends the use of an aqua regia digestion of the residue after the three steps of the extraction procedure for comparison with an aqua regia digestion of the original material. This approach is often referred to as “pseudototal digestion”. This is a vital quality control procedure.

There is an increasing tendency to establish the “mass balance” of a sequential extraction, namely to compare the sum of the steps with the results of a separate total or pseudototal digestion. A comparison with the Tessier procedure showed identical correlation between the metals extracted in the corresponding steps of the BCR and the Tessier procedure.

1.7.2.1 Sequential extraction schemes applied to soil samples**:**

To assess the metal mobility of trace elements in soils on different time scales, a wide range of extraction schemes have been employed [276, 279, 305]. These methods vary with respect to the extraction conditions: chemical nature and concentration of extractants [279], solution/soil ratio, operational pH, and extraction time. If more than one extractant is used, differences occur due to variation in the extraction sequence. The most critical steps are soil sampling, sample preparation, and the selectivity and accuracy of the extraction procedure [276]. As for total metal concentrations, spatial heterogeneity [326], as well as seasonal variation of extractable metal fractions [276] may bias the results. The use of correlation coefficients for choosing extractants for assessment of plant availability of elements needs consideration [327]. For extraction of the exchangeable fraction, almost all possible combinations of major cations with either Cl^-, NO_3^-, or acetate has been used, with concentrations ranging between 0.05 and 1 mol/l, and pH in the neutral range. The solution/soil ratios vary from 4:1 to 100:1, the extraction times between 30 min and 24 h. In ideal systems, the relative exchangeability of trace metals is determined by the affinity of the exchanging cation for the soil solid phase.This affinity increases with increasing valency and decreasing radius of the hydrated cation [305].

Although heterogeneous soil systems may deviate from this ideal behavior, the selectivity of soils for cations was frequently observed to increase according to Na < K < Mg < Ca. Consequently, under comparable conditions (e.g., concentration, extraction time, soil/solution ratio, the efficiency of cations to exchange trace metals usually increases according to Li < Na < K < Mg < Ca < Ba <La) [304]. Because Mg is a harder Lewis acid than Ca, it was reported to displace also specifically adsorbed trace metals [328]. Therefore, Ca- and Ba-salts are regarded as most effective and selective agents in extracting exchangeable trace metals. Unfortunately, both cations may cause serious background problems (interferences) during determination of Pb and other trace metals.

Usually, this can be resolved only by dilution of the extracts by >1:10 prior to measurement decreasing the detection limits by the same ratio [329]. For that reason, the use of easily volatilizable salts (i.e., $MgNO_3$ or NH_4NO_3) has been proposed [282, 288, 330-332]. Compared to 0.1 mol/l solutions of $CaCl_2$ or $BaCl_2$, 1 mol/l NH_4NO_3 in 2.5:1 ratio was found to extract about equal amounts of Al, Fe, Mn, Ni, Pb, V, and Zn, while Cu at concentrations of > 0.1 mol/l, and Cd were less efficiently extracted by 1 mol/l NH_4NO_3[287]. This can be explained by a more effective extraction with $CaCl_2$ through the formation of chlorocomplexes with Cd and Cu. Other anions frequently used are either acetate or nitrate. At equal concentrations, the complexing ability increases in the order nitrate < chloride < acetate. The selectivity for extraction of the unspecifically sorbed (exchangeable) fraction should be decreased in the same order.

The specifically sorbed fraction is explicitly addressed only by few methods. In addition to other differences, the wide range of cations used suggests that most methods do not address any "specifically sorbed" fraction, and, likely even do not extract the same operationally defined solubility class. To extract specifically sorbed trace metals, $Pb(NO_3)_2$ seems to be most adequate, due to its low p*K* (7.7) and large atomic radius, and it is being effective in displacing other trace metals, i.e., Cd (p*K* = 10.1), Ni (p*K* = 9.9), Co (p*K* = 9.7), Zn (p*K* = 9.0) and Cu (p*K* = 7.7), with smaller atomic radius than Pb [333]. Pb $(NO_3)_2$ was found to extract less metal than HAc, probably indicating that the later was more specific [334]. For similar reasons, Cu $(Ac)_2$ was chosen by Mandal et al. [335]. Unfortunately, those trace metals being constituents of the extractants cannot be determined. Therefore, Zeien et al. [288] proposed 1 mol/l NH_4Ac and 1 mol/l NH_4NO_3 in sequence to extract an operationally defined fraction under optimized analytical conditions by using only one cation (NH_4^+) and decreasing the pH throughout the extraction sequence.

Among the extractants most frequently used to dissolve trace metals bound to carbonates are acids such as HCl and HAc (pH = 3–3.5) {(3 < pH < 3.5) would be better}, buffer solutions of HAc/NaAc (pH = 5), and the buffering complexing agent Na_2EDTA, at pH = 4.6. Any of these extractants seems to have some potential to extract carbonates from soils, but is probably neither effective for quantitative dissolution (i.e., CH_3COOH buffers), nor selective (cold diluted acids, i.e., HCl), or both, i.e., Na_2EDTA [305]. Zeien et al. [288] proposed a procedure to dissolve carbonates by adding equivalent amounts of diluted HCl to a 1 mol/l NH_4Ac/HAc buffer, addressing the specifically sorbed and surface occluded trace element fraction of soils with < 5 % carbonates. For extraction of organically bound trace metals, various approaches were used e.g., their release by oxidation or dissolution of the organic matter or through addition of competing, e.g., complexing or chelating ligands.

Among the oxidizing extractants, H_2O_2, either purely or combined with HNO_3 or NH_4Ac, extracts more trace metals from soils than NaOCl [305], i.e., from Fe/Mn-oxides [336]. $K_4P_2O_7$ and $Na_4P_2O_7$ were reported to dissolve organic matter by dispersion and to efficiently complex the released metals [337, 338]. Again, there is evidence from Mössbauer spectrometry [339] and other investigations that, depending on the extraction conditions [340], these extractants dissolve trace metals also from amorphous Fe-oxides [337, 341], or from organo-mineral associations [339, 342]. Accordingly, the variation in extraction parameters with concentrations between 0.1 mol/l and 1 mol/l, solution/soil ratios between 10:1 and 100:1, and extraction times from 1 h to 24 h indicate that results obtained by different procedures are hardly comparable and are likely to extract non-organically bound trace metals to a varying extent. $K_4P_2O_7$ was found to extract more metals when used before Mn-oxide extraction with $NH_2OH*HCl$, while the latter extractant has little effect on the organically bound fraction [334]. Appropriately, pyrophosphate should be used first. Nevertheless, some methods followed the reverse extraction sequence [343, 344].

As an alternative to pyrophosphate salts, some procedures employ NaOH [281] or NaOH/EDTA mixture [345] to extract organically bound trace metals by dissolution of organic matter. The selectivity of these methods is considered low, and the extracted metals may precipitate as hydroxides [305]. Alternative to destruction of the organic ligands, organically bound trace metals may be extracted by competing synthetic chelates (e.g., EDTA or DTPA) [312]. In sequential extractions, EDTA [278] or its ammonium salt [288] was less frequently used than the advantages would suggest. As NH_4EDTA, adjusted with NH_4OH to pH 4.6, was reported to dissolve considerable amounts of amorphous sesquioxides, it may be less selective than some pyrophosphate methods. Nevertheless, it should be considered as an alternative to extractants with alkaline pH (e.g., $Na_4P_2O_7$, $K_4P_2O_7$, NaOH or NaOCl). Thus, NH_4ETDA (pH 4.6) can be fitted in a sequence of extractants with decreasing pH that is thought to increase the selectivity by minimizing adverse effects on each subsequent extraction step (i.e., readsorption or precipitation of trace metal compounds) [288]. Moreover, the procedure is nondestructive to organic matter and organo-mineral associations, thus creating no new surfaces that may cause adsorption of trace metals during subsequent extraction steps as discussed by Beckett [279]. The dissolution of amorphous sesquioxides is probably limited by choosing a reasonable extraction sequence, extracting organically bound trace metals after removal of the most labile oxide fraction (e.g., the Mn-oxides) [307, 334], and by a comparably short extraction time of 90 min, as proposed by Zeien et al. [288]. Accordingly, good correlation were found between organic carbon and NH_4ETDA-extractable metal fractions [346], although there was evidence that EDTA extractants may dissolve trace metals from (amorphous) sesquioxides. Among sesquioxides, the Mn-oxides are most susceptible to changes in pE and pH.

Therefore, trace metals bound to Mn-oxides (i.e., Pb) may be readily mobilized upon changed environmental conditions (e.g., flooding) [343, 347]. For that reason, this environmentally significant fraction is separated prior to Fe- and Al-oxides by most sequential extraction procedures. Essentially, Mn-oxides were extracted by reducing agents, e.g., $NH_2OH*HCl$ or hydroquinone, either pure or mixed with NH_4Ac, HAc, or diluted HNO_3. Some procedures extract Mn-oxides by 0.1 mol/l $NH_2OH*HCl$ in 0.01 mol/l HNO_3 (pH = 2) as initially proposed by Chao [307], or modified, i.e., 0.01 mol/l $NH_2OH*HCl$ in 0.1 mol/l HNO_3 (also reported at pH = 2). Results indicate that the modified method is probably less selective to Mn-oxides [307]. Chao’s method dissolved about 50 % of total Mn and < 1 % of total Fe from highly weathered soils when applied without previous extraction of exchangeable and specifically sorbed trace metals [307], indicating a good selectivity for Mn-oxides.

Conversely, with higher concentrations (i.e., 0.25 mol/l) and higher temperatures during extraction (i.e., 50–100 °C), $NH_2OH*HCl$ extracted considerable amounts of trace metals from sesquioxides with a wide range of crystallinities [279]. As intended by Tessier et al. [281], 0.04 mol/l $NH_2OH*HCl$ in 25 % HAc (pH = 2 at 85 °C for 5 h) actually should extract most of the sesquioxides, including the crystalline fractions. Zeien et al. [288] proposed 0.1 mol/l $NH_2OH*HCl$ in 1 mol/l NH_4Ac (pH 6, 30 min at 20 °C), although Chao [307] selected pH 2 to avoid hydrolysis and subsequent readsorption or precipitation of trace metals. Nevertheless, the procedure of Zeien et al. [288] seems to be comparably selective, hence it dissolves on an average 37 % (0.12–73.9 %) of total Mn, but only 0.02–2.9 % of total Fe from a variety of soils. A negative correlation among Fe and Mn extracted by 0.1 mol/l $NH_2OH*HCl$/1 mol/l NH_4Ac (pH

= 6) indicated that only for low levels of Mn-oxides present in the soil, this reagent dissolved some Fe-oxides up to 2.9 % of total Fe. Since $NH_2OH*HCl$ has little effect on the organically bound metal fraction, it should be applied prior to extractants like $K_4P_2O_7$, $Na_4P_2O_7$, NH_4EDTA [334]. Instead of $NH_2OH*HCl$, some authors [307, 348] proposed a mildly reducing mixture of 0.2 % hydroquinone and 1 mol/l NH_4Ac, dissolving considerably less Mn than $NH_2OH*HCl$ [279]. Applied repeatedly, hydroquinone may be used to partitioning of “easily reducible” and more “resistant” Mn-oxides [278]. In this context, it should be remembered that, along with sorbed fractions, the Mn-oxides are most sensitive to drying procedures prior to extraction, usually causing underestimation of the Mn- oxide fraction upon air-drying. The release of Mn and associated trace metals may be partly reversible, given sufficient time for equilibration after rewetting [272]. Since the required period of equilibration was frequently found to be about one week, the selectivity should be considerably improved by rewetting the soils and allowing them to equilibrate prior to sequential extraction.

Trace metals bound to Fe- and Al-oxides were extracted either by one step or were partitioned in two fractions, referred to as amorphous and crystalline Fe-oxides. Essentially, trace metals bound to amorphous Fe-oxides were removed by various modifications of Tamm’s [349] acid oxalate solution in the dark [279]. In addition to X-ray amorphous Fe-oxides [350], acidic ammonium oxalate in the dark has been claimed to extract fulvic Fe complexes [337, 342] and magnetite [350], but only little crystalline goethite or hematite [351]. This indicates that the selectivity of the method may be satisfactory as long as the extraction time is between 3–48 h [350, 352]. The effects of variation in other extraction parameters, e.g., pH (3–3.5), concentrations of reagents (0.113–0.2 mol/l for $(NH_4)_2C_4O_4$, 0.087–0.2 mol/l for $H_2C_2O_4$) and solution/soil ratio (5:1–100:1) on the extractability of trace metals can hardly be evaluated. Particularly, the great variation in solution/soil ratios leaves doubts on the comparability of these procedures.

Moreover, instead of acidic ammonium oxalate solutions, 0.25 mol/l $NH_2OH*HCl$ + 0.25 mol/l HCl was also used to extract trace metals from amorphous Fe-oxides [353]. To extract either the total amount of Fe-oxides, or the crystalline fraction subsequent to removal of the amorphous Fe-oxides, acid oxalate solutions were frequently employed either under diffuse illumination or UV radiation at 20–100 °C and solution/soil ratios between 10:1 and 50:1 for 0.5–3 h. The concentrations of the $(NH_4)_2C_4O_4*H_2O/H_2C_2O_4$ reagents were either 0.175 mol/l/0.1 mol/l or 0.2 mol/l/0.2 mol/l, occasionally used along with 0.1 mol/l ascorbic acid. This variety of conditions and the pronounced effects of varied illumination and temperature on Fe extractability would suggest that hardly two procedures extract equal amounts of trace metals from soils [279, 350]. Despite variation in extraction parameters, most procedures may fairly selectively remove the crystalline Fe-oxides when employed subsequent to extractions of Mn-oxides, amorphous Fe-oxides, and organic and carbonate fractions [279].

Uncertainties remain as to whether different extraction conditions may result in dissolution of varied amounts of trace elements from clay minerals [354, 355]. Instead of acidic ammonium oxalate, some sequential extraction procedures [353, 356] employ dithionite-citrate-bicarbonate (DCB) extractions according to Mehra et al. [357]. This method was widely used for individual extraction of “free” Fe-oxides, extracting similar amounts of Fe as acidic ammonium-oxalate under illumination, but it was not available as a pure reagent for trace metal analysis [279]. Also, at $pH < 4.5$, DCB precipitates sulfur and Fe-sulfides [358]. Accordingly, other trace metal sulfides may precipitate as well, so trace extractants were usually employed at $pH = 7.8$ as proposed by Mehra et al. [357]. This implies that DCB extractants do not fit into a sequence of extractants with decreasing pH. Summarized, acidic ammonium oxalate ($3 < pH < 3.5$) sequentially employed in the dark and under illumination is obviously adequate for dissolution of trace metals from amorphous and crystalline Fe-oxides.

A list of the most commonly used extraction procedures and the addressed fractions is given in (Table 1.6). The procedures contain in general the extraction steps as described previously (in changed order or more compressed). Slight or significant modifications of these most commonly used procedures are widely reported in the literature. Most extraction procedures address a wide range of heavy metals, but some extraction schemes were developed for specific elements or groups of elements.

Table 1.6 Overview of sequential extraction schemes [256].

Reference	I	II	III	IV	V	VI	VII	VIII	IX	X
Tessier et al. [281]	-	1	-	2	4	3	3	3	-	5
Kersten et al. [359]	-	1	-	2	4	3	3	-	-	5
Hirner [283]	1	2	-	5	3,4,7	5	5	5	5	6
Sposito et al. [282]	-	1	2	4	3	-	-	-	5	-
Shuman [332]	-	1	-	-	2	3	4	5	-	6
Zeien et al. [288]	-	1	2	-	4	3	5	6	-	7
Kotuby-Amacher et al [353]	-	1	2	-	-	3	4	5	-	6
Sims et al. [343]	1	2	-	-	3	4	5	-	6	7
Saha et al. [360]	1	1	-	-	2	3	4	5	-	-
Kuo et al. [356]	-	1	-	-	4	-	2	3	-	4
Liang et al. [361]	1	1	2	-	3,5	3,4	6	-	-	7
Jarvis [362]	-	1	-	-	4	2,3	5	5	-	6
Goldberg et al. [278]	1	1	-	-	2	3	4	4	-	5
Miller et al. [334]	1	2	3	-	4,6	4,5	7	8	-	9
Mandal et al. [335, 363]	1	1	-	-	2	-	3	4	-	-
Murthy [364]	-	1	-	-	1	-	2	3	-	4
McLaren et al. [306]	-	-	-	-	1	2	3	3	-	4
Soon et al. [365]	-	1	2	-	2	-	-	-	-	3
Shuman [366]	-	-	-	-	2	-	3	3	-	4
Rauret et al. [367]	-	1	-	2	4	3	3	3	4	5
McLaren et al. [368][b]	1a	1a	2a	-	1b	2b	2b	2b	-	3b

Chemical species tentatively being extracted: I water-soluble, II unspecifically adsorbed (exchangeable), III specifically adsorbed (sorbed components), IV bound to carbonates, V organically bound, VI Mn-oxides, VII amorphous Fe-oxides, VIII crystalline Fe-oxides, IX sulfides, X: residuals (silicate bound).

Sequence: indicated by Arabic numbers

AWater-soluble fraction determined on separate subsamples by extraction with 0.005 M Ca $(NO3)_2$, soil/solution ratio = 1:10 for 16 h. McLaren and Crawford employed two individual extraction schemes (referred to as a and b). In generally most sequential extraction procedures (SEPs) follow similar fractional degradation with little variation. The information needed from the SEP determines, to some extent, how the extraction is performed with respect to the final fraction, the residual. From a geochemical standpoint, total metal concentration is desired requiring the use of often dangerous reagents. From a biological or agricultural standpoint, less dangerous reagents may be utilized in lieu. The extraction conditions and reagents are listed in (Table 1.7) for the five discussed SEPs.

1.7.3 Tessier Procedure: In the extraction procedure by Tessier et al. [281],1 g of sample is placed in a 50mL tube. The sample is exposed to reagents and shaken (Table 1.7(a)). Each fraction is separated from the supernatant by centrifugation at 10,000 rpm, (12,000 gravity) for 30min. The supernatant is collected for lab analysis. The sediment is rinsed with 8mL of deionized water (DIW) and centrifuged again. For the fourth fraction, a 1 g (dry weight) sample is exposed to 12mL of 5: 1HF-$HClO_4$ acid mixture and evaporated to near dryness. A 10:1 HF-$HClO_4$ acid mixture is added to the sample and again evaporated to near dryness followed by 1mL of $HClO_4$, evaporated until white fumes are visible. The final digestion is performed with 12 NHCl and diluted to 25 ml. In the modified Tessier procedure, [369] analyzed two soils: one with moderate metal contamination and one with heavy contamination. The reagents stay the same but the amounts increase. Fraction one is run as normal.

