FRACTIONATION AND CHARACTERISATION OF HEAVY METALS IN SEWAGE SLUDGES

by

Donna L Lake, B.Sc., G.I.Biol.

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Public Health & Water Resource Engineering
Department of Civil Engineering
Imperial College of Science & Technology
London SW7

ABSTRACT

Literature relating to the fractionation and characterisation of Cd, Cu, Ni, Pb and Zn in sewage sludges and the importance of this concept to their ultimate environmental impact has been reviewed.

A modified sequential chemical extraction technique was used to identify and quantify the different chemical forms in which these metals occur in both liquid and dried forms of raw, activated and digested sludges originating from major UK sewage treatment works. Application of another modified chemical extraction procedure involving progressive acidification to the same sludge samples was also undertaken in order to make a quantitative determination of metal solubilisation under conditions of varying pH. Characteristic fractionation and solubilisation profiles were obtained dependent on the nature of the individual metals rather than on the nature of the individual sludges, although dried and liquid forms differed in their response to both techniques. Residues from the progressive acidification treatment were subjected to sequential extraction in order to elucidate the nature of metal species affected by pH changes.

Sequential chemical extraction was further employed to assess the transitions in metal form which ocurr following anaerobic digestion of both primary and mixed primary sludges in laboratory-scale digesters. The overall effect of anaerobic digestion was to increase the stability of metal forms. Total metal concentration was found to be an important parameter influencing metal distributions during anaerobic digestion.

Membrane filtration was utilised to fractionate Cd, Cu and Pb in various types of sewage sludge according to their association with solids of differing particle size. This technique was further employed in combination with ion-selective electrode potentiometry to assess the influence of particle size on the stability of complexes formed between these metals and sludge solids.

Results obtained have been discussed in relation to the importance of metal form on subsequent sludge disposal.

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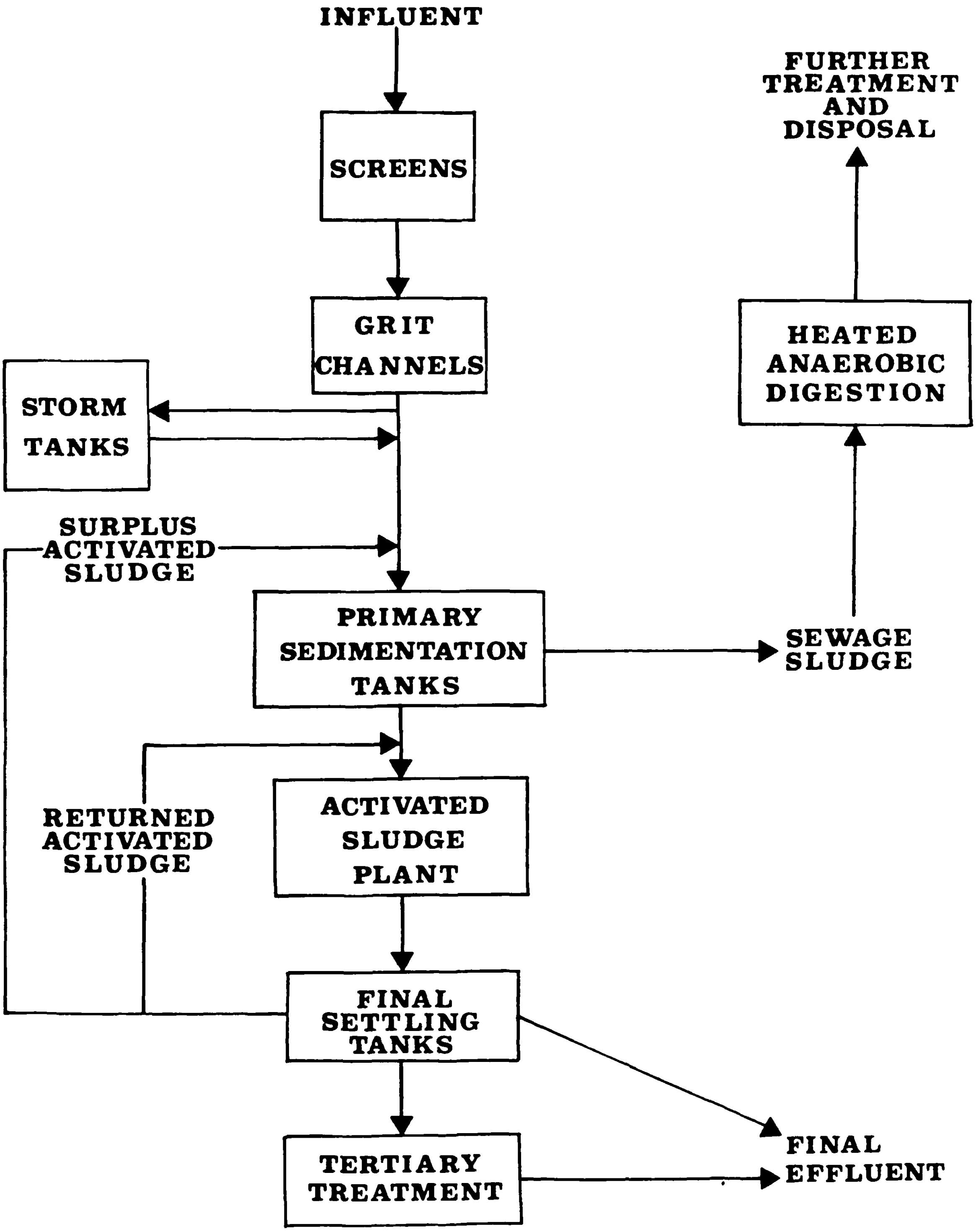
1. INTRODUCTION

Sewage is an inevitable by-product of society which requires appropriate treatment prior to discharge in order to protect the environment in a manner determined by economic, social and political pressures (Tebbutt, 1979; Metcalf & Eddy, 1979; Calcutt & Moss, 1984). Among the objectives of sewage treatment are the removal of floating material, suspended solids, biodegradable organic constituents, specific compounds of nitrogen and phosphorus, pathogenic organisms, organic micropollutants, potentially toxic heavy metals and dissolved inorganic solids (Metcalf & Eddy, 1979). These are achieved through a combination of physical, chemical and biological processes (Tebbutt, 1977; Metcalf & Eddy, 1979). Figure 1 represents a typical sewage treatment scheme incorporating preliminary treatment, primary sedimentation, activated sludge and sludge digestion processes.