The reagent used in fraction two is increased from 8ml to 50ml, with continuous agitation for 5 hrs. The reagent used in fraction three for the heavily contaminated soil is also increased to 50mL with continuous agitation for six hours. Fractions four and five remain unchanged. Rauret et al. [320] determined that an increase in the amount of reagent used increased the concentration of metals extracted for fractions two and three. They determined that the level/type of contamination of the tested sediment had a direct effect on the results obtained and by increasing the amount of used solution from 8ml and 20 ml, respectively, to 50 ml, and were able to extract the maximum amount of metal without saturation.

1.7.4 Community Bureau of Reference (BCR) Procedure.

This procedure is largely similar to that produced by Tessier et al. [281] with the chief difference in the first fraction of the procedure. Instead of evaluating the exchangeable and carbonate bound separately, the BCR procedure combines both in the first fraction [311]. In the BCR procedure, 1 g of sample is placed into a 100 ml tube, exposed to reagents and shaken (see Table 1.7(a)). After each fraction, the solution is centrifuged at 5000 rpm (3000 gravity) for 20 min and the supernatant is collected. The residue is washed in 20 ml of distilled water (D/W) for 15 min, and centrifuged. The residual fraction is not discussed in further detail and it is assumed that the steps closely follow those of Tessier [369, 311].

The BCR procedure was modified by a group of European experts in order to create an accepted protocol that could be used and the results easily reproduced. The modified procedure, again, is largely similar to the original. During fraction one, it was recommended that the sediment remains in suspension at all times during agitation. For fraction 2, the concentration of the reagent used is increased from 0.1mol to 0.5mol. The authors also recommended the addition of a fixed amount of concentrated HNO_3, pH 1.5 during the making of the fraction 2 reagents [370].

1.7.5 Short Extraction Procedure by Maiz:

Maiz et al.[370] conducted a comparison between the Short and Tessier procedures and found that the Short procedure produced strong correlation data for many metals tested. Three grams of residue are placed in a 50 ml tube, exposed to reagents and shaken (Table 1.7(b)). After the first extraction, the solution is centrifuged at 3000 rpm (1000 gravity) for 10 min, the supernatant removed, and analyzed. The sample is then washed in 10 ml of bidistilled water and centrifuged. For the residual fraction, the residue is placed in Teflon tubes with aqua regia—HF acid for an undetermined time [371].

1.7.6 Galàn Procedure:

This procedure is also similar in structure to the Tessier and BCR procedures. However, this procedure was used in extracting metals from soils severely affected by acid mine drainage in Spain such as those seen along the Rio Tinto [372]. Amorphous iron oxy-hydroxides can coat soils resulting is unobtainable data from regularly used techniques such as x-ray diffraction. Initial use of the Gal´an et al. [372] method showed increased accuracy of metals extracted in these soils than the Tessier and BCR methods.

One-half a gram of soil sample is placed into tubes and exposed to reagents (Table 1.7(b)). Centrifugation of the sample, collection of the supernatant, washing, and fraction 4 are analyzed in the same manner as in the aforementioned Tessier extraction.

1.7.7 Geological Society of Canada (GCS) Procedure:

One gram of sample is placed in a 50 ml tube and exposed to reagents and shaken (Table 1.7(c)). In between each fraction samples are centrifuged for 10 min at 1000 g (2800 rpm). The supernatant is collected and the samples are washed in 5 ml of water, centrifuged, adding the wash water to the previous supernatant. Repeat the washing procedure. Prior to performing the fourth fraction (Table 1.7(c)). The amount of time needed to complete the fraction is proportional to the time for the reduction of sample to an appropriate volume [373]. The modified GCS is the most modified of the SEPs. The run time is drastically shortened and the reagents changed.

(Table 1.8) depicts these changes. Benitez and Dubois [374] modified the GCS procedure by testing various reagents at various time in varying order. They determined that no one sequence of events were fully satisfactory for a SEP but recommended one particular method above the others. That experiment was later adapted by Doelsch et al. [375] into the modified GCS.

1.8 PROBLEMS AND LIMITATIONS OF THE SEP:

As long as SEPs have been around there has been controversy over the nonselectivity of the reagents, which may alter surface chemical characteristics of sediments tested, and potential for metals to redistribute among the remaining fractions during the extraction process by sorbing to the freshly exposed surfaces [376, 377].Studies have employed model soils composed of natural mineral and humic acid, or the use of standard addition by adding a pure synthetic component to real sediments prior to extraction. Model soils used by Shan and Chen [376] indicated that redistribution was in fact occurring. Metals collected for fractions 1–3 were less than should have been and for fractions 4-5 were greater. This was a direct indication that as metals were released in the first three fractions they were reattaching to the newly available sites of the next fraction. Metals Cu, Mn, Ni, Pb, V and Zn were collected primarily from the fourth fraction due to the strong complexes these metals tend to form with humic material. Both soil composition and the nature of the metal played a large part in the amount of redistribution that occurred due to different binding sites available and varying binding strengths.

XRD is also a useful tool in characterizing reactivity of silicate clays during the extraction process. Ryan et al. [378] examined samples before extraction, between each phase of extraction and after the extraction was complete to determine if any changes occurred to the soil directly because of the extraction process. They determined that destruction of the octahedral sheet of trioctahedral clays was evident with octahedral Mg-O bonds quite vulnerable to hydrolysis. The significance being that during the first three phases of extraction metals being released do not comprise just the fractions for which they are designed, but can also release metals in structural sites thus skewing results on true bioavailability. This is complemented by a study also utilizing XRD after the fourth, fifth, and sixth fractions of an extraction procedure adapted after Tessier's five step procedure [379]. The mineralogy of the sampled soils varies significantly in a short distance which has an effect on the total metals determined from each sample. They were able to determine metal type that was the main factor controlling the distribution of metals in the Szklary region, Poland.

Table 1.7 (a): Operating conditions for sequential extraction procedures. H Acid mix = 5ml HF, $HClO_4$ 3ml, HNO_3 2ml. t = time, Q = Quantity [ml].

Fraction	t	Temp	Q	Tessier	t	Temp	Q	BCR
Exchangeable	1hr	continuous agitation	8 8	1 g 1mol$MgCl_2$ pH 7.0 or 1mol NaOAc pH 8.2	16hr	$22^{\circ}C \pm 5^{\circ}$ w/constant agitation	40	1 g 0.11 mol CH_3COOH
Bound to Carbonates	5hr	continuous agitation-leached at rm temp.	8	1mol NaOAc pH 5.0 w/acetic acid				
Bound to Iron and Manganese Oxides	6hr	$96^{\circ}C \pm 3$ occasional agitation	20 20	0.3mol $Na_2S_2O_4$+ 0.175 mol Na-citrate + 0.025 mol H-citrate. 0.04 mol NH_2OH*HCl in 25% (v/v) HOAc	16hr	$22^{\circ}C + 5$ w/constant agitation	40	0.1mol NH_2OH*HCl pH 2 with HNO_3
Bound to Organic Matter	2hr 3hr 30 min	$85^{\circ}C \pm 2$ with occasional agitation $85^{\circ}C \pm 2$ with intermittent agitation continuous agitation	3 5 3 5	0.02mol HNO_3 30% H_2O_2 pH 2 with HNO_3 30% H_2O_2 pH 2 with HNO_3 3.2mol NH_4OAc in 20% (v/v) HNO_3-dilute to 20ml	1hr 1hr 1hr 16hr	roomtemp w/occ. Agitation $85^{\circ}C$ degrees $22^{\circ}C+5^{\circ}$ w/constan agitation	10 10 50	8.8mol H_2O_2 pH 2-3 reduce vol. to less than3ml H_2O_2 pH 2-3 reduce vol.to1ml 1mol NH_4OAc pH2 w/HNO_3
Residual			1 Unk	HF-$HClO_4$ 5 : 1 HF-$HClO_4$ 10:1 $HClO_4$ 12NHCl				HF, HNO_3, $HClO_4$

Table 1.7 (b): t = time, Q = Quantity [ml].

Fraction	t	Temp	Q	Maiz-Short	t	Temp	Q	Galàn
Exchangeable	2 hr	rm temp suspend under agitation	10	3 g 0.01 mol $CaCl_2$	1hr	20°C w/continuous agitation	35	0.5 g 1M NH_4OAc pH 5
Bound to Carbonates	4 hr			0.005 mol DTPA				
Bound to Iron and Manganese Oxides		rm temp	2	0.01mol $CaCl_2$ + 0.1mol TEA pH 7.3	6hr	96°C manual agitation every 30 min	20	0.4 M NH2OH*HCl in CH3COOH 25%
Bound to Organic Matter					2hr 3hr	85°C w/ manual agitation every 30 min	3 5 3	0.2M HNO_3 30% H_2O_2, pH 2 30% H_2O_2
Residual				aqua regia-HF acid	30 min 2hr	Continuous agitation	5 10	30% H_2O_2 HF,HNO_3,HCl 10 : 3 : 1

Table 1.7 (c): t = time, Q = Quantity [ml].

Fraction		Form	t	Temp	Q	Canada
Exchangeable	mobile	AEC	6hr		20	1.0mol CH_3CO_2Na pH 5
			6hr		20	1.0 mol CH_3CO_2Na pH 5
Bound to Carbonates		Am Fe ox	2 hr	60°C vortex every 30min	20	20ml 0.25 mol NH_2OH*HCl in 0.05 mol HCl
Bound to Iron and Manganese Oxides			30 min	60°C	20	20ml 0.25 mol NH_2OH*HCl in 0.05 mol HCl
		Cry Fe ox	3hr	90°C vortex every 20min	30	1.0mol NH_2OH*HCl in 25%CH_3CO_2H
Bound to Organic Matter	mobilisible	Org/Sulf	1.5 hr	90°C	30	1.0mol NH_2OH*HCl in 25% CH_3CO_2H
			30 min			750 mg $KClO_3$ and 5ml 12mol HCl vortex and add10mlHCl more
			20 min	90°C	10	15ml H_2O 4molHNO_3
Residual		silicates and residual	Unk	200°C	2	16 mol HNO_3 reduce to 0.5ml
			20 min	90°C	2	12 mol HCl
			1hr	90°C	10	acid mix H
			overnight	Evap70°C		
			last bit	Rai120°C		
					1	12molHCl
			5–10 min		3	16mol HNO_3
					3	H_2O and warm then bring up to 20ml

Table1.8: Operating conditions for the modified GCS extraction procedures using 1 g of sample: t = time, Q = Quantity [ml].

Fraction	t	Temp	Q	Canada Benitez and Dubois [374]
Exchangeable	1.5 hrs 1.5 hrs	25˚C 25˚C	30ml 30ml	0.1mol $NaNO_3$ 0.1mol $NaNO_3$
Adsorbed	1.5 hrs 1.5 hrs	25˚C 25˚C	30ml 30ml	1 mol Na OAc pH5.0w/CH_3COOH 1 M Na OAc pH 5.0w/CH_3COOH
Organic	1.5 hrs 1.5 hrs	25˚C 25˚C	30ml 30ml	0.1mol $Na_4P_2O_7$ 0.1mol $Na_4P_2O_7$
Amorphous Oxyhydroxides	1.5 hrs 1.5 hrs	60˚C 60˚C	30ml 30ml	0.25mol NH_2OH*HCl in 0.5 mol HCl 0.25mol NH_2OH*HCl in 0.5 mol HCl
Crystalline Oxides	1.5 hrs 1.5 hrs	90˚C 90˚C	30ml 30ml	1mol NH_2OH*HCl in 25% H Oac 1mol NH_2OH*HCl in 25% H Oac
Residual	unk			HF, HNO_3, $HClO_4$

2. AIM OF THIS STUDY:-

Speciation of phosphorus was performed using two sequential extraction procedures (modified Chang and Jackson and the BCR procedur) to:

- Compare different forms of phosphorus in soil after 40 years of fertilization strategies. In the period of 40 years, phosphate fertilizer was applied in amounts of 26, 39 and 52 kg P ha^{-1}, but the added amount of urea was limited.
- Establish the most active form of P in a long-term mineral fertilization experiment.
- Monitor the movement of different phosphorus forms along the soil profile.

Multiphase sequential extraction according to modified Tessier procedure (Petrovic *et al.*, 2009) was used in order to:

- Investigate the distribution of metals.
- Establish connections between metals and P in long-term mineral fertilization experiment.
- Investigate of the effects of fertilization on the content of elements in soil and wheat (Triticum aestivum L.).
- Compare wheat yield and amounts of applied fertilizer.
- The elements of interest in our experiment were Al, Cd, Cr, Co, Cu, Fe, Mn, Ni and Pb.

Microwave Digestion system for soil and wheat was used to:

- Evaluation the bioaccumulation factor (BAF) for metals and P.

3. THEORY FOR EXPERIMENTAL METHODS USED:

3.1 CLOSED VESSEL MICROWAVE DIGESTION:

In order to analyze various heterogeneous samples with ICP-OES, they should usually be transferred to homogeneous solutions. In closed vessel microwave digestion, a small amount of sample is digested using various reagents such as mineral acids, under high temperature and pressure. Digestion takes place in closed vessels which are transparent to microwave energy and resistant to acid corrosion [380].

The microwave energy is transferred to heat by the electric polarization and ionic conduction, thus the liquid acid and the vessel in contact with acid is heated. After increasing the temperature and exceeding the acid(s) boiling point, a large amount of gaseous acid is produced. The gaseous acids which cannot properly absorb the microwave energy are condensed in contact with the cold vessel walls and release the energy to the walls. In the next stage which is called “Sustained dynamic thermal no equilibrium”, evaporation and condensation of acids continue and the reaction temperature sustains during the digestion [380].

Closed vessel microwave digestion has several advantages; small amount of reagents is used and the dissolution time is short. The reaction atmosphere is closed and controlled so there is no contamination from the atmosphere and no loss of volatile elements.

Microwave vessels are usually made of Teflon or quartz, so they are appropriate for trace analyses [380]. (Fig 3.1).

Various reagents and mineral acids, usually mixtures, are used to digest various samples.

Nitric acid (HNO_3) in concentrated form is a powerful oxidizing acid. It is the most common acid to oxidize the organic matrices. Aqua regia, a (3:1v/v) mixture of hydrochloric (HCl) and nitric acid, produces strong oxidizing agents which dissolve even noble metals that cannot be dissolved by nitric or hydrochloric acid individually. Warm and concentrated (60-72%) perchloric acid is a powerful oxidizing agent and easily decomposes organic matter. Hydrofluoric acid (HF) is a non-oxidizing agent with high complexing capacity. It is one of the few acids which dissolve silicates. Perchloric and hydrofluoric acid are usually mixed with HNO3. Hydrogen peroxide (H_2O_2) reacts explosively with many organics especially in its concentrated form. The oxidizing power of hydrogen peroxide increases at lower pH [380].

Fig3.1: Closed vessel microwave digestion. [380].

3.2 INDUCTIVELY COUPLES PLASMA ATOMIC EMISSION SPECTROSCOPY (ICP-OES):

Optical Emission Spectrometry. Principles of Quantitative Analysis:

Optical emission spectrometry is one of the oldest physical methods of analysis enabling multielement determinations. In this process, free atoms which are generated by thermal dissociation of the sample material are excited or ionised and excited additionally (several collisions or other processes may be responsible for delivering the required energy). The higher the temperature, the higher is the percentage of excited analyte species (at least, in general) and the higher the emission intensity. The Boltzmann equation relates the temperature (T) with the number of atoms in an energy state E_0 and an excited state Eq, provided that the source is in a so- called thermal equilibrium, as:

$$n^*/n_0 = g_q / g_0 e^{-(Eq - E_0)/k_B T}$$

Where n_0 is the number of atoms in the energy level E_0, n* the number of atoms in an energy state Eq, k_B the Boltzmann's constant, g_q and g_0 the statistical weights for each energy state (Eq and E_0) and T the temperature in Kelvin.

The flames commonly used as atomisers have temperatures in the range 2000–3000K allowing for the analysis of elements such as Na, and K by OES. The flame temperatures are not high enough to excite many other elements, so other atomisers such as spectroscopic plasmas have to be used.

Linear (straight-line) relationships can be easily achieved between the emission intensity of a given transition and the total atomic concentration of the element in the atomisation/excitation system. However, under certain conditions, spectral lines from resonance transitions can display a phenomenon called self-absorption, giving rise to non-linearity in calibration graphs at high concentrations. If changes to the experimental setup cannot correct the problem, it will cause difficulties in classical linear calibration, although they can be solved by multivariate calibration techniques [381]. (Fig3.2).

Fig3.2: Inductively Couples Plasma Atomic Emission Spectroscopy (ICP-OES). [381].

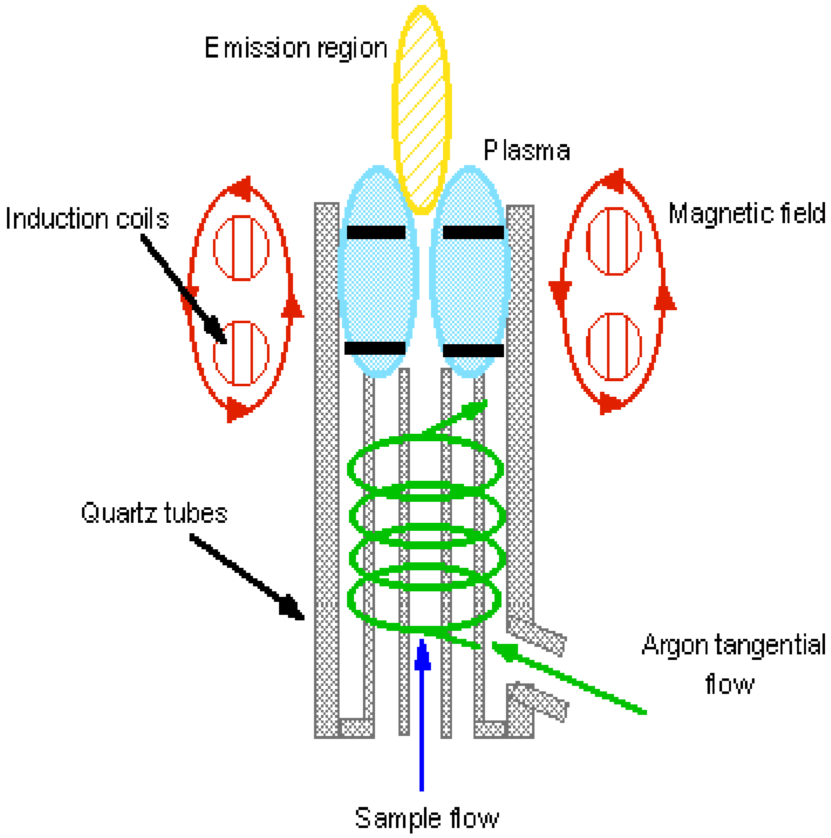
3.2.1 Atomic Emission Spectrometry:

Flames and plasmas can be used as atomisation/excitation sources in OES.