Influent raw sewage undergoes preliminary treatment to remove coarse solids (through utilisation of screens or comminutors) and grit, which might otherwise adversely affect subsequent treatment processes and mechanical equipment. Storm tanks serve to reduce excessive variations in flow rate to the sewage treatment process, since hydraulic overloading may produce a deterioration in effluent quality (Steel & McGhee, 1979).

Primary treatment has traditionally implied a sedimentation process to remove suspended solids by settling under near quiescent conditions. For this purpose, large sedimentation tanks of various designs are employed, most treatment plants incorporating mechanically desludged tanks of circular or rectangular design with radial and horizontal flows respectively (Metcalf & Eddy, 1979; Downing, 1983). Generally, retention times in primary sedimentation tanks are short; approximately one or two hours at peak flow with surface loading rates of between 1.0 and 2.5 $m^3m^{-2}h^{-1}$ (Tebbutt, 1977; Steel & McGhee, 1979). Primary sedimentation is, however, only 60 to 70% efficient in the removal of suspended solids (Matthews, 1984); this being due to the low settling velocity of some particles, which may be attributable to

Figure 1 Flow diagram of a conventional sewage treatment process employing activated sludge



either their size or specific gravity. Colloidal particles, for example, are considered to have virtually no settling velocity (Tebbutt, 1977; Degremont, 1979). However, chemicals may be added to remove colloidal solids or for the precipitation of phosphorus compounds during primary settling (Steel & McGhee, 1979). Primary sedimentation thus effects the removal of settleable solids from sewage and concentrates these solids in a smaller volume, as raw primary sludge. In addition, primary sedimentation produces an effluent suitable for secondary or biological treatment.

Aerobic biological oxidation is usually employed for secondary treatment; microorganisms metabolise and remove soluble, colloidal and finely suspended particulate organic matter from the settled sewage (or primary effluent) and are inturn removed from the secondary effluent during 'final sedimentation'. This principle is basic to all secondary biological treatment processes, although its application may vary as either attached (fixed film) growth or suspended processes (Metcalf & Eddy, 1979; Steel & McGhee, 1979). Attached growth processes are seen in the trickling or percolating filter and rotating biological contactors. Suspended growth processes include the activated sludge process and oxidation ponds. However, the two most commonly employed methods of secondary treatment are biological filtration and the activated sludge process, each serving approximately 40% of the population of the UK (Downing, 1983).

The percolating filter operates by trickling settled sewage over a biofilm held on an inert medium, such as granite, clinker, or plastic. The biofilm metabolises most of the remaining impurities in the settled sewage and the effluent leaves the filter in a suitably purified state. However, as the polluting matter is converted into biomass so excess biomass, often termed 'humus', is shed thus maintaining the biofilm in a state of equilibrium. The humus sludge is separated from the effluent by final sedimentation, and is then frequently co-settled with primary sludge (Downing, 1983; Matthews, 1984).

The activated sludge process offers the advantages of higher organic loadings and a requirement for less land area over the percolating filter method (Downing, 1983; Hawkes, 1983). The conventional activated sludge process consists of a rectangular aeration tank, a sedimentation tank and a sludge-return line (Figure 1). In general practice, recycled activated sludge flocs are mixed with the influent settled sewage at the inlet of the aeration tank to maintain a mixed liquor suspended solids concentration typically in the range of 2000 to 8000 mgl^{-1} (Hawkes, 1983). The 'mixed liquor' is subsequently aerated by releasing a large volume of compressed air through porous diffuser domes installed at the base of the aeration tank. Aeration serves to maintain a dissolved oxygen concentration of 1 to 2 mgl^{-1} in the mixed liquor suspension, as required for the respiratory activity of the aerobic microorganisms, and also to maintain the biomass flocs in a continuous state of agitated suspension, which ensures maximum contact with the organic waste. The microbial flocs thus achieve rapid degradation of the organic wastes and provide a good quality sludge with rapid settling characteristics (Tebbutt, 1977; Hawkes, 1983).

Separation of the activated sludge biomass from the purified effluent is achieved by settlement within final sedimentation tanks. Settled activated sludge has a highly water-retentive gel structure and is usually recycled as a slurry containing between 0.5 and 2.0% dry solids (Hawkes, 1983). Surplus activated sludge, representing the biosynthesised fraction of the waste, is withdrawn from the returned activated sludge as it is recycled. In the conventional activated sludge process, for every kg of biochemical oxygen demand (BOD) removed, 0.5 to 0.8 kg dry weight of sludge is synthesised (Hawkes, 1983). Similar to humus sludge, excess activated sludge is frequently cycled back to the primary sedimentation tank to produce primary co-settled sludge.

The ultimate aim of such treatment processes is to produce an effluent of a quality suitable for discharge to the selected receiving water. The normal standard attained is an effluent with a suspended solids concentration of 30 mgl^{-1} and a BOD of 20 mgl^{-1} or better (Tebbutt, 1977; Downing, 1983). However, where more stringent or specific criteria are defined for limiting effluent discharge, such as the 10:10:10 standard (concentration of suspended solids, the 5-day BOD and the concentration of ammoniacal nitrogen not exceeding 10 mgl^{-1}) required for discharge to rivers used as a source of potable water supply (Downing, 1983), tertiary treatment may be necessary to achieve this. Irrigation over grass plots or passage through lagoons, micro-stainers or sand filters may be utilised in the production of higher quality effluents (Matthews, 1984).

In achieving effluents of a quality suitable for discharge to receiving waters, the various pollutants are concentrated to form sewage sludges. The production of sewage sludge in the European Economic Community (EEC) exceeds 5.9×10^6 tonnes of dry solids annually (Calcutt & Moss, 1984), to which the UK contributes 1.22×10^6 tonnes (Matthews _et al._, 1984; Davis, 1984). While it is unlikely that sludge production in the UK will increase significantly in the near future (Healey, 1984), production in other EEC countries, such as France and Italy, is expected to increase substantially above current rates, as indicated in Table 1 (Calcutt & Moss, 1984). It has been estimated by Matthews (1983) that sludge production in the EEC as a whole will increase by about 50% over the next decade, while production in the United States, currently at 4.5×10^6 tonnes per annum, is expected to double over a similar period (Bunch, 1982a).