Electrically generated plasmas produce flame-like atomisers with significantly higher temperatures and less reactive chemical environments compared with flames. The plasmas are energised with high-frequency electromagnetic fields (radiofrequency or microwave energy) or with direct current. By far the most common plasma used in combination with OES for analytical purposes is the inductively coupled plasma (ICP).

The main body of an ICP consists of a quartz torch (15–30mm in diameter) made of three concentric tubes (see Figure 3.3) and surrounded externally by an induction coil that is connected to a radiofrequency generator commonly operating at 27 MHz. An inert gas, usually argon, flows through the tubes. The spark from a Tesla coil is used first to produce ‘seed’ electrons and ions in the region of the induction coil. Subsequently the plasma forms, provided that the flow patterns are adequate inside the torch, giving rise to high-frequency currents and magnetic fields inside the quartz tube. The induced current heats the support gas to a temperature of the order of 7000–8000K and sustains the ionisation necessary for a stable plasma. Usually, an aerosol from the liquid sample is introduced through the central channel transported by an argon flow of about 11 min^{-1}. A much higher Ar flow velocity (about 101 min^{-1}) is introduced tangentially to prevent overheating. Because of efficient desolvation and volatilisation in the ICP, this atomiser/excitation source is commonly applied for the analysis of dissolved samples. The high temperatures and the relative long residence time of the atoms in the plasma (2–3 ms) lead to nearly a complete solute vaporisation and high atomisation efficiency. Accordingly, although matrix and inter-element effects should be relatively low, it has been observed that sometimes they are significant. Further, the high excitation capacity of this source gives rise to very rich spectra, so a careful assessment of potential spectral interferences is essential. On the other hand, the ICP emission frequently has an important background due to bremsstrahlung (i.e. continuous radiation produced by the deceleration of a charged particle, such as an electron, when deflected by another charged particle, such as an atomic nucleus) and to electron–ion recombination processes.

For a given ICP-OES instrument, the intensity of an analyte line is a complex function of several factors. Some adjustable parameters that affect the ICP source are the radiofrequency power coupled into the plasma (usually about 1 KW), the gas flow rates, the observation height in the lateral-viewing mode and the solution uptake rate of the nebuliser. Many of these factors interact in a complex fashion and their combined effects are different for dissimilar spectral lines. The selection of an appropriate combination of these factors is of critical importance in ICP-OES. [381].



(Fig 3.3 Torch for ICP-OES) [381].

4. EXPERIMENTAL SECTION:

4.1 REAGENTS:

All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany).

4.1.1 Phosphorus: A stock standard solution of orthophosphate (100 mg P/L) was prepared from KH_2PO_4. Various solutions were prepared by dilution of the stock phosphorus solution.

4.1.2 Trace elements: Calibration standards were prepared from multielement standard stock solutions. These multielement standards and blanks were prepared in the same matrix as the extracting reagents in order to minimize the matrix effects.

The content of phosphorus and metals in all extracts were determined by inductively coupled plasma atomic emission spectrometry, iCAP 6500 Duo (Thermoscientific, United Kingdom).

4.1.3 Chemicals: Several chemicals with the following qualities were used in different stages of the experimental work:

4.1.3.1 Modified Chang and Jackson procedure for the phosphorus sequential extraction. (Manojlovic et al. 2007)**[382]:**

- NH_4Cl 1M (water soluble P).
- NH_4F 0.5M (AL bound P).
- NaCl Saturated.
- NaOH 0.1M (Fe bound P phase 3), (occluded P phase 5).
- Na-citrate + 1g Na-dithionite 0.3M (ducible P).
- H_2SO_4 0.25M (Ca bound P).

4.1.3.2 Community Bureau of Reference (BCR) Sequential Extraction procedure for the phosphorus (BCR (Ruban et al., 2001)) [383]:

- NaOH 1M (NaOH-P), (Inorganic P).
- HCl 3.5M (HCL-P), (conc. HCL-P).
- HCl 1M (Organic P).
- NaCl 1M

4.1.3.3 Sequential Extraction of Metals: modified Tessier procedure (Petrovic et al., 2009):

Ammonium-acetate 1M (Exchangeable).

- HCl 0.6M (Residual).
- Hydroxylamine hydrochloride, 0.2M + HCl 0.02M (Bound to carbonates and easily reducible).
- Oxalic acid 0.2M + ammonium oxalate 0.2M (Moderately reducible).
- Hydrogen peroxide (30%), Ammonium acetate, 3.2M (Organic-sulphide).
- HCl conc.

4.1.3.4 BCR sequential extraction Protocol for trace metals**:**

- Acetic acid, 0.11M
- Hydroxylamine hydrochloride, 0.5M
- Hydrogen peroxide, 300 mg/g, 8.8M
- Ammonium acetate, 1M

4.1.3.5 Microwave Total digestion procedure for trace metals:

- HNO_3 65%.
- HCl 37%.
- HF 40%.
- H_3BO_3 5%.

4.1.4 Gas:

Quality of the gas used in the ICP-OES was the argon (Ar) (99.999% Ar).

4.1.5 Water quality:

The type of water was used has resistance >18.0 MΩ cm (at 25 °C).

4.1.6 Multi-element standard solutions:

Standards for metals were prepared from multielement standard stock solutions (1000 mgL^{-1}). These multielement standard solutions and blanks were prepared in the same matrix as the extracting reagents in order to minimize the matrix effects. Multi-element standard solutions used to make the inter-calibration and calibration solutions are listed in Appendix A.

4.2 APPARATUS:

Centrifuge, balance.

Polyethylene centrifuge tubes.

Porcelain crucibles.

Test-tubes.

Pipettes with different volumes

Shaker

4.3 SAMPLES:

4.3.1 Site description and sample background:

The investigation was conducted at the Varna experimental station, 44°41′38″ and 19°39′10″ (near Belgrade, Serbia), where a wide range of different fertilization treatments has been undertaken since 1968.

4.3.2 Soil samples

The soil type is Stagnosol, a loam textured Pseudogley developed on Pliocene loam and clay materials under aquatic conditions at 109 m above sea level. Average annual precipitation of the site is 705 mm, and the average temperature is 12°C (Table 4.1):

Table 4.1. Climatic characteristics of the Varna experimental station, Serbia:

Year	Month												sum for the year
	1	2	3	4	5	6	7	8	9	10	11	12	
Mean air temperature (°C)													
2005	0.0	-2.9	4.4	11.1	16.9	19.2	21.5	19.7	17.3	11.6	4.9	2.2	10.5
2006	-1.4	0.9	5.6	12.5	16.4	19.6	22.8	19.1	17.5	13.1	7.1	2.7	11.3
2007	5.2	5.8	8.6	13.0	18.5	22.0	22.6	22.3	14.3	10.4	3.7	0.3	12.2
2008	2.0	4.5	7.6	12.4	17.2	21.2	21.6	21.7	15.4	12.6	7.4	4.0	12.3
avg. (1964-2002)	0.0	2.4	7.0	11.7	17.2	20.4	21.9	21.5	17.0	11.7	6.1	1.5	11.6
Precipitation, mm													
2005	37.3	75.7	56.6	63.3	78.4	97.8	112.9	138.5	60.8	16.3	38.9	82.4	859.1
2006	38.8	46.7	98.9	89.8	59.5	115.0	38.7	165.2	24.9	32.2	29.7	55.8	795.2
2007	53.6	47.1	75.6	4.3	90.6	75.3	38.9	67.0	106.4	128.8	110.8	48.6	846.9
2008	27.8	11.3	62.8	40.5	37.8	40.0	33.8	18.4	76.5	11.2	45.8	46.4	452.2
avg. (1964-2002	48.2	41.3	46.1	54.6	62.0	84.6	72.1	57.7	56.7	58.4	60.5	61.4	701.0

The experiment was arranged as a randomized block design, with each treatment randomized in three blocks for a total of 12 plots (5 by 11 m). Composite samples of five soil subsamples were taken from two depths: surface (0-30 cm) and subsurface (30-60 cm) layers in spring 2008.

Three different amounts of $NH_4H_2PO_4$ fertilizer (MAP) were applied during 40 years experiment (26, 39, and 52 kg P/ha) in combination with a constant amount of N (urea, 60 kg/ha) and K (KCl, 50 kg/ha).

The mineralogical composition of the studied soil was: illite (50-70%), vermiculite (10-30%), and other clay minerals (kaolinite, chlorite, feldspat, quartz, and amphibolites). The cultivated cops were winter wheat (Triticum aestivum L.) and corn (Zea maize L.) with crop residues removed. The soil cultivation was performed by a standard plowing to 25 cm depth.

The fertilizer applied during this period was from several sources and contained trace elements as shown in (Table 4.2):

Table 4.2: Content of trace elements in the applied monoammonium phosphate:

Element	**As**	**Hg**	**F**	**Cr**	**Cu**	**Zn**	**Cd**	**Ni**	**Pb**	**Fe**
Concentration mg kg^{-1}	4–15	0.003–0.005	19,600–26,7	15–315	10–20	10–38	3–16	4–39	10–20	4000–5000

Soil pH was determined with a glass electrode pH meter in a 1:2.5 water solution. Soil total C and N were measured with an elemental CNS analyzer, Vario model EL III (ELEMENTAR Analysasysteme GmbH, Hanau, Germany; Nelson and Sommers, 1996) [384], Available P and K were determined by the Al-method of Egner–Riehm (Enger and Riehm, 1958) [385] where 0.1 M ammonium lactate (pH = 3.7) was used as an extract. After the extraction, K was determined by flame emission photometry and P by spectrophotometry after color development with ammonium molybdate and $SnCl_2$(Enger and Riehm, 1958). Soil Ca and Mg were extracted by ammonium acetate and determined with a SensAA Dual atomic adsorption spectrophotometer (GBC Scientific Equipment Pty Ltd, Victoria, Australia; Wright and Stuczynski, 1996) [386].

Determination of CEC was done by steam distillation method after the treatment with 1 M ammonium acetate (Sumner and Miller, 1996) [387]. Exchangeable Al was determined by the titration method after Sokolov: extraction with 1 M KCl (1:2.5) followed shaking for 1 h and titration with 0.01 M NaOH (Jakovljević et al., 1985) [388]. Trace elements were determined with an ICAP 6300 ICP optical emission spectrometer (Thermo Electron Corporation, Cambridge, UK), after the soils were digested with concentrated HNO_3 for extraction of hot acid-extractable forms, and by diethylenetriaminepentaacetic acid (DTPA) for extractableelements (Soltanpour et al.,

1996) [389]. The F content was determined by ion-selective electrode, after the soil had been fused with NaOH for total F and after extraction with water for available F (Frankenberger et al., 1996) [390]. Soil granulometric composition was performed using the pipette method (Day, 1965) [391].

4.3.3 Soil reference materials: The certified reference material CRM 684 (river sediment extractable phosphorus, BCR, Community Bureau of Reference, Brussels, Belgium) and the certified reference material CRM 701 (Lake Sediment Extractable Trace Elements, BCR, Community Bureau of Reference, Brussels, Belgium) were used to confirm the accuracy of the employed method..

4.4 PROCEDURES:

4.4.1 Phosphorus Sequential Extraction Methods:

Modified Chang and Jackson and the Community Bureau of Reference (BCR) Sequential Extraction procedures:

A portion of each soil sample (air dried) was subjected to sequential extraction. Speciation of phosphorus was carried out using two sequential extraction procedures: modified Chang and Jackson and BCR procedure. Details are given in (Table 4.3):

Table 4.3: Reagent and corresponding P fractions for two sequential extraction procedures used in this study:

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Manojlovic et al. 2007 [382]	1M NH_4Cl Water soluble P	0.5 M NH_4F Al - P	0.1 M NaOH Fe - P	0.3 M Nadithionite, Na citrate Reducible P	0.1 M NaOH Occluded P	0.25 M H_2SO_4 Ca - P
BCR (Ruban et al., 2001)[383]	1M NaOH NaOH - P	3.5 M HCl HCl - P	1M NaOH Inorganic P	1M HCl + calcination Organic P	3.5 M HCl +calcination Conc HCl -P	

4.4.2 Sequential Extraction of Metals (Multiphase sequential extraction according to modified Tessier procedure (Petrovic *et al.*, 2009): The soil was subjected to a 5-phase sequential extraction [392] in order to study the substrates (Fe, Al, Mn and Ca) of phosphorus and metals. Also it was used in order to investigate the distribution of metals. Details are given in (Table 4.4).

Table 4.4: Reagent in the sequential extraction procedure used to study substrates metals (Fe, Al, Mn and Ca) and phosphorus:

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5
Petrovic et al. 2009 [392]	1 M CH_3COONH_4 Exchangeable	0.1 M $NH_2OH\cdot HCl$ Bound to carbonates and easily reducible	0.2 M $(NH_4)_2C_2O_4$ and 0.2 M $H_2C_2O_4$ Moderately reducible	30 % H_2O_2 + 3.2 M CH_3COONH_4 Organic sulphide	6M HCl Residual

4.4.3 The BCR three stage extraction procedure of metals:

The procedure recommended by the Standards, Measurements and Testing programme of the European Union (SM& T–formerly BCR), for the determination of heavy metals in soils. The BCR sequential extraction procedure is given in Table 4.5

Table 4.5: The BCR three stage extraction procedure of metals:

Extraction step	Reactive / concentration / pH	Solid phase
1	Acetic acid: CH_3COOH (0.11 mol L^{-1}), pH 2.85	Exchangeable, water and acid soluble (e.g., carbonates)
2	Hydroxylammonium chloride: $NH_2OH\cdot HCl$ (0.1 mol L^{-1}) at pH 2	Reducible (e.g., iron/manganese oxides)
3	Hydrogen peroxide: H_2O_2 (8.8 mol L^{-1}), followed by ammonium acetate: CH_3COONH_4 (1.0 mol L^{-1}) at pH 2	Oxidisable (e.g., organic substance and sulphides)

4.4.4Soil Microwave Digestion system:

I- Soil Partial Digestion:

Sample amount: 0.5 g

Reagents: 8 ml of HNO_3 65%, 5 ml of HCl 37%, 1.5 ml of HF 40%

Procedure:

1. Place a TFM vessel on the balance plate, tare it and weigh of the sample.
2. Introduce the TFM vessel into the HTC safety shield.
3. Add the acids; if part of the sample stays on the inner wall of the TFM vessel, wet it by adding acids drop by drop, then gently swirl the solution to homogenize the sample with the acids.

4. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench.
5. Insert the segment into the microwave cavity and connect the temperature sensor
6. Run the microwave program to completion.
7. Cool the rotor by air or by water until the solution reaches room temperature.
8. Open the vessel and transfer the solution to a marked flask.

II- Soil Total Digestion :(only after application Soil Partial Digestion):

Sample amount: 0.5 g.

Reagents: 1 ml of HF 40%, 5 ml of H_3BO_3 5%.

Procedure: The same procedure like Soil Partial Digestion.

4.4.5 harvested wheat (Triticum aestivum) Microwave digestion system:

For pseudo-total metal content determination, one gram of wheat sample (grain or stalk) was digested directly into each pre-cleaned vessel and 7 mL of % 65 HNO_3 and 1 mL of %33 H_2O_2 then were added; then the vessels were placed inside a rotor of the microwave digestion system and finally submitted to a microwave digestion program. After cooling, the digest was filtered, through a Whatman type 41 filter and finally diluted to 50 mL with distilled water. The sample solutions were stored in plastic containers and finally the content of metals was determined by ICP-OES.

4.5 STATISTICAL ANALYSIS USED IN THIS STUDY:

In order to quantitatively analyze and confirm the relationships between microelement contents in soil samples and added amount of phosphorus, the Pearson correlation analysis was applied to the obtained data. The Pearson correlation coefficient is a common parameter used to quantify the strength of linear association between the pairs of variables by calculating a summary index. Definition of Pearson correlation coefficient could be found in any standard textbook of statistics [393].

5 RESULTS AND DISCUSSION:

5.1 SEQUENTIAL EXTRACTION OF METALS: MODIFIED TESSIER PROCEDURE (PETROVIC ET AL., 2009):

First Phase (exchangeable):

The content of following elements Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Pb extracted after sequential extraction first phase are given in (Table5.1). The (Figures 5.1) shows the correlation coefficient between the amount of added phosphorus and content of each metal for first phase. The amount of added phosphorus was correlated with content of each metal and these correlation coefficient and statistical significance of the relationships (p-values) were presented in (Table5.2).

Results for determination the total concentration of elements obtained in grain and stalk samples for various amounts of phosphate fertilizer are given in (Tables 5.3 and 5.4).Wheat yield per ha was presented in (Table 5.5).

In this phase the increase of amount of added phosphorus significantly caused the increase of the content of each metal in soil (high correlation coefficients which are given in (Table 5.2). Low correlation coefficient was found only between phosphorus and cobalt (0.2940), while lead showed high (-0.9281) negative correlation. The long-term fertilization with monoammonium-phosphate obviously had influence on the total content of elements such as Al, Cd, Co, and Cr.