Raw sewage sludges typically contain between 1 and 7% dry solids and are usually highly putrescible and offensive (Lester _et al._, 1983). Approximately 70% of the solids content is of an organic (volatile) nature, consisting principally of lignin-humus, water-soluble polysaccharides, greases, waxes, oils, resins, hemicellulose, cellulose, acid-resistant proteins, nitrogen and sulphur compounds, vitamins and fatty acids (Sommers _et al._, 1977; Strachan _et al._, 1983; Matthews, 1984); the remaining 30% predominantly comprising inorganic (non-volatile) crystalline mineral components, such as quartz, calcite, dolomite and kaolinite (Silviera _et al._, 1977; Matthews, 1984). In addition, raw sewage sludges will contain a variety of heavy metals, organic micropollutants and pathogenic organisms. Consequently, treatment of such sludges is frequently required to pro-

Table 1 Production and disposal of sewage sludge within the EEC
(After Calcutt & Moss, 1984)

	Current sludge production		Use of disposal routes				
Member state	DS per annum (tonnes$\times10^3$)	% of estimated maximum	Agricultural land[a] (%)	Landfill[b] (%)	Incineration (%)	Dumping at sea (%)	Discharge via pipelines (%)
Belgium	70	45	15	83	2	0	0
Denmark	130	85	45	45	10	0	0
France	840	50	30	50	20	0	0
West Germany	2200	80	39	49	8	0	0
Greece	3	<5	0	100	0	0	0
Republic of Ireland	20	20	4	51	0	45	
Italy	1200	50	20[c]	55[c]	5	0	0
Luxembourg	11	70	90	10	0	0	0
Netherlands	230	70	53	32	3	0	13

[a] Includes small quantities for horticulture, allotments and gardens
[b] Includes small quantities for land reclamation and forests
[c] 20% unspecified

duce more stable and less offensive sludges of reduced volume and/or pathogenic content, which are more amenable to disposal.

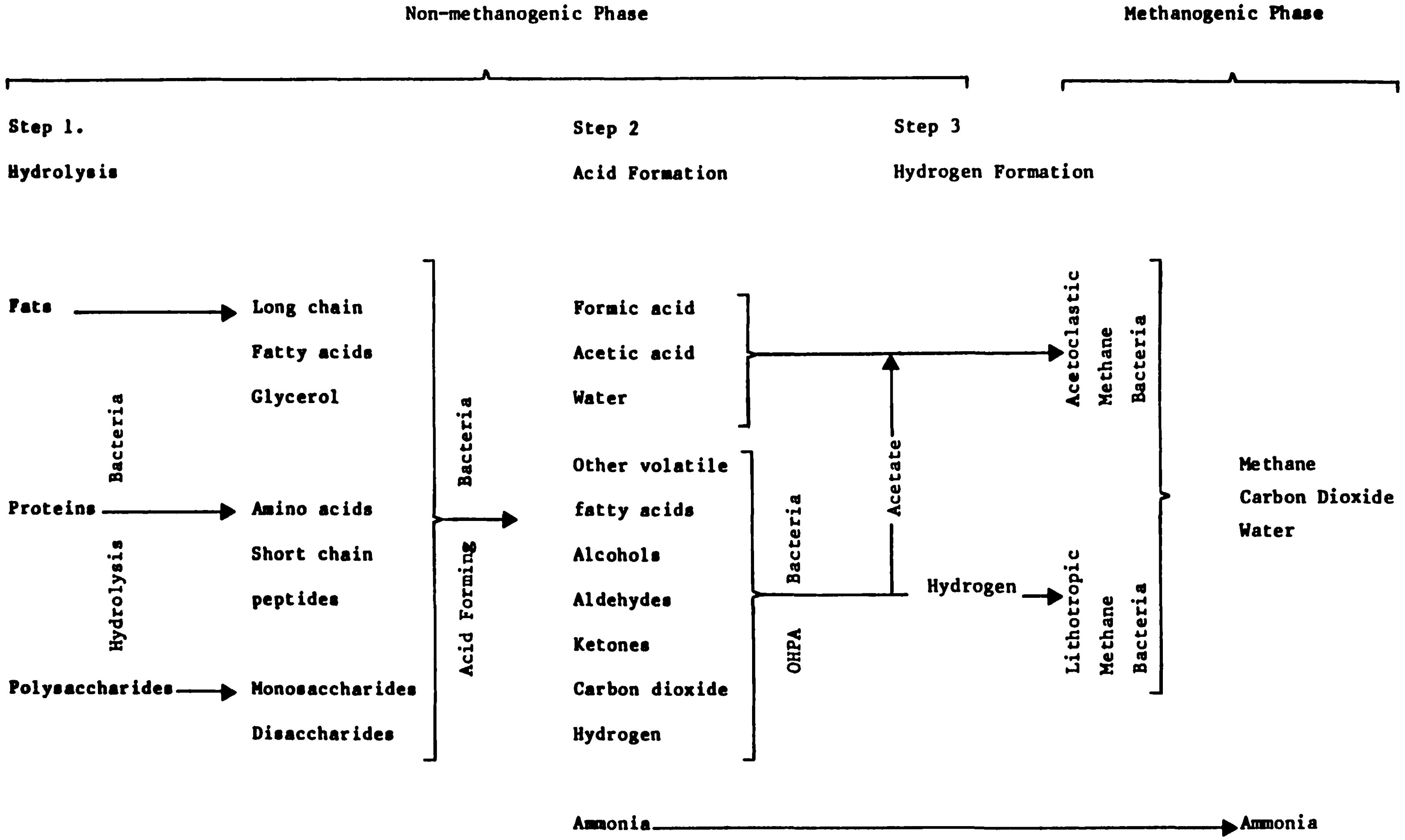
Sludge treatment and disposal may account for up to 50% of the operating costs of a sewage treatment facility (Department of the Environment & National Water Council, 1981; Lester et al., 1983). Methods of sludge treatment used singly or in combination prior to disposal include: thickening by stirring or flotation; digestion, either aerobically or anaerobically; composting with domestic refuse; conditioning by the addition of chemicals, such as synthetic organic polyelectrolytes, lime or ferrous sulphate; dewatering, on drying beds, in filter presses, by vacuum filtration, centrifugation or by heat drying and incineration in multiple hearth or fluidised bed furnaces (Downing 1983; Lester et al., 1983; Matthews, 1984). The most widely used sludge treatment process is anaerobic digestion (Lester et al., 1983), approximately 50% of the total sludge produced in the UK being stabilised by this process (Mosey, 1983).

Anaerobic digestion is ideally suited to the treatment of a high strength waste such as sewage sludge; the major advantages of this process being its relatively low energy and nutrient requirements, its capacity for utilisation of high organic loading rates, its lack of nuisance odour, its low production of excess (stabilised) sludge and its release of economically valuable methane gas as a by-product (Parker et al., 1981; Forday & Greenfield, 1983). Conventional anaerobic digestion cannot, however, provide a complete treatment since digester performance in terms of chemical oxygen demand (COD) removal and solids destruction is limited by the discharge of all of its biomass production in its own effluent (Mosey, 1981). Normal works practice is to feed primary or co-settled sludge to a heated mixed digester reactor maintained in the mesophilic temperature range of 25 to 38°C (Mosey, 1983). Putrefaction and methane fermentation occur simultaneously in the same reaction vessel; typical retention times for mesophilic sewage works digesters being in the order of 20 to 40 days (Mosey, 1983).

Traditionally anaerobic digestion has been considered a two stage process, namely, a non-methanogenic followed by the methanogenic stage. However, it is now recognised that the first stage, for which a variable collection of both obligate and facultative anaerobic bacteria are required, may include as many as three steps (Mosey, 1982; Forday & Greenfield, 1983). The first step involves the hydrolysis of the fats, proteins and polysaccharides present in the sludge to produce long chain fatty acids, glycerol, short chain peptides, amino acids, monosaccharides and disaccharides. During the second step (acid formation), these soluble organic compounds are further degraded to yield a range of low molecular weight volatile fatty acids, including formic and acetic acids, ketones, aldehydes and alcohols in addition to carbon dioxide and hydrogen. Of these intermediates, only hydrogen, formic acid and acetic acid can be utilised directly as substrates by the methanogenic bacteria (Lester et al., 1983), the remainder requiring conversion to assimilable intermediates by the obligatory hydrogen producing acetogenic (OHPA) bacteria (Verstraete et al., 1981). This conversion process constitutes the third step. Some bacteria are able, however, to undertake both steps 1 and 2 and to produce hydrogen, formic acid and acetic acid, thus eliminating the requirement for step 3 (Lester et al., 1983). During the final phase, strictly anaerobic methanogenic bacteria convert the lower volatile acids to methane, carbon dioxide and other trace gases, which mix to form a gaseous fuel known variously as digester gas, sludge gas or 'Biogas'. These stages of anaerobic digestion are summarised in Figure 2.

As a result of the equilibrium which exists between the phases, volatile acid levels are low in a working digester, often less than 300 mgl^{-1} expressed as acetic acid (Department of the Environment, 1979). The almost complete microbial fermentation of carbohydrates during anaerobic digestion results in a 60 to 75% reduction in volatile solids, the resulting organic matter consisting of a mixture of microbial tissue, lignin, cellulose, lipids, organic nitrogen compounds and humic acid-like materials (Baldwin et al., 1983). Following anaerobic digestion, the stabilised sludge may be disposed of directly or treated further using secondary digesters (at ambient temperature), sedimentation tanks or lagoons, from which the super-

Figure 2 Phases of anaerobic digestion



natant liquor, which may constitute 30% of the sludge volume and 15% of the organic load on the works, may be withdrawn and returned to the head of the works.

Treatment of sewage sludge is frequently influenced by the final disposal option selected. In the UK, 65% of the total sludge produced is disposed to land, 31% is dumped at sea and 4% is incinerated. Of the sludge disposed to land, 60% is applied to agricultural and horticultural land while the remainder is used principally for land reclamation and land fill (Davis, 1984; Matthews, 1984). Sludge application to agricultural land may have beneficial effects by adding plant nutrients and fertilising the soil in addition to improving soil structure (Sterritt & Lester, 1980a; Department of the Environment & National Water Council, 1981; Coker, 1983). It has been estimated that only 1.2% of the 12 x 10^6 hectares of land used for agricultural purposes in the UK receives sewage sludge (Healey, 1984), indicating the potentially large areas of land available for increasing use of this disposal option. A comparison of the UK disposal figures with those reported for other EEC countries (Table 1) indicates that the UK is virtually isolated in its use of sea dumping as a sludge disposal option, while simultaneously emphasising the predominance of land disposal throughout the EEC. In the USA the virtual cessation of ocean dumping of sludge is likely to have increased the utilisation of land disposal in excess of the 55% previously reported (Bunch, 1982b).

Sewage sludge disposal is governed by both economic and environmental considerations; the optimum disposal route being influenced by local conditions. Potential environmental hazards associated with sewage sludge disposal have been defined principally in terms of odour, pathogens, trace organic compounds and heavy metals (Department of the Environment & National Water Council, 1981; Jamieson, 1981; Davis, 1984; Healey, 1984). Heavy metals present in sewage sludge are of major concern due to their non-biodegradability and their toxic properties. Disposal of heavy metal contaminated sludges may therefore have potentially adverse effects on human health, the ecology of the receiving environment and on surface and ground-water quality (Lester et al., 1983).

'Heavy' metals have been defined as those possessing a specific gravity greater than five (Lapedes, 1978; Lewin & Beckett, 1980; Coker & Matthews, 1983; Cottenie & Verloo, 1984; Matthews, 1984). The range of elements described by such terminology includes the metals Cd, Cu, Ni, Pb and Zn (Stover et al., 1976; Lewin & Beckett, 1980; Sterritt & Lester, 1980a; Jamieson, 1981; Lester, 1983; McBride & Bouldin, 1984). These five heavy metals are considered to be of primary concern with respect to sludge disposal (Silviera & Sommers, 1977; Sposito et al., 1982a; Davis, 1984), due to their specific toxicity and their ubiquity which results in elevated sludge concentrations, typically exceeding soil concentrations by two orders of magnitude or more (Davis, 1980). All five metals are included in the United States' Environmental Protection Agency (USEPA) list of priority pollutants (Fisher, 1978; Anthony & Breimhurst, 1981) and in the EEC 'black and grey' lists of dangerous substances discharged to the aquatic environment (European Economic Community, 1976). While Cu, Ni, Pb and Zn are incorporated in the 'grey' list, or list II, Cd occurs in the 'black list', or list I (European Economic Community, 1976). All of these metals have been examined for their potential carcinogenicity; Cd, Ni and Pb having been found to induce cancer in animals or man (Becking, 1981).