Table 5.2: The Pearson’s correlation coefficients (r) for added phosphorus and various substrates from first phase:

	Correlation coefficients. r	P values
P-Al 1	0.9339	0.0660
P-Cd1	0.7398	0.2600
P-Co1	0.2940	0.7100
P-Cr 1	0.8789	0.1210
P-Cu1	0.9937	0.0060
P-Fe 1	0.7262	0.2740
P-Mn1	0.6772	0.3230
p-Ni 1	0.9929	0.0070
P-Pb1	-0.9281	0.0720

Table 5.3: The total concentration of elements in the grain and standard deviation (SD)

Element	0 kgPha^{-1}		26 kgPha^{-1}		39 kgPha^{-1}		52 kgPha^{-1}	
	(µg/g)	SD	(µg/g)	SD	(µg/g)	SD	(µg/g)	SD
Al	6.22	1.79	2.98	0.76	2.80	0.69	2.82	0.36
Cd	0.11	0.01	0.10	0.01	0.11	0.01	0.11	0.01
Co	0.05	0.01	0.04	0.02	0.04	0.01	0.04	0.01
Cr	0.11	0.02	0.09	0.01	0.09	0.01	0.08	0.01
Cu	3.41	0.15	3.37	0.16	3.40	0.79	3.54	0.69
Fe	48.45	4.49	42.29	4.22	41.29	2.54	40.63	3.74
Mn	47.90	2.78	43.80	3.45	43.79	3.16	43.24	2.80
Ni	0.63	0.03	0.58	0.10	0.60	0.18	0.56	0.07
P	2995.96	50.18	2904.81	117.74	3035.22	81.15	3080.22	60.05
Pb	0.36	0.18	0.38	0.13	1.25	0.88	1.41	0.89

Table5. 4: The total concentration of elements in the stalk and standard deviation (SD)

Element	0 kgPha^{-1}		26 kgPha^{-1}		39 kgPha^{-1}		52 kgPha^{-1}	
	(µg/g)	SD	(µg/g)	SD	(µg/g)	SD	(µg/g)	SD
Al	94.25	12.40	24.28	8.89	17.33	6.79	14.02	5.75
Cd	0.34	0.06	0.30	0.02	0.27	0.16	0.23	0.02
Co	0.09	0.08	0.02	0.02	0.01	0.01	0.01	0.01
Cr	0.78	0.43	0.35	0.11	0.27	0.04	0.25	0.04
Cu	2.59	1.24	2.05	0.40	1.79	0.39	1.68	0.25
Fe	194.07	149.70	48.28	29.70	28.28	6.47	22.25	5.64
Mn	122.66	14.26	94.35	20.13	96.97	17.05	95.09	8.29
Ni	0.57	0.22	0.29	0.04	0.35	0.08	0.33	0.09
P	554.56	113.35	340.66	30.66	430.54	49.90	487.14	123.65
Pb	1.03	0.33	0.70	0.37	0.68	0.43	0.89	0.66

Table 5.5: Wheat yield per ha

Amount of applied P $kgPha^{-1}$	Wheat yield ($kgha^{-1}$)				
	I	II	III	IV	Average
0	478.18	400	400	433.33	427.88
26	1883.76	2166.67	2266.67	1933.33	2062.61
39	1981.2	2000	2333.33	2000	2078.63
52	2046	2200	1866.67	1733.33	1961.54

I, II, III and IV are four measurements from different plots.

Table 5.1: Concentration of metals extracted after modified Tessier Sequential Extraction : Phase 1: Ammonium Acetate 1M

Fertilizer	**Depth**	***NO***	**Al mg/g**	**Ca mg/g**	**Cd µg/g**	**Co µg/g**	**Cr µg/g**	**Cu mg/g**	**Fe mg/g**	**Mg mg/g**	**Mn mg/g**	**Ni mg/g**	**P µg/g**	**Pb mg/g**	**Zn mg/g**
0 kg/ha	***0-30***	***A***	**0.1800**	**1.5802**	**0.0203**	**0.1455**	**0.1839**	**0.0019**	**0.0383**	**0.3056**	**0.0493**	**0.0005**	**0.0000**	**0.0020**	**0.0015**
	30-60	***B***	**0.0164**	**1.5838**	**0.0100**	**0.1733**	**0.0748**	**0.0017**	**0.0040**	**0.3326**	**0.0492**	**0.0006**	**0.0000**	**0.0035**	**0.0012**
26 kg/ha	***0-30***	***C***	**0.1429**	**1.4980**	**0.0347**	**0.2345**	**0.1641**	**0.0024**	**0.0277**	**0.2777**	**0.0609**	**0.0007**	**0.0000**	**0.0014**	**0.0024**
	30-60	***D***	**0.3607**	**1.6061**	**0.0674**	**0.1401**	**0.2715**	**0.0020**	**0.3741**	**0.3376**	**0.0478**	**0.0007**	**0.0000**	**0.0014**	**0.0027**
39 kg/ha	***0-30***	***E***	**0.0259**	**1.6491**	**1.1142**	**0.2500**	**0.3596**	**0.0044**	**0.0075**	**0.3033**	**0.0524**	**0.0008**	**5.6550**	**0.0012**	**0.0040**
	30-60	***F***	**0.1005**	**1.7628**	**1.8677**	**0.2338**	**0.4678**	**0.0018**	**0.0211**	**0.3532**	**0.0464**	**0.0009**	**0.0000**	**0.0013**	**0.0026**
52 kg/ha	***0-30***	***G***	**0.5963**	**1.6102**	**1.8564**	**0.3576**	**0.6088**	**0.0028**	**0.0396**	**0.3061**	**0.0584**	**0.0011**	**24.5012**	**0.0012**	**0.0022**
	30-60	***H***	**0.7297**	**1.8463**	**1.3025**	**0.2070**	**0.7426**	**0.0022**	**0.1471**	**0.3795**	**0.0455**	**0.0010**	**2.7693**	**0.0011**	**0.0021**

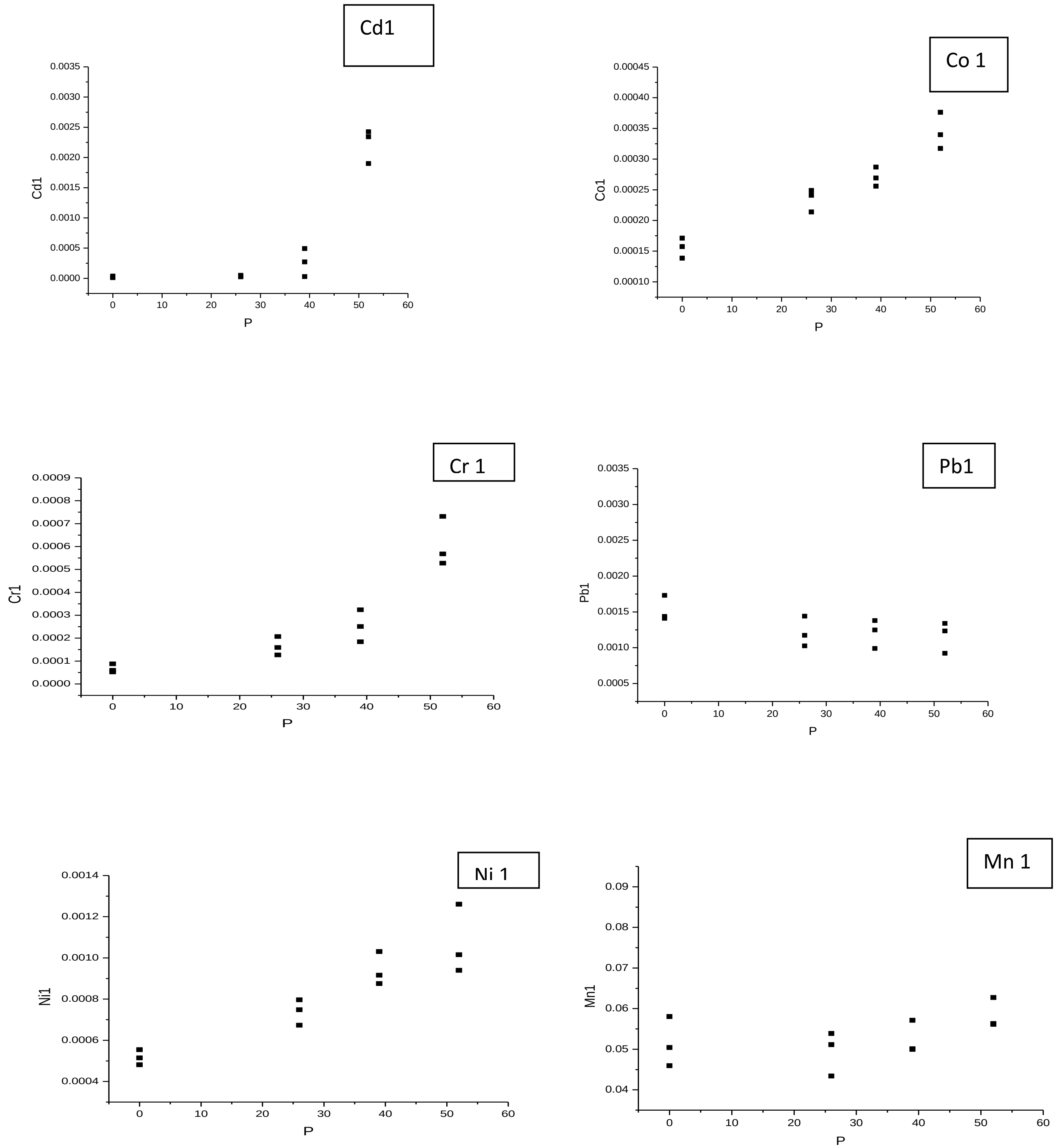


Fig 5.1: the correlation coefficient between the amount of added phosphorus and content of each metal for first phase

Second phase (bound to carbonate fraction):

content of following elements Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Pb extracted after sequential extraction second phase are given in (Table5.6). The (Figures 5.2) shows the correlation coefficient between the amount of added phosphorus and content of each metal for second phase. The amount of added phosphorus was correlated with content of each metal and these correlation coefficient and statistical significance of the relationships (p-values) were presented in (Table5.7).

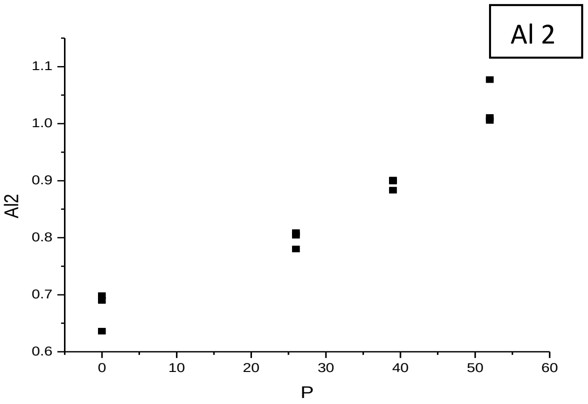
In this phase similar increase of the content of each metal was also observed with the exception of copper (0.2292) and lead (-0.2270). High correlation (0.9806) between applied phosphorus and aluminum from second phase (Al 2) was expected since the aluminum was a typical representative of clay minerals. In acidic mineral soils, as in our case, P is fixed mainly by aluminum and iron [394].

Table 5.7: The Pearson’s correlation coefficients (r) for added phosphorus and various substrates from second phase:

	Correlation coefficients. r	P values
P-Al2	0.9806	0.0190
P-Cd2	0.9814	0.0190
P-Co2	0.9415	0.0590
P-Cr2	0.9718	0.0280
P-Cu2	0.2292	0.7710
P-Fe2	0.9986	0.0010
P-Mn2	0.9207	0.0790
P-Ni2	0.9197	0.0800
P-Pb2	-0.2270	0.7730

Table 5.6: Concentration of metals extracted after modified Tessier Sequential Extraction : Phase 2: $NH_2OH.HCl$ 0.2M + HCl 0.02 M

Fertilizer	Depth	*NO*	Al mg/g	Ca mg/g	Cd mg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mg mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	*0-30*	*A*	0.6747	0.2785	0.0002	0.0027	0.0009	0.0056	1.2117	0.0501	0.1918	0.0016	0.1545	0.0050	0.0078
	30-60	*B*	0.8785	0.2699	0.0002	0.0037	0.0013	0.0068	1.4519	0.0777	0.2373	0.0023	0.1352	0.0049	0.0194
26 kg/ha	*0-30*	*C*	0.7977	0.2866	0.0002	0.0027	0.0010	0.0057	1.3637	0.0525	0.1957	0.0014	0.2380	0.0046	0.0029
	30-60	*D*	1.0186	0.2623	0.0002	0.0042	0.0014	0.0066	1.4695	0.0796	0.2790	0.0023	0.1757	0.0048	0.0027
39 kg/ha	*0-30*	*E*	0.8944	0.2951	0.0002	0.0030	0.0010	0.0056	1.3376	0.0499	0.2360	0.0016	0.2875	0.0042	0.0030
	30-60	*F*	1.1392	0.2896	0.0002	0.0037	0.0014	0.0066	1.4394	0.0855	0.2704	0.0025	0.1996	0.0044	0.0027
52 kg/ha	*0-30*	*G*	1.0312	0.2944	0.0002	0.0040	0.0011	0.0056	1.3258	0.0569	0.2674	0.0018	0.3619	0.0045	0.0032
	30-60	*H*	1.2754	0.2738	0.0002	0.0049	0.0015	0.0069	1.4714	0.0920	0.3336	0.0026	0.2147	0.0044	0.0028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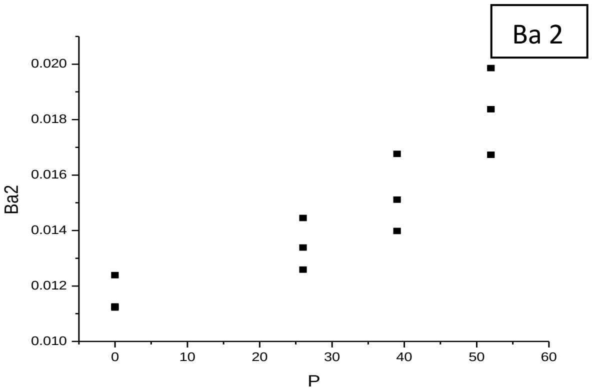
P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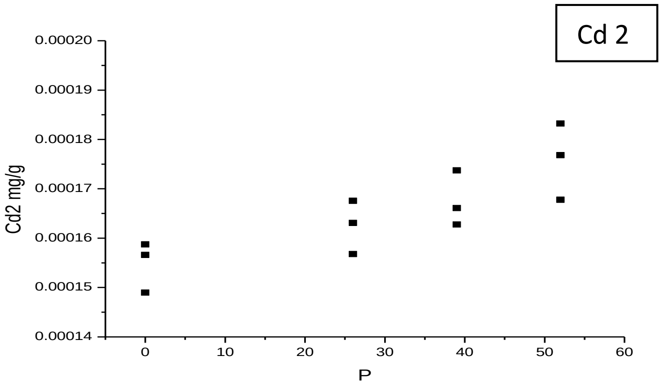
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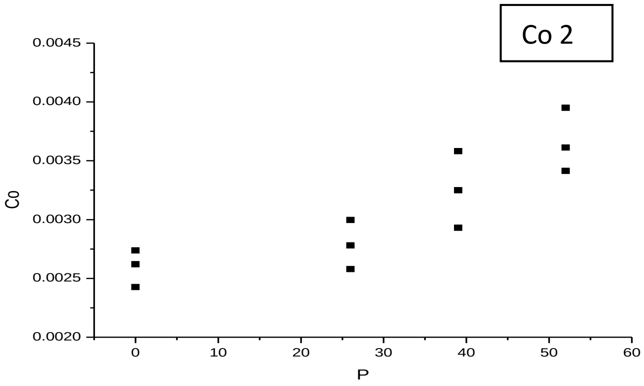
40
50
60
P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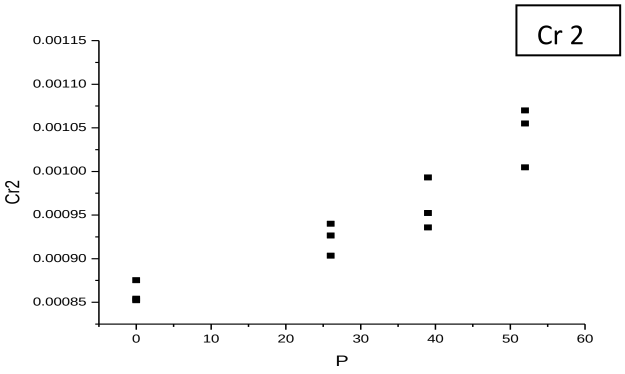
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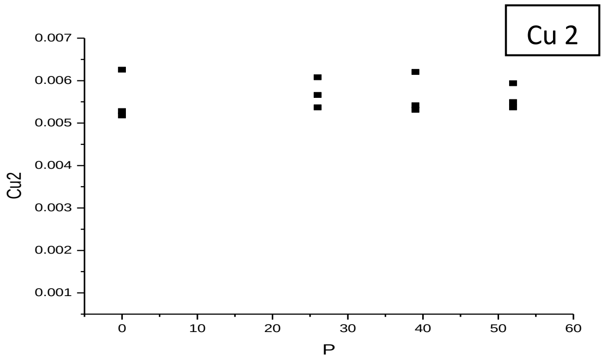
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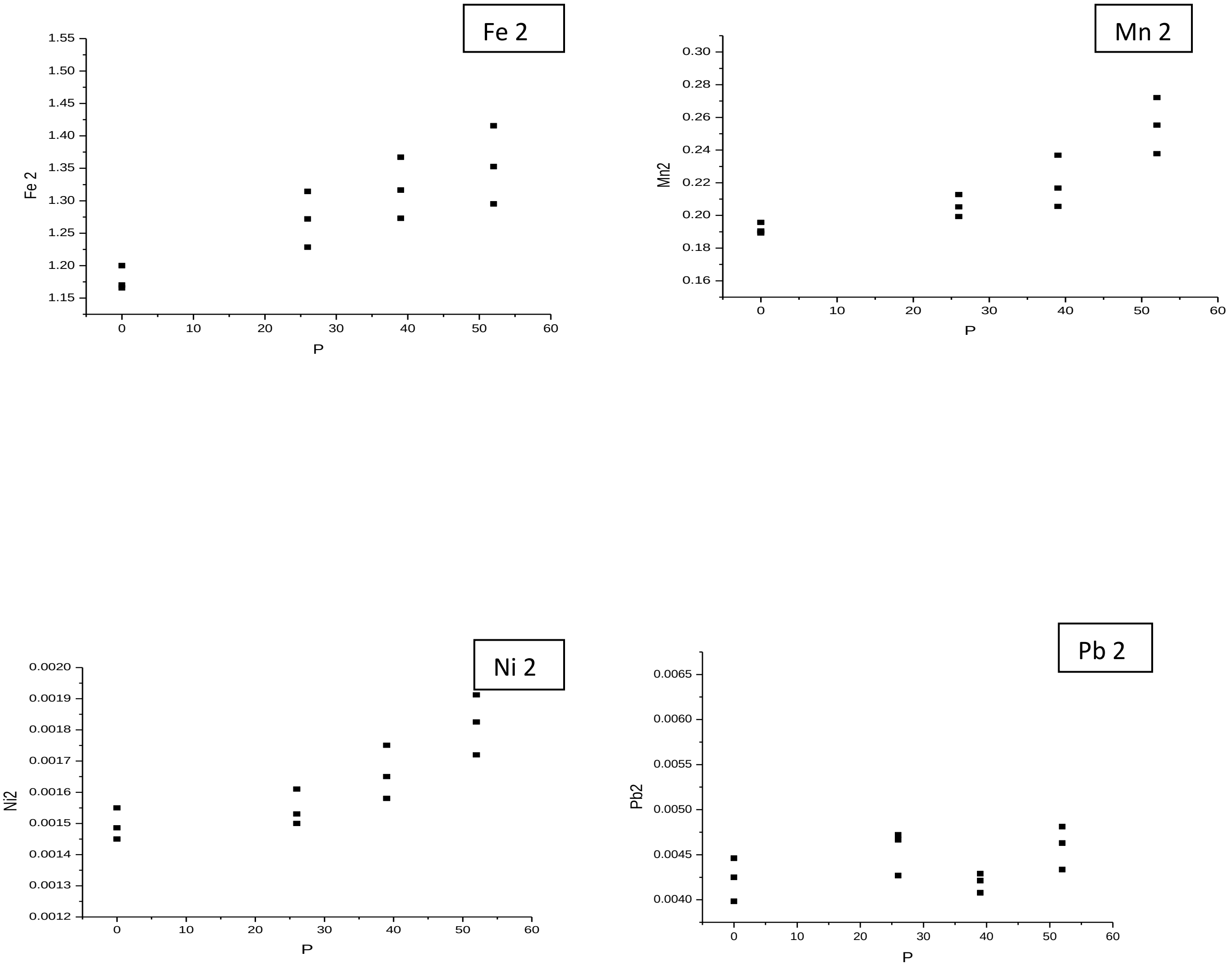


Fig 5.2: the correlation coefficient between the amount of added phosphorus and content of each metal for Second phase

Third phase (moderately reducible):

content of following elements Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Pb extracted after sequential extraction third phase are given in (Table5.8). The (Figures 5.3) shows the correlation coefficient between the amount of added phosphorus and content of each metal for third phase. The amount of added phosphorus was correlated with content of each metal and these correlation coefficient and statistical significance of the relationships (p-values) were presented in (Table5.9).