Toxicity has been defined as the ability of a chemical substance to produce injury on reaching a susceptible site in or on an organism (Chalmers, 1981). It is generally recognised that the absorption of inorganic compounds depends to a great extent on their solubility; metals in solution being most readily absorbed (Oehme, 1978). The toxic action of many heavy metals is primarily a result of their affinity for complexing or chelating groups, such as thiol groups, in biological molecules (Vallee & Ulmer, 1972; Lerch, 1980). Typically they cause disruption of protein structure and function leading to enzymic and metabolic disorders (Vallee & Ulmer, 1972). The toxicity of Cd, Cu, Ni, Pb and Zn to plants (Brown & Jones, 1975; Foy et al., 1978), animals (Schroeder & Darrow, 1973; Dulka & Risby, 1976) and man (Dulka & Risby, 1976; Green, 1978; Beliles, 1979; Ryan et al., 1982) has been extensively reviewed. The concentrations of these metals which may produce noticeable acute toxic effects would only arise from incidents of gross pollution. However, chronic toxic effects from long-term exposure to relatively low concentrations of bioaccumulative heavy metals may represent a more serious hazard with respect to sewage sludge disposal (Sterritt & Lester, 1980a; Bunch, 1982b).

In addition to their limiting effects on sludge disposal, Cd, Cu, Ni, Pb and Zn are the metals most likely to exert deleterious effects on biological sludge treatment processes, causing a reduction in process performance (Davis, 1980). The toxicity of these metals may be affected by many factors including pH, temperature, other toxic substances, chelating agents and compounds that form precipitates (Anthony & Breimhurst, 1981). Anaerobic digestion is more susceptible to the toxic effects of heavy metals than aerobic systems, due to the narrow spectrum of species present (Barth, 1975; Anthony & Breimhurst, 1981). Although the sensitivity of anaerobic digestion to toxic inhibition was once attributed to the methanogens (Stein & Malone, 1980), it has since been recognised that the OHPA population is of particular significance in governing the efficiency and stability of the digestion process (Verstraete _et al_., 1981).

It is well established that heavy metals inhibit anaerobic digestion by inactivating a wide range of enzymes, essentially through reacting with their sulphydryl functional groups (Kugelman & McCarty, 1965; Mosey & Hughes, 1975). However, the diagnosis of such toxic inhibitory effects is rendered difficult by the complexity of the sludge matrix and the phenomena of stimulation, antagonism, synergism and acclimation (Kugelman & Chin, 1971). Consequently, experiments concerned with the toxic action of heavy metals to anaerobic digestion have generally been undertaken under controlled conditions with single metals.

Total concentrations of Cd, Cu, Ni, Pb and Zn reported by Hayes & Theis (1978) to be inhibitory and toxic to anaerobic digestion are presented in Table 2; inhibitory and toxic concentrations being defined respectively as those at which gas production first declined and at which a 70% reduction in gas production occurred. Considerable variation has been reported, however, in the toxicity of individual metals to anaerobic digestion when determined as the total metal concentration (Masselli et al., 1961; McDermott et al., 1963; 1965; Hayes & Theis, 1976; 1978), indicating the importance of metal form to toxic inhibition. Insoluble metal forms are generally regarded to be of less toxicological significance than soluble forms (Hayes & Theis, 1978; Sterritt & Lester, 1984a), and the importance of sulphides and carbonates produced under the reducing conditions of digestion in the precipitation of metal ions and their consequent detoxification has been demonstrated (Mosey et al., 1971; Mosey, 1976). Threshold inhibitory concentrations of soluble phase Cd, Cu and Zn reported by Anthony & Breimhurst (1981) are also presented in Table 2.

Table 2 Metal concentrations inhibitory and toxic to anaerobic digestion (mgl^{-1})

Metal	Soluble inhibitory concentration[1]	Total inhibitory concentration[2]	Total toxic concentration[2]
Cd	0.02	<20	>20
Cu	1 - 10	40	70
Ni	-	10	30
Pb	-	340	>340
Zn	5 - 20	400	600

[1] Anthony & Breimhurst (1981)

[2] Hayes & Theis (1978)

Mosey (1976) proposed that where prediction of toxic inhibition to anaerobic digestion has to be based on the total concentration of heavy metals present the concentrations of Cd, Cu, Ni, Pb and Zn, when summed on an equivalent weight basis and expressed relative to the sludge solids, could be used to represent the total metal load of the sludge (K) in the pH range 6.9 to 7.3. From an appraisal of published toxicity data, Mosey (1976) suggested that digester failure is possible when K exceeds 400 $meqkg^{-1}$ and almost certain when K exceeds 800 meqkg^{-1}. Failure of the digestion process may create difficulty in the further treatment of and the disposal of the septic waste, while restoration of digestion is likely to be an additional economic burden (Department of the Environment, 1973).

The presence of significant quantities of heavy metals in sewage sludge is due to their association with settleable solids during primary and secondary sewage treatment. Heavy metals present in sewage may originate from a variety of sources, which may be categorised as domestic, industrial or urban run-off (Lester, 1983; Matthews, 1984; Spiegel et al., 1985). While some heavy metals, such as Cu and Zn, may occur in appreciable concentrations in sewage of domestic origin, the most significant input is likely to be from industrial sources (Davis, 1980; Department of the Environment & National Water Council, 1981). Many industrial uses of heavy metals involve the discharge of metal-laden effluents to the sewerage system, the ubiquity of Cd, Cu, Ni, Pb and Zn in industrial effluents being emphasised in Table 3. Wide ranging variations of a cyclic, seasonal or irregular nature may occur in industrial discharges of these metals to sewers, depending on the type of manufacturing operations employed (Chalmers, 1981). Shock loads of heavy metals from industrial sources have frequently been observed to enter sewage plants (Oliver & Cosgrove, 1974; Funke, 1975). Other factors influencing the heavy metal loading to a sewage treatment plant include wet weather, the time of day or night (Roberts et al., 1977), the day of the week and the season of the year (Beckett, 1980). Typical concentrations of Cd, Cu, Ni, Pb and Zn found in influent raw sewages are presented in Table 4. Such values demonstrate that up to ten-fold variations in the concentrations of each of these metals may occur in raw sewage, while simultaneously emphasising the predominance of Zn in this matrix.