In this phase by modified Tessier procedure (moderately reducible) showed which elements were bounded to Fe-Mn oxides and hydroxides. The only one positive correlation was with aluminum (0.9975) which could be explained by fixation phosphorus with aluminum. The addition of phosphorus fertilizer resulted in the decrease of metal content.

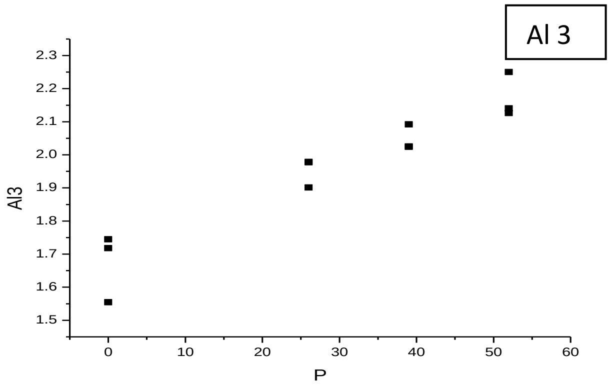
Iron and manganese probably went to available fractions, primary in second which is easy reducible fraction. The part of these mobilized microelements also went to exchangeable fraction. In addition, some of these microelements were adopted of wheat. While some of microelements were sink in deeper soil layers.

Table 5.9: The Pearson’s correlation coefficients (r) for added phosphorus and various substrates from third phase:

	Correlation coefficients. r	P values
P-Al3	0.9975	0.0030
P-Cd3	-0.4522	0.5480
P-Co3	-0.9993	0.0010
P-Cr3	-0.9985	0.0840
P-Cu3	-0.9000	0.1000
P-Fe3	-0.9536	0.0440
P-Mn3	-0.9563	0.0550
P-Ni3	-0.9895	0.0100
P-Pb3	-0.9956	0.0040

Table 5.8: Concentration of metals extracted after modified Tessier Sequential Extraction : Phase 3: Oxalic Acid + NH_4 Oxalate 0.4 M

Fertilizer	Depth	NO	Al mg/g	Ca mg/g	Cd µg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mg mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	*0-30*	*A*	1.6725	0.0549	0.5183	0.0027	0.0085	0.0356	12.3715	0.3137	0.2416	0.0036	0.6454	0.0145	0.0114
	30-60	*B*	1.8790	0.0585	0.0495	0.0025	0.0066	0.0363	12.4037	0.3432	0.2405	0.0035	0.6019	0.0129	0.0104
26 kg/ha	*0-30*	*C*	1.9525	0.0576	0.0826	0.0027	0.0076	0.0372	12.7659	0.3422	0.2543	0.0034	0.7486	0.0140	0.0095
	30-60	*D*	2.0634	0.0589	0.0747	0.0025	0.0071	0.0336	11.4981	0.3379	0.2359	0.0035	0.6017	0.0123	0.0091
39 kg/ha	*0-30*	*E*	2.0473	0.0620	0.0661	0.0025	0.0070	0.0331	11.1663	0.3420	0.2261	0.0034	0.7035	0.0122	0.0119
	30-60	*F*	2.2390	0.0576	0.1059	0.0026	0.0084	0.0350	11.9346	0.3668	0.2388	0.0039	0.6500	0.0125	0.0097
52 kg/ha	*0-30*	*G*	2.1722	0.0661	0.0398	0.0023	0.0069	0.0301	10.4917	0.3180	0.2042	0.0033	0.8159	0.0129	0.0088
	30-60	*H*	2.3440	0.0577	0.0839	0.0026	0.0086	0.0335	12.0716	0.3672	0.2399	0.0038	0.7499	0.0131	0.0092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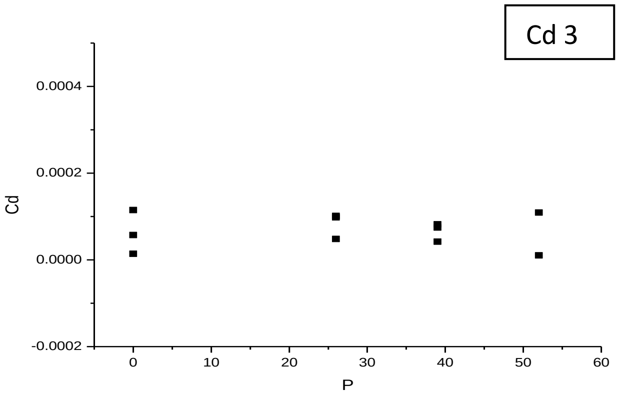
40
50
60
P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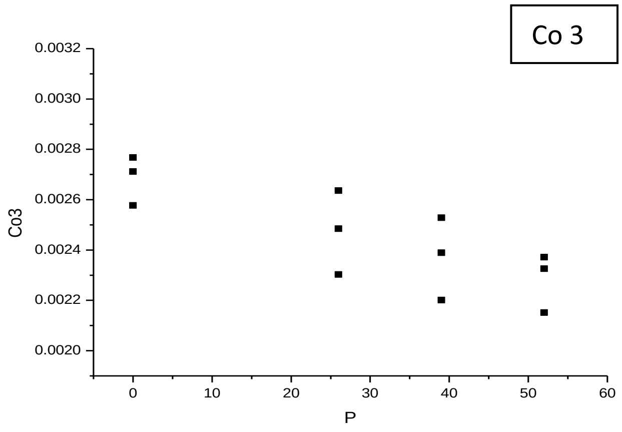
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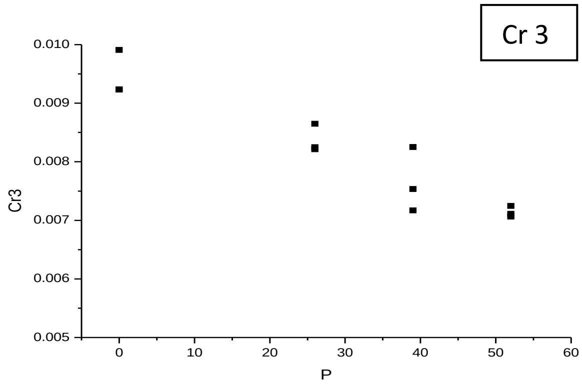
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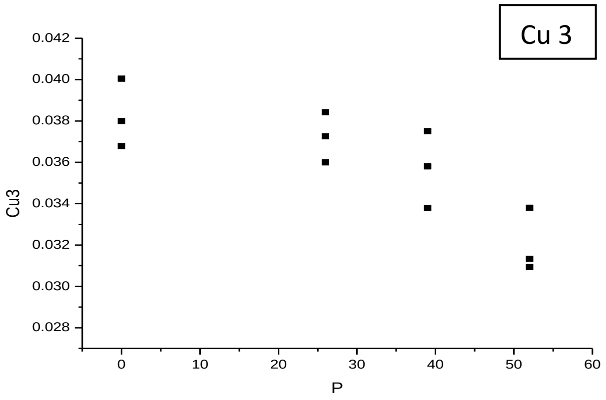
P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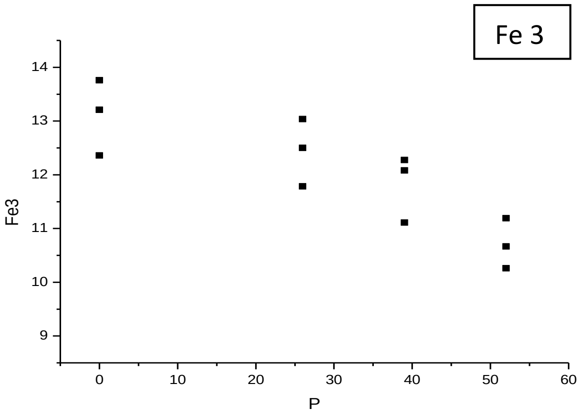
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P

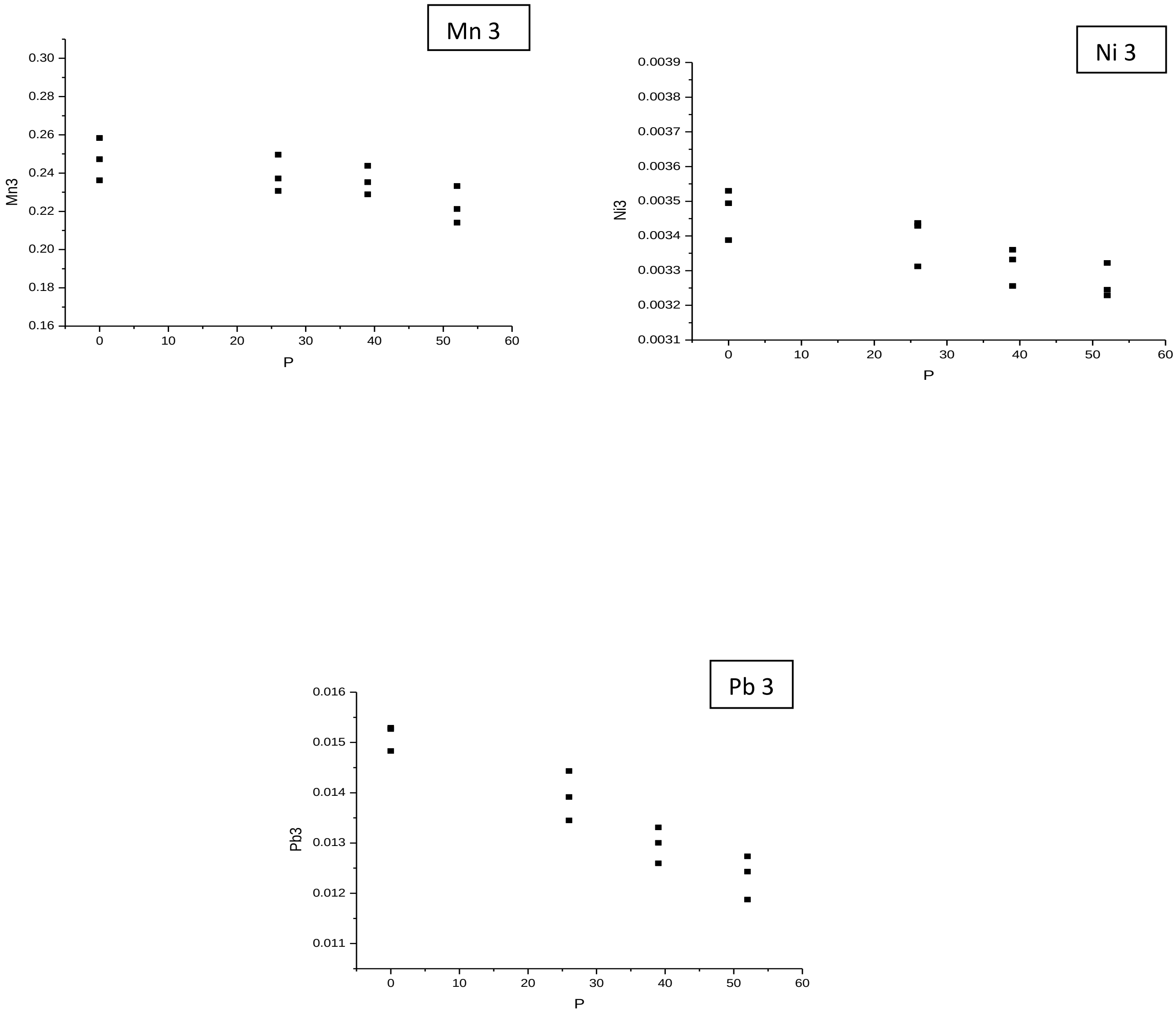


Fig 5.3: the correlation coefficient between the amount of added phosphorus and content of each metal for Third phase

Fourth phase (organic):

content of following elements Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Pb extracted after sequential extraction fourth phase are given in (Table5.10). The (Figures 5.4) shows the correlation coefficient between the amount of added phosphorus and content of each metal for fourth phase. The amount of added phosphorus was correlated with content of each metal and these correlation coefficient and statistical significance of the relationships (p-values) were presented in (Table5.11).

This phase (organic) showed the least sensitivity on amount of applied phosphorus fertilizer. It is noted, decreasing of content extracted aluminum and especially nickel with high negative correlation factor (-0.9799). Cobalt (0.7194) and copper (0.8761) had good correlations with added phosphorus as we expected because of their ability to form sulphide.

Table 5.11: The Pearson’s correlation coefficients (r) for added phosphorus and various substrates from fourth phase:

	Correlation coefficients. r	P values
P-Al4	-0.7638	0.2360
P-Cd4	0.5944	0.4060
P-Co4	0.7194	0.5730
P-Cr4	0.6787	0.3210
P-Cu4	0.8761	0.1240
P-Fe4	-0.5012	0.4990
P-Mn4	-0.4318	0.5680
P-Ni4	-0.9799	0.0200
P-Pb4	0.2345	0.7600

Table 5.10: Concentration of metals extracted after modified Tessier Sequential Extraction : Phase 4: NH_4 Acetate 3.2 M + H_2O

Fertilizer	Depth	NO	Al mg/g	Ca mg/g	Cd mg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mg mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	*0-30*	*A*	0.7871	0.5761	0.0011	0.0009	0.0032	0.0013	0.1689	0.7317	0.0200	0.0047	0.0000	0.0009	0.0054
	30-60	*B*	0.3877	0.4727	0.0016	0.0008	0.0030	0.0012	0.0244	0.5659	0.0145	0.0040	0.0000	0.0010	0.0045
26 kg/ha	*0-30*	*C*	0.5678	0.5393	0.0006	0.0008	0.0032	0.0011	0.0463	0.5742	0.0164	0.0040	0.0000	0.0012	0.0041
	30-60	*D*	0.4449	0.5731	0.0006	0.0008	0.0026	0.0012	0.0343	0.6124	0.0145	0.0041	0.0000	0.0018	0.0033
39 kg/ha	*0-30*	*E*	0.8424	0.5315	0.0020	0.0009	0.0036	0.0013	0.0729	0.6152	0.0170	0.0044	0.0000	0.0010	0.0050
	30-60	*F*	0.8768	0.5926	0.0011	0.0007	0.0034	0.0011	0.0392	0.5402	0.0141	0.0039	0.0000	0.0010	0.0044
52 kg/ha	*0-30*	*G*	0.5310	0.5266	0.0014	0.0008	0.0029	0.0013	0.0402	0.5274	0.0142	0.0036	0.0000	0.0007	0.0038
	30-60	*H*	0.3551	0.5426	0.0004	0.0007	0.0023	0.0009	0.0190	0.6031	0.0151	0.0038	0.0000	0.0004	0.0020