It is essential that high concentrations of heavy metals in raw sewage entering a sewage treatment plant are removed during the treatment processes, since the discharge of metal-laden effluents to water courses may contaminate the hydrosphere, affect the ecology of rivers or the use of river water, or ultimately, may have effects on human health, especially in areas where water re-use is practiced (Wilson,

Table 3 Major industrial sources of Cd, Cu, Ni, Pb and Zn in sewage (Compiled from Dean *et al.*, 1972; Barnhart, 1978; Patterson, 1979)

	Cd	Cu	Ni	Pb	Zn
Mining & Metallurgy	X	X	X	X	X
Electroplating & Metal finishing	X	X	X	X	X
Paints & Dyes	X	X	X	X	X
Pharmaceuticals		X			X
Batteries	X		X	X	X
Chemical Manufacturing	X	X	X	X	X
Textiles	X	X	X		X
Electrical & Electronic		X	X	X	X
Explosives		X		X	
Pesticides				X	X
Leather		X			X
Rubber & Plastics	X				X
Petroleum & Coal	X	X	X	X	X
Fertilizer	X	X	X	X	X

Table 4 Mean concentrations of Cd, Cu, Ni, Pb and Zn in raw sewage (mgl^{-1})

Reference	[1]	[2]		[3]	[4,5]	[6]
		(a)	(b)			
Metal						
Cd	0.006	0.004	0.005	0.012	0.006	0.033
Cu	0.310	0.066	0.467	0.194	0.163	0.168
Ni	0.330	0.063	0.046	0.098	0.158	0.115
Pb	0.230	0.024	0.210	0.052	0.040	0.051
Zn	2.400	0.322	0.290	0.252	1.650	2.070

[1] Oliver & Cosgrove (1974) - 1 Canadian sewage treatment works
[2] Callahan *et al.* (1979) - 2 USA treatment works;
(a)= low industrial flow (b)= high industrial flow
[3] Feiler *et al.* (1979) - 1 USA sewage treatment works
[4] Lester *et al.* (1979) - 1 UK sewage treatment works
[5] Stoveland *et al.* (1979) - " " " "
[6] Wukasch *et al.* (1979) - 1 USA sewage treatment works

1976). A wide range of removal efficiencies have been observed for individual heavy metals during sewage treatment, this being a consequence of varying operational and environmental conditions. This reflects the complexity of interactions which determine the distribution of each metal species and their resultant behaviour. Results obtained from six of the numerous studies conducted to assess the removal efficiencies of Cd, Cu, Ni, Pb and Zn during sewage treatment are summarised in Table 5. Although considerable variation in percentage removals is displayed for each of these metals, some trends are apparent. All five heavy metals have been reported to be removed with an efficiency in excess of 50%, Pb having consistently the highest and Ni the lowest percentage removals.

Heavy metals entering a sewage treatment works may be partially removed during primary sedimentation (Lester, 1981; Rossin et al., 1983). Such removal may occur as either settling of precipitates or association of heavy metals with settleable solids. The principal determinants of metal removal during primary sedimentation are the efficiency of suspended solids removal and the chemical species of the metal (Lester, 1983). According to Sterritt & Lester (1984a), approximately 30 to 40% of most heavy metals are removed from raw sewage during primary treatment. Metals removed may exist principally in insoluble forms; removal efficiencies for soluble forms of Cu, Ni, Pb and Zn having been reported to be less than 1% (Oliver & Cosgrove, 1974).

Removal of soluble metal forms is almost entirely achieved during the secondary or biological treatment stage. The activated sludge process is crucial in ensuring minimal concentrations of heavy metals in final effluents, and may achieve higher removal efficiencies for most metals, with the exception of Ni, than percolating filter treatment (Stones, 1977). Possible removal mechanisms have been outlined by Brown & Lester (1979) as:

i) physical trapping of precipitated metals in the sludge floc matrix;
ii) binding of soluble metal to extracellular polymers;

Table 5 Typical percentage removal efficiencies of Cd, Cu, Ni, Pb and Zn in complete sewage treatment

Reference Metal	[1] (a)	 (b)	[2]	[3] (a)	 (b)	[4,5]	[6]
Cd	80	78	35	60-65	55-100	89	81
Cu	73	71	55	86	81	96	85
Ni	16	18	45	59	33	70	29
Pb	93	91	65	58-100	-	92	95
Zn	77	78	60	64	81	94	89

[1] Oliver & Cosgrove (1974) (a) 4 weeks study (b) 3 day study
[2] Roberts _et_ _al_. (1977)
[3] Feiler _et_ _al_. (1979) (a) large industrial contribution (b) domestic and commercial contribution
[4] Lester _et_ _al_. (1979)
[5] Stoveland _et_ _al_. (1979)
[6] Wukasch _et_ _al_. (1979)

iii) accumulation of soluble metal by the cell;
iv) volatilisation of metal to the atmosphere.

Thus, during activated sludge treatment, heavy metals initially present as soluble forms may be removed by association with the settleable biomass, while further removal of insoluble metals may also occur by association with this biomass. Efficient flocculation and settling is critical to the removal of all metal forms during secondary treatment (Lester, 1983). The wide ranges of removal efficiencies obtained for Cd, Cu, Ni, Pb and Zn during activated sludge treatment together with the major factors influencing their removals have been reviewed by Brown & Lester (1979) and Lester (1983).

The environmental distribution of heavy metals from many sources is therefore mediated and, to a degree, controlled by sewage treatment. Since less than 1% of the total flow of sewage to a works is produced as sludge (Lester et al., 1983) and this typically contains more than 50 to 80% of the total quantity of Cd, Cu, Ni, Pb and Zn entering the works, these metals are concentrated to a significant degree. It has been estimated that approximately 7000 ty^{-1} of these five metals are incorporated into sludges in the UK (Lester, 1983; Rossin et al., 1983). The concentrations of these metals present in sewage sludges vary widely, however, according to the geographical location, type and quantity of industrial input and the efficiency of removal achieved by treatment processes (Berrow & Webber, 1972; Horvath et al., 1981; Speigel et al., 1985). Typical concentrations found in surveys conducted in the UK (Berrow & Webber, 1972; Department of the Environment & National Water Council, 1981; Sterritt & Lester, 1981) and the USA (Bunch, 1982b; Mumma et al., 1984; Spiegel et al., 1985) are presented in Table 6; the variability in total concentrations of individual metals being demonstrated by the wide ranges encountered and the divergent mean values. Some cases of extremely high concentrations (1.6 to 5.0% w/w) of Cu, Pb and Zn have been recorded, but in general, the mean concentrations of these metals are in the order of 0.1 to 0.4% (w/w), while those of Cd and Ni are typically one to two orders of magnitude lower.