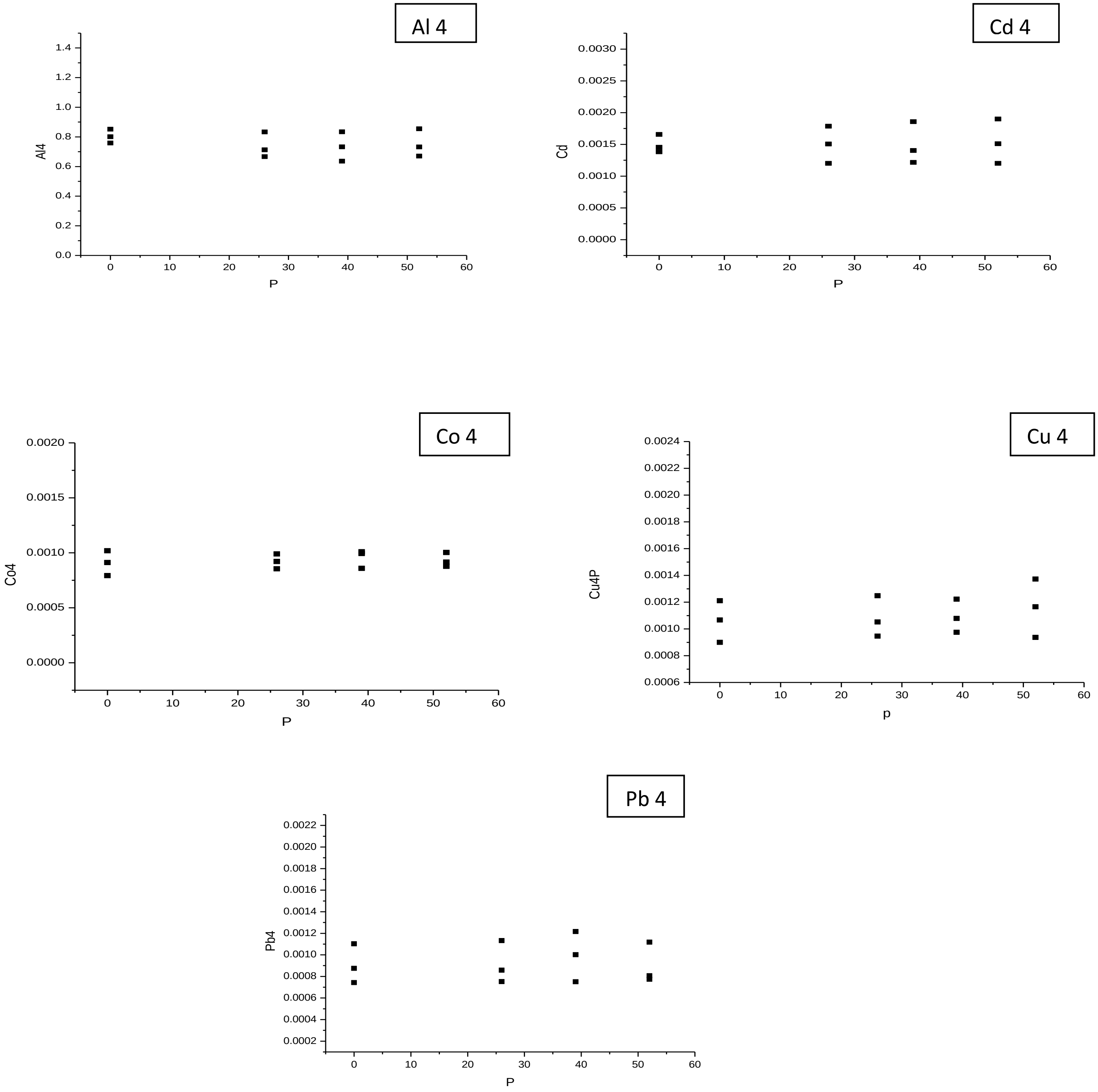


Fig 5.4: the correlation coefficient between the amount of added phosphorus and content of each metal for Fourth phase

Fifth phase:

Content of following elements Al, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Pb extracted after sequential extraction fifth phase are given in (Table5.12). The (Figures 5.5) shows the correlation coefficient between the amount of added phosphorus and content of each metal for fifth phase. The amount of added phosphorus was correlated with content of each metal and these correlation coefficient and statistical significance of the relationships (p-values) were presented in (Table5.13).

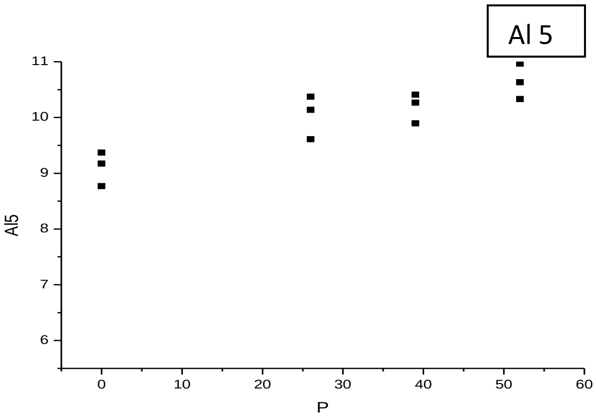
In this phase, the increasing of amount applied phosphorus fertilizer brought to increase incorporation some of elements in mineral matrix. The high correlation coefficient was found with aluminum, cadmium and chromium, 0.9883, 0.9991 and 0.9851, respectively. This phase is not available in common condition of exploitation. It is very important because there is no mobilization of these elements in available form.

Table 5.13 The Pearson's correlation coefficients (r) for added phosphorus and various substrates from fifth phase:

	Correlation coeficients. r	P values
P-Al5	0.9883	0.0120
P-Cd5	0.9991	0.0010
P-Co5	0.8033	0.1970
P-Cr5	0.9851	0.0150
P-Cu5	0.6280	0.3720
P-Fe5	0.6995	0.7874
P-Mn5	0.8567	0.1430
P-Ni5	-0.5379	0.4620
P-Pb5	0.8259	0.1740

Table 5.12: Concentration of metals extracted after modified Tessier Sequential Extraction : Phase 5: HCl 6 M :

Fertilizer	**Depth**	***NO***	**Al mg/g**	**Ca mg/g**	**Cd mg/g**	**Co mg/g**	**Cr mg/g**	**Cu mg/g**	**Fe mg/g**	**Mg mg/g**	**Mn mg/g**	**Ni mg/g**	**P mg/g**	**Pb mg/g**	**Zn mg/g**
0 kg/ha	***0-30***	***A***	**9.1036**	**0.2281**	**0.0008**	**0.0025**	**0.0218**	**0.0394**	**9.6787**	**2.1807**	**0.0519**	**0.0137**	**0.1472**	**0.0316**	**0.0386**
	30-60	***B***	**10.0396**	**0.2806**	**0.0008**	**0.0028**	**0.0235**	**0.0392**	**9.6774**	**2.4838**	**0.0545**	**0.0117**	**0.1393**	**0.0317**	**0.0377**
26 kg/ha	***0-30***	***C***	**10.0402**	**0.2701**	**0.0008**	**0.0029**	**0.0241**	**0.0410**	**10.0939**	**2.5489**	**0.0579**	**0.0135**	**0.1628**	**0.0345**	**0.0403**
	30-60	***D***	**10.2175**	**0.2717**	**0.0008**	**0.0029**	**0.0247**	**0.0399**	**9.8855**	**2.5683**	**0.0545**	**0.0154**	**0.1457**	**0.0333**	**0.0407**
39 kg/ha	***0-30***	***E***	**10.1889**	**0.2806**	**0.0008**	**0.0028**	**0.0243**	**0.0401**	**9.8941**	**2.5425**	**0.0579**	**0.0129**	**0.1577**	**0.0333**	**0.0397**
	30-60	***F***	**10.2565**	**0.2642**	**0.0008**	**0.0029**	**0.0246**	**0.0400**	**9.9477**	**2.6378**	**0.0555**	**0.0137**	**0.1493**	**0.0334**	**0.0388**
52 kg/ha	***0-30***	***G***	**8.6387**	**0.2318**	**0.0007**	**0.0025**	**0.0207**	**0.0341**	**8.5590**	**2.1528**	**0.0478**	**0.0120**	**0.1414**	**0.0284**	**0.0359**
	30-60	***H***	**10.3451**	**0.2411**	**0.0008**	**0.0030**	**0.0256**	**0.0407**	**10.0598**	**2.6247**	**0.0554**	**0.0129**	**0.1572**	**0.0343**	**0.0411**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Al5
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10
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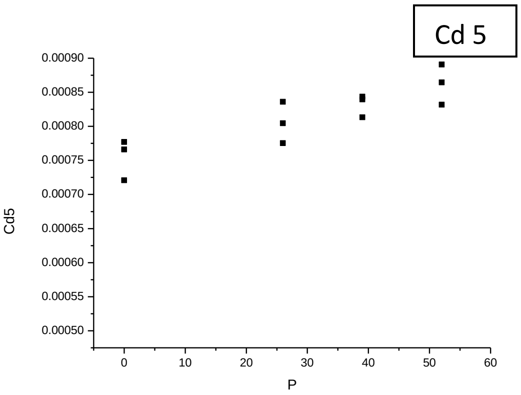
8
7
6
0
10
20
30
40
50
60
P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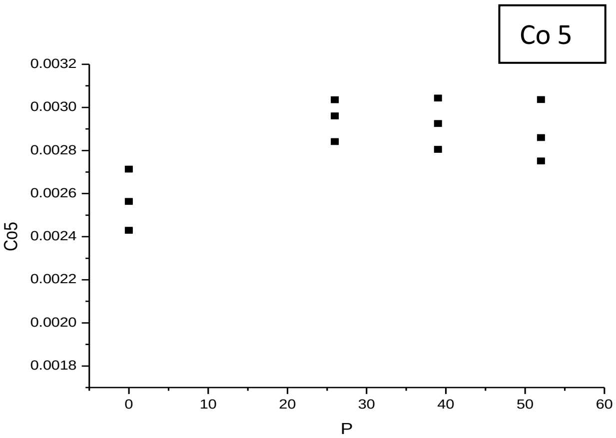
0
10
20
30
40
50
60
P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Co5
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0
10
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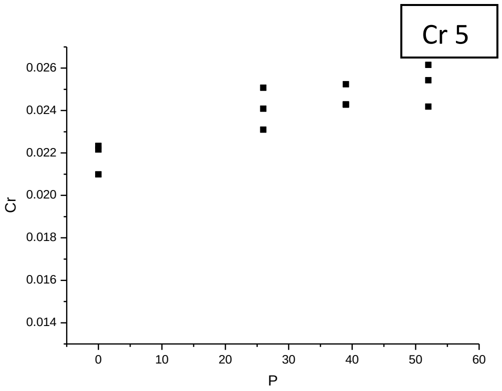
30
40
50
60
P


Cr 5
Cr
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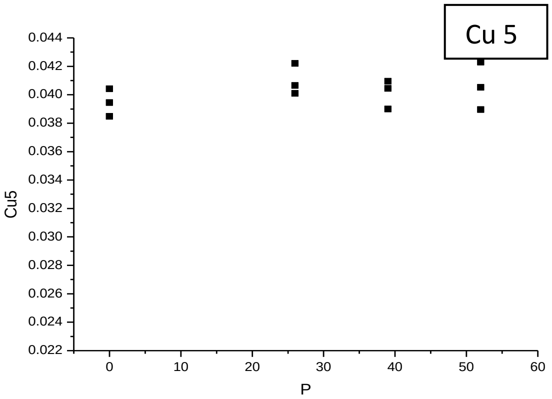
0.024
0.022
0.020
0.018
0.016
0.014
0
10
20
30
40
50
60
P


Cu 5
Cu5
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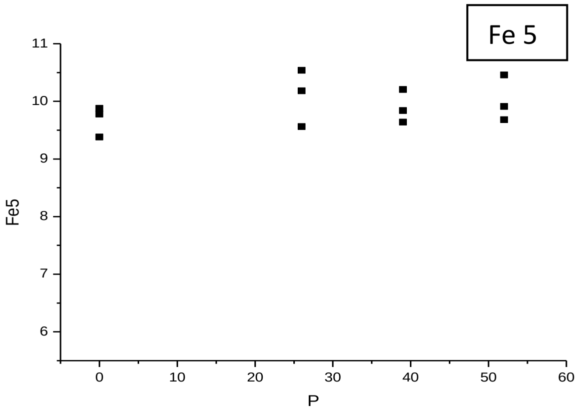
0.040
0.038
0.036
0.034
0.032
0.030
0.028
0.026
0.024
0.022
0
10
20
30
40
50
60
P


Fe 5
Fe5
11
10
9
8
7
6
0
10
20
30
40
50
60
P

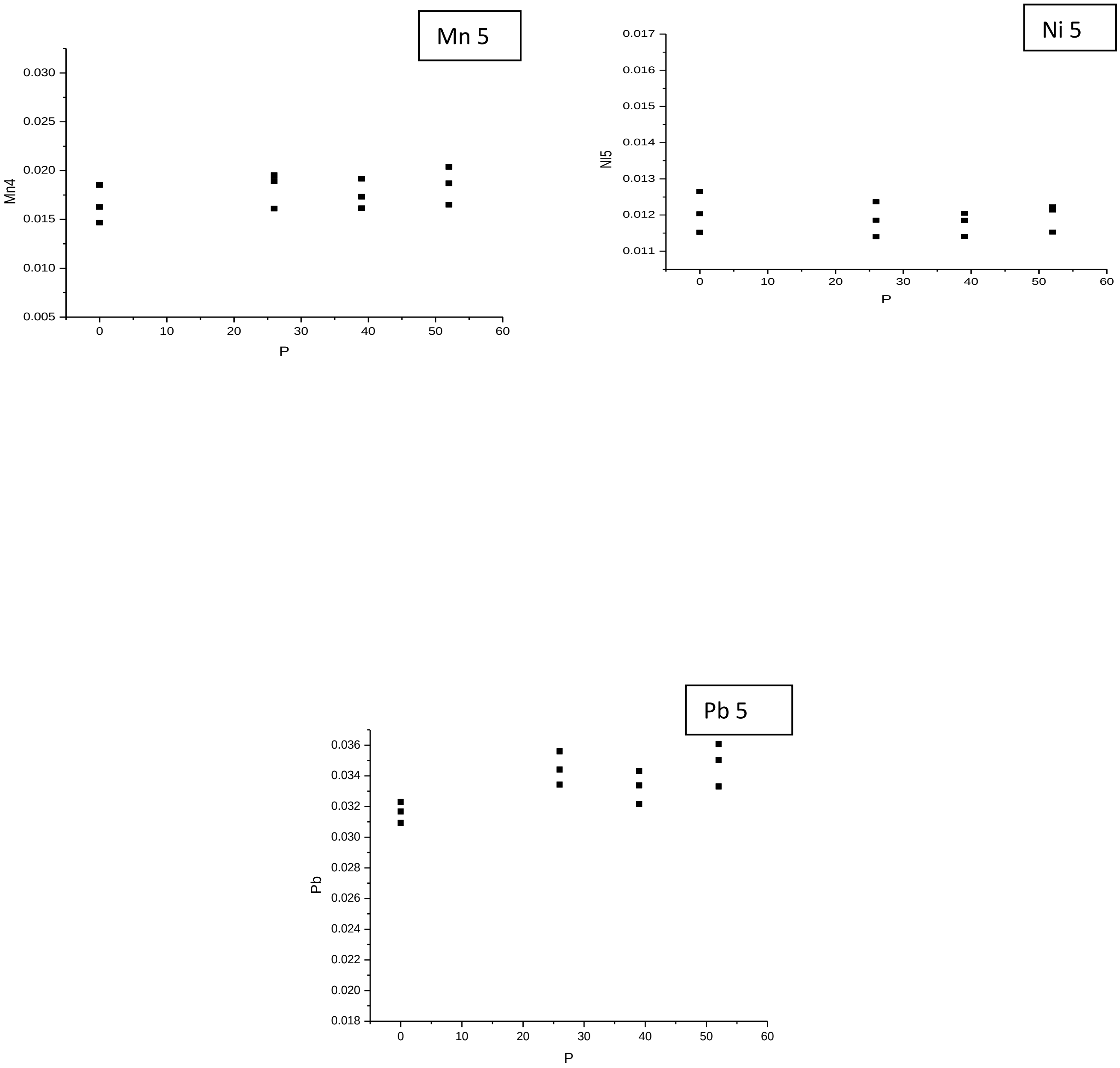


Fig 5.5: the correlation coefficient between the amount of added phosphorus and content of each metal for Fifth phase

The standard reference material (wheat flour NIST SRM 1567a) was used for calibrating instrument and evaluating the reliability of analytical method for this determination.

In acidic mineral soils, as in our case, lead was slightly adopted by the wheat. The great power of root to accumulate the lead could be kind of protection for above-ground part of plants [395]. It was also found that lead content in soil samples was much higher than in plants. The lead content in plant increased significantly with the increase of amounts of applied fertilizer. It suggested that the amount of fertilizer must be controlled.

It is known that application of phosphorus fertilizers increase the content of cadmium in soil samples [396]. The increase of cadmium concentration with amount of applied fertilizer was evident in Table (5.14). On the other hand, the concentration of cadmium in plants (grain or stalk) was very similar (Table 5.3 and 5.4). The content of copper and nickel in soil samples (Table 5.14) was much higher than in plants. It suggested that copper and nickel participated in the formation of insoluble complexes with organic matter and thus they were retained in the soil. The characteristic of soil had positive effect on their absorption prevention [397].

When the total amount of adopted microelements (mgha^{-1}) was compared with the amount of applied phosphorus (kgPha^{-1}) it was observed that for adoption of most microelements the maximum was achieved for the lowest amount of added P (26 kgha^{-1}). This is presented on Figure (5.6).

When the 26 kgha^{-1} of phosphorus was added the yield of wheat yield was considerably increased. Further increase of added fertilizer did not significantly increase the yield (Table 5.5).

The highest applied average amounts (52 kgPha^{-1}) even resulted in the reduction of wheat yield. It should be noted that the amount of nitrogen fertilizer was constant and it certainly influenced the low yields. A very important factor to be considered was a dry year and the lack of irrigation systems.

Table 5.14: Concentration of metals extracted after sequential extraction:

Concentration of Al extracted after sequential extraction (mg/g on dry soil)						
Phosphate fertilizer kgPha^{-1}	I phase	II phase	III phase	IV phase	V phase	Total
0	0.0234	0.6747	1.6725	0.8039	9.1036	12.2781
26	0.1121	0.7977	1.9525	0.7375	10.0402	13.6400
39	0.0997	0.8944	2.0473	0.7343	10.1889	13.9646
52	0.1911	1.0312	2.1722	0.7521	10.6425	14.7891
Concentration of Cd extracted after sequential extraction (µg/g on dry soil)						
0	0.020	0.155	0.062	1.497	0.755	2.489
26	0.035	0.162	0.083	1.497	0.805	2.582
39	0.264	0.168	0.066	1.490	0.832	2.820
52	2.223	0.176	0.040	1.537	0.862	4.838
Concentration of Co extracted after sequential extraction (µg/g on dry soil)						
0	0.156	2.597	2.687	0.908	2.567	8.913
26	0.235	2.787	2.473	0.921	2.943	9.359
39	0.271	3.253	2.373	0.954	2.923	9.774
52	0.344	3.657	2.283	0.931	2.883	10.099
Concentration of Cr extracted after sequential extraction (µg/g on dry soil)						
0	0.066	0.860	9.460	3.417	21.830	35.633
26	0.164	0.923	8.370	3.183	24.087	36.727
39	0.253	0.960	7.650	3.393	24.603	36.860
52	0.609	1.040	7.140	13.267	25.257	47.312
Concentration of Cu extracted after sequential extraction (µg/g on dry soil)						
0	1.887	5.573	38.273	1.060	39.443	86.237
26	2.427	5.703	37.227	1.082	40.987	87.425
39	2.623	5.643	35.697	1.092	40.127	85.182
52	2.790	5.600	32.020	1.159	40.590	82.159

Concentration of Fe extracted after sequential extraction (mg/g on dry soil)						
0	0.012	1.179	13.108	0.069	9.679	24.047
26	0.018	1.272	12.440	0.046	10.094	23.869
39	0.014	1.319	11.822	0.073	9.894	23.122
52	0.040	1.354	10.706	0.040	10.017	22.157
continued						
Concentration of Mn extracted after sequential extraction (mg/g on dry soil)						
0	0.051	0.192	0.247	0.017	0.016	0.524
26	0.049	0.206	0.239	0.018	0.018	0.530
39	0.052	0.220	0.236	0.017	0.018	0.543
52	0.058	0.255	0.223	0.017	0.019	0.571
Concentration of Ni extracted after sequential extraction (µg/g on dry soil)						
0	0.517	1.497	3.470	4.967	12.067	22.517
26	0.739	1.547	3.393	4.500	11.877	22.056
39	0.940	1.660	3.317	4.450	11.767	22.134
52	1.073	1.820	3.263	4.267	11.967	22.390
Concentration of Pb extracted after sequential extraction (µg/g on dry soil)						
0	1.527	4.230	15.127	0.906	31.630	53.419
26	1.213	4.553	13.933	0.913	34.477	55.090
39	1.207	4.193	12.970	0.990	33.280	52.640
52	1.164	4.593	12.343	0.900	34.800	53.801

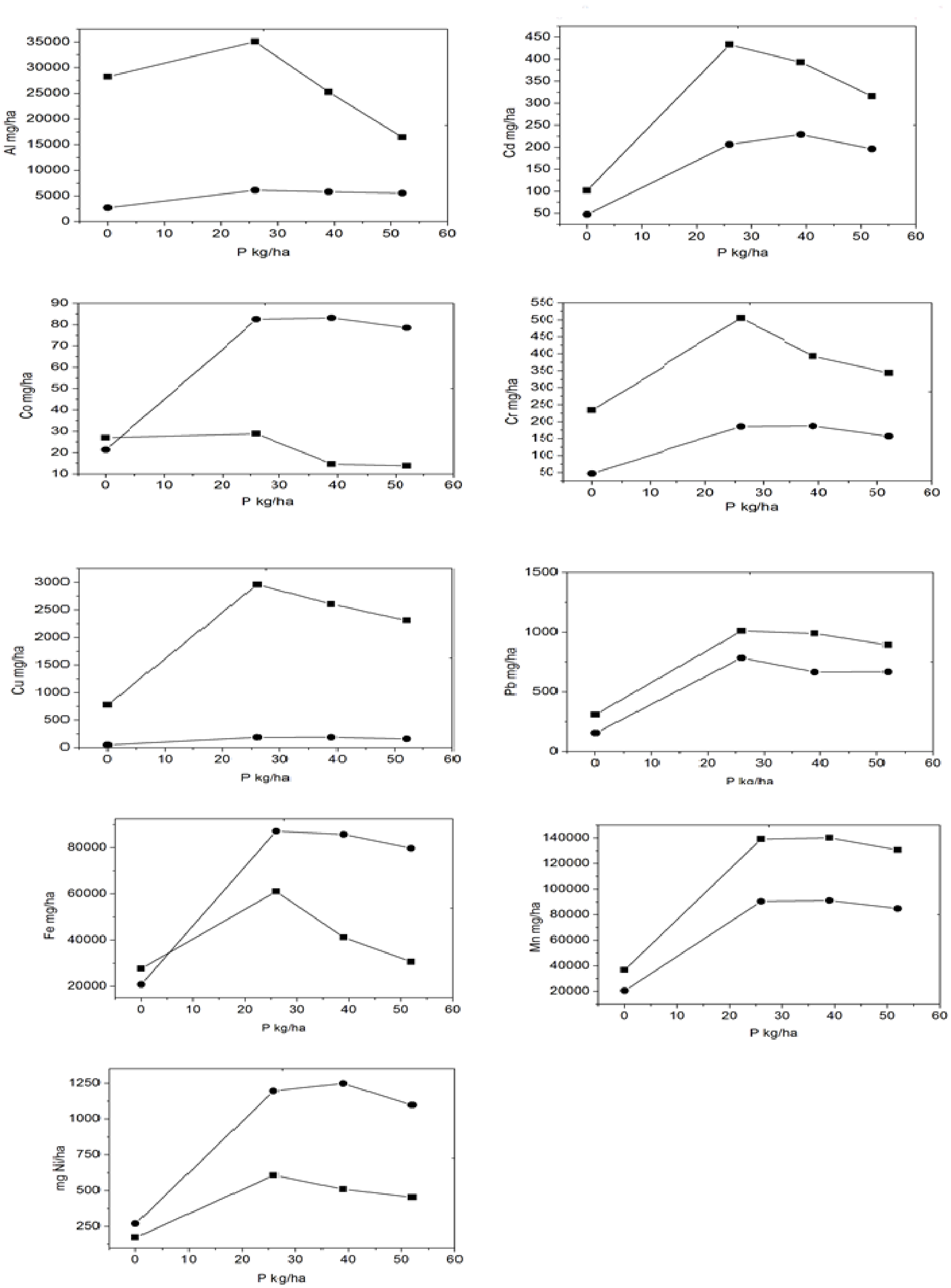


Fig 5.6 the total amount of adopted microelements (mgha^{-1}) compared with the amount of applied phosphorus (kgPha^{-1}).

5.2 MODIFIED CHANG AND JACKSON PROCEDURE FOR THE PHOSPHORUS SEQUENTIAL EXTRACTION (MANOJLOVIC ET AL. 2007):

The obtained results are presented in Table (5.15)

Phosphorus extractable by NH_4Cl (water-soluble P): The content of phosphorus soluble in ammonium chloride solution (NH_4Cl) was low, about 1 % of the total content of mineral phosphorus. Fertilization with mineral phosphorus increased the content of water-soluble phosphorus in comparison with the control sample (6 times for sample treated by 52 kg P ha^{-1}, 0–30 cm depth). This significant increase was directly related to the accumulation of P [398] and was influenced by the processes of saturation of free spaces for adsorption of P in soil [399].The increase of water soluble P was lower for samples of 30–60 cm depth, which could be explained by P fixation during through-soil leaching.

Phosphorus bound to aluminum (Al–P):

Phosphorus bound to aluminum (Al–P) represented a significant part of the mineral phosphorus. The recorded relative increase of Al–P versus the other mineral forms of P (from 16.08 % in the control to 34.51 % in the treatment with 52 kg P ha^{-1}) indicated the strong effect of fertilization exactly on this form of phosphorus. This was confirmed by the significant absolute increase of Al–P according to the applied fertilizer (three times more in 52 kg P ha^{-1} compared to the control). In acidic mineral soils, as in the present case, P is fixed mainly by aluminum and iron while in alkaline soils by calcium [400].The phosphorus movement along the depth was very limited and slow due to its strong binding. However, the increase in the absolute and relative values (from 14.35 % in the control to 19.18 % in the treatment with 52 kg P ha^{-1}), as well as the high coefficient of correlation between the first and second depths, indicate its mobility.

Phosphorus bound to iron (Fe–P):

Phosphate bound to iron represented a very small fraction (about 0.1 %) of the mineral Al forms, probably due to its binding to reducible and occluded forms of P, similarly as the labile fraction of Fe–P is included in the Al–P phase. However, in spite of the small amounts, application of P-fertilizer increased its content compared to the control for the first depth, 0–30 cm (Table 5.15). The percentage of Fe–P compared to the other mineral forms changed very little.

Table **(5.15):** Sequential analysis by a modified Chang and Jackson method. Forms of phosphorous in Stagnosol in a long-term phosphate fertilization experiment; m in mg kg^{-1}; w – ratio to control; w_{min} – content of mineral P in %; a, b, c, d, - the identical letters show no significant difference between the different fertilization treatments

kg P ha^{-1}	Treatment																	
	NH_4CL-P			AL-P			Fe-P			Reducible P			Occluded P			Ca-P		
	m	w	w_{min}	m	w	w_{min}	m	w	w_{min}	m	w	w_{min}	m	w	w_{min}	m	w	w_{min}
Depth: 0-30																		
0	3.08a	1.0	0.8	63.77a	16.1	1.0	0.30a	0.07	1.0	143.08a	36.1	1.0	46.94ab	11.8	1.0	139.35a	35.1	1.0
26	5.35a	1.7	1.2	100.80b	23.4	1.6	0.54b	0.13	1.8	125.04a	29.1	0.9	32.57a	7.6	0.8	165.75a	38.5	1.2
39	9.96b	3.2	0.6	130.16c	23.8	2.0	0.44ab	0.08	1.5	195.57b	35.7	1.4	67.37c	12.3	1.4	144.50a	26.4	1.0
52	19.38c	6.3	1.1	194.88d	34.5	3.1	0.46b	0.08	1.5	176.07b	31.2	1.2	60.70bc	10.7	1.3	126.33a	22.4	0.9
P	<0.01			<0.001			<0.001			<0.01			<0.05					
Depth: 03-60																		
0	2.69a	1.0	0.8	48.50a	14.4	1.0	0.30a	0.09	1.0	120.27a	35.6	1.0	33.93a	10.0	1.0	132.15a	39.11	1.00
26	3.71a	1.4	0.9	63.33ab	15.9	1.3	0.41a	0.10	1.4	130.44a	32.6	1.1	44.73a	11.2	1.3	157.02a	39.29	1.19
39	5.38a	2.0	1.4	78.96b	19.8	1.6	0.31a	0.08	1.0	172.10b	43.1	1.4	56.67b	14.2	1.7	137.91a	34.51	1.04
52	4.67a	1.7	1.1	83.78b	19.2	1.7	0.28a	0.06	0.9	116.37a	26.6	1.0	47.47a	10.9	1.4	184.15a	42.17	1.39
P		-			<0.01						<0.01			<0.05				
Correlation cofficient between two depths	0.518($P<0.05$)			0.876($P<0.01$)			0.405			0.405			0.254			0.385		

Reducible phosphorus (R-P):

The reducible-P was well represented in the fraction compared to other mineral forms of P (about 30 %). Noticeably, the portion of this P *versus* the other forms in the first depth was the greatest in the control treatment, while in the higher fertilizer treatments; it was similar to the values of Al–P (Table 5.15). The absolute content of R-P in soil between treatments was different. However, no clear tendency of the increase in its content for each treatment was observed, especially for the second depth.

Occluded phosphorus (O-P):

The occluded phosphorus fraction represented about 10 % of the mineral forms of P. Fertilization resulted in irregular increases in the content of occluded P, probably due to irregularities of the field conditions and the processes that influence the content of O-P. Changes in the content of O-P were not detected.

Phosphorus bound to calcium (Ca–P):

Phosphorus bound to calcium was a significant fraction within the studied soil. For the second depth, its portion in the mineral forms of P was the greatest (42.17 % in the treatment with 52 kg P ha^{-1}). The absolute content of Ca–P did not show significant differences for both depths (Table 5.15). The absence of the effect of P-fertilization in acidic soils was also recorded in another paper. [401] A possible explanation lies in the fact that Ca–P was localized within the mineral apatite and thus, weakly changeable. However, the relative increase of Ca–P for the second depth comparing to the other mineral P forms indicated a significantly reduced binding of phosphorous with Al in the second depth.

5.3 COMMUNITY BUREAU OF REFERENCE (BCR) SEQUENTIAL EXTRACTION PROCEDURE FOR THE PHOSPHORUS (BCR (RUBAN ET AL., 2001)):

The BCR analysis is not a classic sequential analysis. Less specific means for isolation of the first two fractions are used in comparison with the Chang and Jackson method. The share of NaOH–P (Al–Fe–Mn bound P) and HCl–P (Ca–P) fractions increased for both depths and depended on the amount of added fertilizer (Table 5.16). The results obtained for the fertilized samples indicated the leaching of the NaOH fraction from the first to the second depth.

The fraction of organic phosphorous (Org-P) represented about 20–30 % of the sum of the inorganic and organic P. The relative amount of organic P decreased from 28.9 to 20.3 %, correspondingly to the application rate of the fertilizer in the first depth, while in the second depth, this decrease was not linear (Table 5.16). However, for the second depth, the two highest rates of treatment with fertilizer resulted in higher contents of Org-P in comparison with the first depth. The absolute content of Org-P was not changed, which indicates that phosphorus from the fertilizer did not transform to organic P.

The content of concHCl-P showed a linear increase with depth through both layers, with the increase in the greater depth indicating greater fixation of P.

The certified reference material CRM 684 (river sediment extractable phosphorus, BCR, Community Bureau of Reference, Brussels, Belgium) was used to confirm the accuracy of the employed method. The results of phosphorous determination in the reference material showed very good agreement with the declared value (Table 5.17).

Table 5.17: Results of the BCR method. (Forms of phosphorous in the certified reference material (CRM 684)):

Phosphorous forms	NaOH-P	HCl-P	Inorganic P	Organic P	Conc. HCl-P
Declared, mg kg^{-1}	550±21	536±26	1113±24	209±9	1373±35
Found, mg kg^{-1}	587±11	530±11	1145±10	224±5	1401±10

Table **(5.16):** Results of the BCR method. Forms of phosphorous in Stagnosol in a long-term phosphate fertilization experiment; m in mg kg^{-1}; w – ratio to control; w_{IP+OP} – sum of IP + organic P, %; a, b, c - the identical letters show no significant difference between the different fertilization treatments

kg P ha^{-1}	Treatment											
	NaOH-P		HCL-P		IP			Organic P			Conc.HCL P	
	m	w	m	w	m	w	w_{IP+OP}	m	w	W_{IP+OP}	m	w
Depth: 0-30												
0	523.33a	1.00	366.7a	1.00	990.0a	1.00	71.0	403.3	1.0	28.9	892.0a	1.0
26	806.67b	1.54	533.3b	1.45	1200.0ab	1.21	76.3	373.3a	0.9	23.7	1127.7ab	1.3
39	1033.33b	1.97	683.3c	1.86	1473.3bc	1.49	79.6	376.7	0.9	20.6	1188.3b	1.3
52	1003.33b	1.97	753.3c	2.05	1600.0c	1.61	79.7	406.7	1.1	20.3	1374.0c	1.5
P	<0.001		<0.001		<0.05			<0.01				
Depth: 30-60												
0	583.33a	1.00	340.0a	1.00	946.7a	1.00	71.0	386.7ab	1.0	29.0	813.7a	1.0
26	890.00b	1.52	420.0a	1.23	1020.0a	1.08	74.5	350.0a	0.9	25.6	958.3ab	1.2
39	1210.00c	2.07	546.7b	1.61	1093.3a	1.15	73.7	390.0ab	1.1	26.3	1048.7bc	1.3
52	1196.67c	2.05	586.7b	1.64	1226.7a	1.30	72.0	476.7b	1.2	28.0	1068.0c	1.3
P	<0.01		<0.05		-				- -			
Correlation cofficient between two depths	0.901(*P*<0.01)		0.865(*P*<0.01)		0.483			-0.075			0.799(*P*<0.01)	

5.4 DETERMINATION OF SUBSTRATES OF METALS AND PHOSPHORUS:

The results for the phosphorus sequential extraction methods were compared to the results for the sequential extraction of metals and a better assessment of substrates (Fe, Al, Mn and Ca) associated with phosphorus was thereby provided.

The most important correlations found between the contents of the different phosphorus forms and corresponding substrates are presented in (Tables 5.18 and 5.19).

The very high correlation between the applied P and aluminum from the second and the third phase confirms the importance of the aluminum present in soils for the chemodynamics of phosphorus. Regardless of the applied phosphorus extraction method, very high correlations between contents of aluminum and most phosphorus forms were obtained.

Aluminum from the second phase (aluminum II) is a typical representative of the clay minerals present in the examined samples and it could be extracted by an acid hydroxyamine solution, together with manganese oxides and the associated form of P. The role of aluminum II and manganese II confirmed the good correlation with different forms of P (Tables 5.18 and 5.19).

Oxalate/oxalic acid (the extraction solution for phase III) is very efficient in the extraction of different forms of phosphorus associated with colloid amorphous aluminum oxides and iron oxides. This fraction in the examined samples represents a significant substrate of phosphorus and the highest increasing content of phosphorus due to fertilization. This is confirmed by the very high correlation factor.

Although most of iron oxides are present in phase III (there is no strong correlation with phosphorus), their role in the chemodynamics of phosphorus should not be ignored, especially because aluminum has a tendency to co-precipitate with iron oxide.

In addition, considering the soil type, the low correlations with calcium were expected.

Table 5.18: The correlation of extracted phosphorus by the modified Chang and Jackson method with various substrates:

Parameter	Correlation coefficient, *r*	*P* value
Added P / Al II	0.9663	0.000
NH_4Cl-P / Al II	0.8502	0.000
Al-P / Al II	0.9037	0.000
Added P / Mn II	0.6911	0.013
NH_4Cl-P / Mn II	0.6599	0.020
Al-P / Mn II	0.7380	0.006
Al-P / Al III	0.8255	0.001
Red-P / Al III	0.5778	0.049
Added P / Al III	0.9546	0.000

Table 5.19: The correlation of extracted phosphorus by the BCR method with various substrates:

Parameter	Correlation coefficient, *r*	*P* value
NaOH-P / Al II	0.8936	0.000
HCl-P / Al II	0.9120	0.000
IP / Al II	0.8931	0.000
concHCl-P / Al II	0.8879	0.000
NaOH-P / Mn II	0.6383	0.025
HCl-P / Mn II	0.7321	0.007
NaOH-P / Al III	0.9122	0.000
HCl-P / Al III	0.8945	0.000
IP / Al III	0.8709	0.000
concHCl-P / Al III	0.8721	0.000

5.5 BIOACCUMULATION FACTOR (BAF):

The Bioaccumulation factor (BAF) is a competent technique developed to assess the level of the metal in the plant as a fraction of the soils total. Also Chemical bioaccumulation factors (BAF) are considered a simple tool to estimate the bioaccumulation of contaminants in a particular organism in a specific medium.