Table 6 Typical concentrations of Cd, Cu, Ni, Pb and Zn found in UK and USA sewage sludges (mgkg^{-1} dry solids)

Reference	[1]		[2]		[3]		[4]		[5]		[6]	
Metal	range	mean	range	mean	range	mean	range	mean	range	mean	range	mean
Cd	<60-1500	<200	0.4-183	29	2-110	25	ND-1100	88	3-198	41	0.04-14.6	6.3
Cu	200-8000	970	36-2889	613	170-2080	721	45-16000	1250	126-7729	1546	0.03-8763	1667
Ni	20-5300	510	5-3036	188	16-2020	290	ND-2800	410	29-800	259	0.2-930	140
Pb	120-3000	820	19-3538	550	28-45400	1550	80-26000	1940	80-676	326	2.0-472	190
Zn	700-49000	4100	199-19000	1820	94-9210	1930	50-28360	3480	475-10900	2213	4.4-1000	450

[1] Berrow & Webber (1972) - 42 UK sludge samples analysed
[2] DOE/NWC (1981) - 2400 UK sludge samples analysed
[3] Sterritt & Lester (1981) - 40 UK sludge samples analysed
[4] Bunch (1982b) - Typical values from the USEPA 'STORET' data system
[5] Mumma *et al.* (1984) - 30 sludge samples analysed
[6] Spiegel *et al.* (1985) - 30 USA sludge samples analysed

Background concentrations of heavy metals found in uncontaminated soils similarly show considerable variation, ranging from 0.01 to 0.7 $mgkg^{-1}$ for Cd, 2 to 100 $mgkg^{-1}$ for Cu, 5 to 500 $mgkg^{-1}$ for Ni, 2 to 200 $mgkg^{-1}$ for Pb and 10 to 300 $mgkg^{-1}$ for Zn (Swaine, 1955; Bowen, 1966; Berrow & Webber, 1972). Such variation primarily reflects differences in the nature of underlying parent material, the principal determining factors being the selective incorporation of particular elements in specific minerals during igneous-rock crystallisation, relative rates of weathering and the modes of formation of sedimentary rocks (Mitchell, 1964; Berrow & Burridge, 1979). Despite such variation, however, it is apparent in comparing this soil data with data presented for sewage sludges (Table 6) that total concentrations of Cd, Cu, Ni, Pb and Zn tend to be much higher in sludges than in soils. Therefore, any addition of sludge to agricultural land would almost certainly result in increased concentrations of these metals accumulating in the soil. Repeated sludge applications could consequently lead to toxic effects on crops or bioaccumulation to potentially hazardous concentrations in the food chain (Hyde, 1976; Mitchell _et al._, 1978; Sterritt & Lester, 1980a; Webber _et al._, 1984; Bray _et al._, 1985).

In an attempt to minimise such risks, many countries have formulated guidelines to regulate heavy metal additions to agricultural soils from sewage sludge application (USEPA, 1977; Federal Register, 1979; Thormann, 1979; Commission of the European Communities, 1980a;b; Department of the Environment & National Water Council, 1981; Evans _et al._, 1981), reviews of which have been presented by Matthews (1984) and Webber _et al._ (1984). While varying considerably in detail, such guidelines are generally based on one or more of the following control strategies:

i) limitation of the heavy metal concentrations in the sludge applied to agricultural land;
ii) limitation of the heavy metal loadings to agricultural land;
iii) limitation of the heavy metal concentrations in the soil.

In the UK guidelines (Department of the Environment & National

Water Council, 1981), maximum permissible additions of twelve potentially toxic heavy metals in sewage sludge to uncontaminated non-calcareous soils over a period of at least thirty years are specified. Maximum additions of Cd, Cu, Ni, Pb and Zn are recommended as 5, 280, 70, 1000 and 560 $kgha^{-1}$ respectively. Additions of the phytotoxic metals Cu, Ni and Zn are subject, however, to an overriding limitation in terms of the 'Zn equivalent' concept, which supposes that Cu is twice and Ni eight times as toxic as Zn on a weight basis and that the toxicities are additive (Chumbley, 1971). Hence, it is recommended (Department of the Environment & National Water Council, 1981) that the maximum addition of Zn equivalent in sewage sludge to uncontaminated non-calcareous soils should be limited to 560 $kgha^{-1}$ over a period of thirty years or more. There is evidence to suggest, however, that the toxicities of Cu, Ni and Zn may not be additive and that the use of the Zn equivalent equation greatly underestimates the concentrations of sludge-borne metals that can be safely applied to agricultural soils (Davis & Beckett, 1978; Beckett & Davis, 1982; Davis _et al_., 1985).

It is apparent that conflicting information concerning acceptable heavy metal loadings to soil has resulted in widely different recommendations between some countries. The maximum annual loading for Cd, for instance, is 15 gha^{-1} in Denmark and Sweden, 20 gha^{-1} in Finland and the Netherlands and 30 gha^{-1} in Norway, which may be compared with 167 gha^{-1} for the UK, 280 gha^{-1} for Canada (Ontario) and 1250 $g\ ha^{-1}$ for the USA (Davis & Coker, 1980). The proposed EC Directive on sludge disposal to land (Commission of the European Communities, 1982) has been regarded as a step towards standardisation of guidelines currently followed by member countries (Webber _et al_., 1984), but according to Matthews (1983), unamended implementation of the Directive would result in increased expenditure and, possibly, less sludge utilisation on agricultural land in the UK.

Guidelines and legislation designed to limit heavy metal additions to soil are based almost exclusively on total metal concentrations. While total metal content may indicate the degree of environmental contamination, it is not, however, a sufficient criterion for assessment of the potential bioavailability of the metals. A better understanding of the effects of heavy metals on crops can only be attained from a detailed knowledge of the metal speciation (defined as the distribution of a metal between different physicochemical species (Florence, 1982)) and the response of the plants to each species (Sterritt & Lester, 1980a). In addition speciation will determine metal mobility and hence potential contamination of groundwater following sludge application (Emmerich et al., 1982a).