In this study, the Bioaccumulation Factor (BAF) was used to estimate the bioavailability of (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, P, and Pb) in the Grain and Wheat.

It was calculated as the ratio of the element concentration in the Grain and Wheat (in mg g^{-1} dry wt) to the total soil element content (in mg g^{-1} dry wt).

The BAF of metals was calculated by: $BAF=C_{\text{Grain or Wheat}}/C_{\text{soil}}$

$C_{\text{Grain or Wheat}}$ and C_{soil} are metals concentration in the Grain and Wheat (mg g^{-1}) and soil (mg g^{-1}).

Total concentration of selected metals in Grain and Wheat is given in Tables (5.3, 5.20), and the Total concentration of selected metals in soil total digestion is given in Table(5.21).

Table (5.20): Total concentration of selected metals in Wheat:

Eelement	concentration (μg/g)			
Al	100.47	27.26	20.13	16.84
Cd	0.45	0.40	0.38	0.34
Co	0.14	0.06	0.05	0.05
Cr	0.89	0.44	0.36	0.33
Cu	6.00	5.42	5.19	5.22
Fe	242.52	90.57	69.57	62.88
Mn	170.56	138.15	140.76	138.33
Ni	1.20	0.87	0.95	0.89
P	3,550.52	3,245.47	3,465.76	3,567.36
Pb	1.39	1.08	1.93	2.30

Bioaccumulation factor (BAF) was calculated as metal concentration ratio of Grain and Wheat to fraction of the soil total. And the results from this study as shown in Table (5.22). Table (5.22): Bioaccumulation factor (BAF) of the metals in the grain and wheat:

elements	species	0 kg/ha	26 kg/ha	39 kg/ha	52 kg/ha
Al	grain	0.0009	0.0005	0.0004	0.0005
	wheat	0.014	0.004	0.003	0.003
Cd	grain	0.1571	0.1667	0.1833	0.1833
	wheat	0.643	0.667	0.633	0.567
Co	grain	0.0071	0.0060	0.0067	0.0063
	wheat	0.020	0.009	0.008	0.008
Cr	grain	0.0023	0.0019	0.0021	0.0018
	wheat	0.018	0.009	0.008	0.008
Cu	grain	0.0756	0.0771	0.0736	0.0801
	wheat	0.133	0.124	0.112	0.118
Fe	grain	0.0003	0.0002	0.0003	0.0002
	wheat	0.001	0.001	0.000	0.000
Mn	grain	0.1371	0.1201	0.1366	0.1378
	wheat	0.488	0.379	0.439	0.441
Ni	grain	0.0529	0.0475	0.0526	0.0467
	wheat	0.101	0.071	0.083	0.074
P	**grain**	**7.5962**	**6.4782**	**6.4197**	**5.9076**
	wheat	**9.002**	**7.238**	**7.330**	**6.842**
Pb	grain	0.0136	0.0162	0.0483	0.0597
	wheat	0.052	0.046	0.075	0.097

Bioaccumulation factors (BAF) obtained for (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb) were all less than 1.00 implying no bio-accumulation. However, Bioaccumulation factors (BAF) greater than 1.00 obtained for P evidently indicated that the P was highly bio-accumulated and bio-magnified.

5.21 Total soil digestion: Concentration of metals extracted after total digestion:

fertilizer	depth	*NO*	Al mg/g	Ca mg/g	Cd mg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mg mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	*0-30*	*A*	7.2404	2.6678	0.0007	0.0070	0.0488	0.0451	184.0010	0.7495	0.3495	0.0119	0.3944	0.0265	0.0337
	30-60	*B*	7.4441	2.6064	0.0006	0.0072	0.0490	0.0463	185.9780	0.8159	0.3770	0.0126	0.3680	0.0278	0.0334
26 kg/ha	*0-30*	*C*	6.1584	2.6249	0.0006	0.0067	0.0476	0.0437	176.7985	0.6255	0.3647	0.0122	0.4484	0.0235	0.0356
	30-60	*D*	6.2689	2.1969	0.0006	0.0062	0.0434	0.0445	166.2827	0.4866	0.3153	0.0119	0.3585	0.0241	0.0342
39 kg/ha	*0-30*	*E*	6.6489	2.1695	0.0006	0.0060	0.0426	0.0462	164.8974	0.4596	0.3205	0.0114	0.4728	0.0259	0.0328
	30-60	*F*	5.7349	2.3101	0.0006	0.0067	0.0453	0.0468	170.1649	0.4588	0.3239	0.0125	0.3923	0.0232	0.0363
52 kg/ha	*0-30*	*G*	5.7783	2.4332	0.0006	0.0063	0.0440	0.0442	166.5299	0.4501	0.3138	0.0120	0.5214	0.0236	0.0348
	30-60	*H*	5.9069	2.4108	0.0006	0.0065	0.0462	0.0468	173.4814	0.4768	0.3144	0.0128	0.4315	0.0247	0.0365

5.6 BCR SEQUENTIAL EXTRACTION:

The results of first phase (exchangeable) water and acid soluble species showed that the concentrations of metalas after extraction are between (0.0003 mg/g for Co - 0.0277 mg/g for Mn). As we see at (table 5.23).

Table 5.23: Concentration of metals extracted after BCR Sequential Extraction : Phase 1: Actic Acid 0.11M

fertilizer	depth	No	Al mg/g	Cd µg/g	Co mg/g	Cr µg/g	Cu mg/g	Fe mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb µg/g	Zn mg/g
0 kg/ha	*0-30*	A	0.0893	0.2014	0.0005	0.0707	0.0005	0.0050	0.0357	0.0010	0.0381	0.1137	0.0015
	30-60	B	0.0747	0.1547	0.0004	0.0595	0.0005	0.0041	0.0315	0.0012	0.0254	0.1046	0.0008
26 kg/ha	*0-30*	C	0.1072	0.1884	0.0006	0.0712	0.0005	0.0081	0.0427	0.0011	0.0519	0.1231	0.0012
	30-60	D	0.0873	0.1464	0.0004	0.0817	0.0005	0.0044	0.0297	0.0012	0.0363	0.0807	0.0008
39 kg/ha	*0-30*	E	0.1095	0.1970	0.0005	0.0717	0.0005	0.0052	0.0353	0.0011	0.0627	0.0960	0.0014
	30-60	F	0.0861	0.1375	0.0004	0.0701	0.0004	0.0030	0.0257	0.0012	0.0447	0.0946	0.0006
52 kg/ha	*0-30*	G	0.1093	0.2131	0.0005	0.0768	0.0004	0.0050	0.0372	0.0012	0.0792	0.0800	0.0012
	30-60	H	0.0774	0.1156	0.0003	0.0838	0.0004	0.0026	0.0277	0.0011	0.0467	0.0888	0.0006

The results of secound phase (reducible species e.g. bound to Fe/Mn oxyhydroxides) showed that the concentrations of metalas after extraction are between (0.0015 mg/g for Ni - 4.9329 mg/g for Fe). As we see at (table 5.24).

Table 5.24: Concentration of metals extracted after BCR Sequential Extraction : Phase 2: $HONH_2Cl$ 0.5M

fertilizer	depth	No	Al mg/g	Cd µg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	***0-30***	**A**	**1.6654**	**0.0739**	**0.0041**	**0.0086**	**0.0203**	**4.5580**	**0.2055**	**0.0019**	**0.2734**	**0.0188**	**0.0063**
	30-60	**B**	**1.7008**	**0.0553**	**0.0044**	**0.0089**	**0.0212**	**4.6127**	**0.2288**	**0.0025**	**0.2435**	**0.0182**	**0.0057**
26 kg/ha	***0-30***	**C**	**1.5205**	**0.0497**	**0.0042**	**0.0089**	**0.0118**	**4.7579**	**0.2248**	**0.0015**	**0.2535**	**0.0145**	**0.0045**
	30-60	**D**	**1.5236**	**0.0393**	**0.0052**	**0.0093**	**0.0084**	**5.0321**	**0.2809**	**0.0020**	**0.1707**	**0.0139**	**0.0039**
39 kg/ha	***0-30***	**E**	**1.4879**	**0.0524**	**0.0038**	**0.0088**	**0.0082**	**4.7733**	**0.2094**	**0.0015**	**0.2535**	**0.0142**	**0.0046**
	30-60	**F**	**1.5834**	**0.0373**	**0.0048**	**0.0093**	**0.0088**	**4.9557**	**0.2418**	**0.0020**	**0.1845**	**0.0146**	**0.0044**
52 kg/ha	***0-30***	**G**	**1.5490**	**0.0589**	**0.0039**	**0.0088**	**0.0084**	**4.7095**	**0.2120**	**0.0015**	**0.2946**	**0.0150**	**0.0048**
	30-60	**H**	**1.6019**	**0.0482**	**0.0051**	**0.0094**	**0.0090**	**4.9329**	**0.2675**	**0.0023**	**0.2076**	**0.0153**	**0.0045**

The results of secound phase (oxidisable species e.g. bound to Fe/Mn organic matter or sulfides) showed that the concentrations of metalas after extraction are between (0.000 mg/g for Cd - 1.1877 mg/g for Al). As we see at (table 5.25).

Table 5.25: Concentration of metals extracted after BCR Sequential Extraction : Phase 3: Ammonium Acetate 1M

fertilizer	depth	No	Al mg/g	Cd mg/g	Co mg/g	Cr mg/g	Cu mg/g	Fe mg/g	Mn mg/g	Ni mg/g	P mg/g	Pb mg/g	Zn mg/g
0 kg/ha	*0-30*	A	1.0643	0.0000	0.0005	0.0063	0.0008	0.3382	0.0082	0.0025	0.5688	0.0015	0.0038
	30-60	B	0.9323	0.0000	0.0004	0.0050	0.0006	0.3130	0.0068	0.0021	0.4920	0.0013	0.0027
26 kg/ha	*0-30*	C	1.0748	0.0000	0.0005	0.0066	0.0007	0.3620	0.0076	0.0024	0.6079	0.0016	0.0035
	30-60	D	1.1877	0.0000	0.0005	0.0061	0.0007	0.4775	0.0094	0.0027	0.6487	0.0015	0.0036
39 kg/ha	*0-30*	E	1.0707	0.0000	0.0005	0.0064	0.0008	0.3190	0.0081	0.0026	0.6056	0.0013	0.0039
	30-60	F	1.1401	0.0000	0.0004	0.0058	0.0007	0.3157	0.0079	0.0027	0.5770	0.0014	0.0034
52 kg/ha	*0-30*	G	1.1152	0.0000	0.0005	0.0066	0.0008	0.4923	0.0077	0.0026	0.6925	0.0015	0.0038
	30-60	H	1.3013	0.0000	0.0005	0.0064	0.0007	0.6185	0.0091	0.0028	0.7011	0.0019	0.0037

The certified reference material CRM 701 (Lake sediment extractable Trace Elements, BCR, Community Bureau of Reference, Brussels, Belgium) was used to confirm the accuracy of the employed method. The results of Trace Elements determination in the reference material showed very good agreement with the declared value.

Table 5.26: Results of the BCR method. Forms of Trace Elements in the certified reference material (CRM 701)

Extraction step	Element	Certified value mg/kg	Uncertainty mg/kg	Found, mg/kg
First step	Cd	7.34	0.35	7.3
	Cr	2.26	0.16	2.16
	Cu	49.3	1.7	48.3
	Ni	15.4	0.9	14.14
	Pb	3.18	0.21	3.19
	Zn	205	6	200
secound step	Cd	3.77	0.28	4
	Cr	54.7	2.0	47.2
	Cu	124	3	127
	Ni	26.6	1.28	27
	Pb	126	3	124.6
	Zn	114	5	117
Third step	Cd	0.274	0.055	0.1
	Cr	143	7	146
	Cu	55.2	3.7	55.44
	Ni	15.3	0.9	15.21
	Pb	9.28	1.92	7
	Zn	45.7	3.4	46.72

6 CONCLUSIONS:

- Long-term fertilization in the period if 40 years with monoammonium-phosphate (MAP) influenced the total content elements such as Al, Cd, Co, and Cr in soil. The total content of these elements increased with amount of applied P fertilizer, but did not lead to the increase of adoption by plant. Al, Cd and Cr were retained in the stalk where their content was higher than in the grain. On the contrary, cobalt was equally distributed in the grain and stalk.
- It was clear that the adsorption of phosphorus by soils is finite. Some of phosphorus adsorbed on soils during phosphorus adsorption processes could be also desorbed and released into surface waters when environmental conditions change. The release of soil phosphorus could become a dominant process and threaten the health of aquatic ecosystems.
- The yield of wheat yield was considerably increased when the 26 kgha^{-1} of phosphorus was added. Further increase of added fertilizer did not significantly increase the yield and therefore it was wasteful in terms of agricultural production. The highest applied amount (52kgP/ha) resulted in the reduction of wheat yield. On the other hand it was not environmentally friendly.
- Long-term fertilization with monoammonium-phosphate (MAP) influenced the content of all forms of P except P bound to Ca as determined by a modified Chang and Jackson method. The 40-year long fertilization period did not affect the content of organic P in soils. On the contrary, the results obtained by the BCR method showed that the contents of NaOH–P and HCl–P fractions were increased, as well as leaching along the soil profile.
- Fertilization considerably increased the content of available P, especially of P bound to Al. Application of higher amounts of P-fertilizer resulted in the dominance of the Al–P fraction in the studied soil. A regular influence of fertilization on the second depth (30–60 cm) was established only for Al–P.
- The application of mineral P fertilizer on the soil produced a significant increase in the bioavailable phosphorus. Most of this phosphorus was bound to clay minerals (hydrated oxides of aluminum and manganese from second and third phase) and remained in bioavailable forms.

- The BAF obtained for (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb) were less than 1.00 and were considered normal. The BCF obtained for P were however greater than 1.00 indicating that P was highly bioaccumulated and bio-magnified in the grain and wheat samples.

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Appendix A

Certificate of Analysis

Multi-Element Plasma Standard Solution 4, Specpure

Item code: 42885

Lot: HC825498

Matrix: dilute HNO_3

Analysis

Elements	con mg/l
Ag (Silver)	1011 ± 10
Al (Aluminium)	1008 ± 10
B (Boron)	1011 ± 10
Ba (Barium)	1022 ± 10
Bi (Bismuth)	1011 ± 10
Ca (Calcium)	1011 ± 10
Cd (Cadmium)	1007 ± 10
Co (Cobalt)	1007 ± 10
Cr (Chromium)	1011 ± 10
Cu (Copper)	1007 ± 10
Fe (Iron)	1006 ± 10
Ga (Gallium)	1003 ± 10
In (Indium)	1007 ± 10
K (Potassium)	1011 ± 10
Li (Lithium)	1010 ± 10
Mg (Magnesium)	1008 ± 10
Mn (Manganese)	1011 ± 10
Na (Sodium)	1009 ± 10
Ni (Nickel)	1009 ± 10
Pb (Lead)	1009 ± 10
Sr (Strontium)	1008 ± 10
Tl (Thallium)	1010 ± 10
Zn (Zinc)	1008 ± 10

Author's Biography

Naser Ramdan R. Amaizah was born on December 19th 1973 in S. Khamis Libya.

He graduated from the Faculty of art and science, Department of Chemistry, University of Al-Mergheb, in 1996.

He received his Master degree in Chemistry from the Department of Chemistry, University of Al-Mergheb, in 2004.

From 1996 to 2004 he was employed at the Al-Mergheb University, Chemistry Department as an assistant researcher.

Since 2004 he has been employed at the Al-Mergheb University, Chemistry Department as an assistant lecturer.

Prilog:

Bibliografija kandidata kategorisana prema kriterijumima Ministarstva za nauku Republike Srbije

M 21 – Radovi objavljeni u vrhunskim časopisima međunarodnog značaja

- Jelena Mutic, Dragan Manojlovic, Renata Kovacevic, Jelena Trifunovic, **Naser Ramdan Amaizah**, Ljubisa Ignjatovic, "Feasibility of the internal standardization in direct determination of arsenic in wine samples by inductively coupled plasma atomic emission spectrometry“ Microchem. J., **98** (2011) 11-14; ISSN 0026-265X IF_{2011}=3.048

M 22 - Radovi objavljeni u časopisima međunarodnog značaja

- Amaizah Naser Ramdan, Cakmak Dragan B, Saljnikov Elmira, Roglic Goran M, Kokovic Nikola, Manojlovic Dragan D, „Effect of waste Al-phosphate on soil and plant“ Plant soil environ. 2013 59 (3):130-135; ISSN 1214-1178, IF_{2011}=1,040

M 23 - Radovi objavljeni u časopisima međunarodnog značaja

- **Naser Ramdan Amaizah,** Dragan Cakmak, Elmira Saljnikov, Goran Roglić, Vesna Mrvić, Dragan Manojlović „Fractionation of Soil Phosphorus in a Long-Term Phosphate Fertilization“ J. Serb. Chem. Soc**.** 77(7) (2012) 971-981; ISSN 1820-7421 IF_{2011}: 0.879

Прилог 1.

Изјава о ауторству

Потписани Naser Ramdan Amaizah

број уписа 35/2008

Изјављујем

да је докторска дисертација под насловом

„Toxic microelements: bioavailability and mobilization in soil after 40 years of phosphorous fertilization“

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
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Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Naser Ramdan Amaizah

Број уписа 35/2008

Студијски програм doktor hemijskih nauka

Наслов рада „Toxic microelements: bioavailability and mobilization in soil after 40 years of phosphorous fertilization"

Ментор prof Dragan Manojlović

Потписани ______

изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

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У Београду, 31. 5. 2013.

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

„Toxic microelements: bioavailability and mobilization in soil after 40 years of phosphorous fertilization“

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство
2. Ауторство - некомерцијално
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2. **Ауторство – некомерцијално**. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела.

3. **Ауторство - некомерцијално** – без прераде. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела. У односу на све остале лиценце, овом лиценцом се ограничава највећи обим права коришћења дела.

4. **Ауторство - некомерцијално** – делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца не дозвољава комерцијалну употребу дела и прерада.

5. **Ауторство – без прераде**. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца дозвољава комерцијалну употребу дела.

6. **Ауторство** - делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца дозвољава комерцијалну употребу дела и прерада. Слична је софтверским лиценцама, односно лиценцама отвореног кода.