The principal physicochemical forms in which heavy metals are likely to occur in aerobic and anaerobic sewage sludges have been classified by Gould & Genetelli (1975) as:

i) soluble a) ionic b) organic complexes c) inorganic complexes;
ii) co-precipitates in metal oxides;
iii) precipitates;
iv) adsorbates a) physical b) chemisorption c) clay lattice;
v) organometallic complexes a) simple complexes b) chelates;
vi) biological residues.

The distribution of metals between the specific forms varies widely according to the chemical properties of the individual metal and the characteristics of the sludge, which are a function of the physical and chemical properties imposed by the particular sludge treatment process. These include such parameters as pH, temperature, oxidation-reduction potential, the presence of complexing agents and the concentrations of precipitant ligands (Gould & Genetelli, 1978a).

Unfortunately, analytical limitations imposed by interferences, selectivity and sensitivity have so far precluded a complete differentiation of metals between *all the specific chemical forms* in the complex sludge matrix. However, a number of schemes have been proposed to segregate metals into several operationally defined fractions on the basis of particle size and extractability in various chemical reagents.

Metal forms in sludges have most frequently been investigated by selective chemical extraction techniques. Such techniques incorporate either a single reagent to extract an individual metal fraction (Jenkins & Cooper, 1964; Berrow & Webber, 1972; Bloomfield & Pruden, 1975; Bradford et al., 1975; Lagerwerff et al., 1976; Stover et al., 1976; Bloomfield & McGrath, 1982; Adams, 1985) or a series of reagents to remove metals from various component fractions in sequence (Stover et al., 1976; Silviera & Sommers, 1977; Förstner et al., 1981; Emmerich et al., 1982b; Fraser & Lum, 1983; Legret et al., 1983; Steinhilber & Boswell, 1983; Oake et al., 1984). Mechanisms of extraction are generally based on dissolution of solids, reversal of chemical reactions, formation of stable non-adsorbed complexes and/or displacement of adsorbed or ion-exchangeable metals (Pickering, 1981; Cottenie & Verloo, 1984).

Many of the single chemical extractant techniques applied to sewage sludge, which involve either shaking of a sludge sample in reagent at a known sludge solids to volume ratio over an optimum time period or continuous leaching of columns of sludge, were based on techniques originally developed for use in soil chemistry. Simple aqueous extractions have generally been employed to investigate the occurrence of water-soluble heavy metals in sewage sludges. This fraction is considered to approximate the concentration of metal in the solution phase and hence that most readily available for plant uptake (Viets, 1962; Cox & Kamprath, 1972; Worthington, 1979; Soon & Bates, 1982; Cottenie & Verloo, 1984; Matthews, 1984). Lagerwerff et al. (1976) demonstrated that <1% of Pb and approximately 2% of Cu were leached by tap water from columns of air-dried digested sludge, while 11% of Cd and 36% of Zn were water-soluble (Lagerwerff et al., 1976). However, following repeated percolation of columns of dried digested sludge with distilled water, Jenkins & Cooper (1964) calculated the total Cu, Ni and Zn extracted to be no greater than 0.3, 14.3 and 1.7% respectively; reductions in the water solubility of these metals following successive percolations being attributed to the development of anaerobic conditions. Bloomfield & Pruden (1975) similarly observed considerable reductions in water-soluble forms of Cu, Ni and Zn following anaerobic incubation for three months, although Cd and Pb increased in solubility from <0.1 to 7% and <0.2 to 0.6% respectively.

Subsequent aeration increased the water-solubility of Cu and Zn but had no corresponding effect on Cd, Ni and Pb. In contrast, however, consistently low water solubilities following anaerobic incubation for one week have been reported for Cd (<0.1%) and Pb (<0.03%) while Cu, Ni and Zn solubilities were <0.1 to 6.2%, 1.5 to 9.2% and <0.01 to 0.8% respectively (Stover et al., 1976). It is apparent from these observations that metals in sludge generally exhibit low water solubilities, with Ni consistently emerging as the most and Pb as the least water-soluble metal. Considerable variation in metal solubility between sludges has been reported, reflecting differences in sludge properties.

Extraction techniques based on metal chloride solutions and neutral acetate salts, which have frequently been utilised in the displacement of exchangeable metal forms from soils (Adams, 1965; Cox & Kamprath, 1972; McLaren & Crawford, 1973; Elsokarry & Lagg, 1978; Cavallaro & McBride, 1984; Cottenie & Verloo, 1984) and sediments (Tessier et al., 1979; Meguellati et al., 1983) have also been applied to sewage sludges (Lagerwerff et al., 1976; Förstner et al., 1981; Adams, 1985). Exchangeable forms have been defined as cations which are easily interchangeable between the soil solution and negatively charged sites on the surface of soil colloids (Doyle et al., 1978) and hence may be available for uptake by plants. Extraction of air-dried digested sludge with 0.06 M $CaCl_2$ solution resulted in <1% of Pb and <2% of Cu being leached, while the extractability of Cd and Zn was greater, at 46 and 81% respectively (Lagerwerff et al., 1976). Using 1.0 M ammonium acetate (NH_4OAc) in the removal of exchangeable forms from activated sludges, Förstner et al. (1981) similarly observed the extractabilities of Cd and Zn to be relatively greater than those of Cu and Pb, using the term 'metal pairs' to denote similarity in the behaviour of Cu-Pb and Zn-Cd respectively. However, Zn displaced from artificially metal-loaded sewage sludges by $BaCl_2$ was reported to represent only 3.9% of the total (Adams, 1985), while 4.2% of the Cu and 2.8% of the Ni was $BaCl_2$-extractable. Although chloride reagents have been preferred to neutral acetate salts in extracting exchangeable cations from soils (Gibbs, 1973; McLaren & Crawford, 1973), objections have been raised regarding their suitability in determining this form due to the formation of dissolved metal-chloro complexes (Doner, 1978; Calmano & Förstner, 1983).

Extraction techniques based on acidification or chelating organics, which have been used to determine 'available' forms of metals in soils (Wear & Evans, 1968; Norvell & Lindsay, 1969; 1972; Trierweiler & Lindsay, 1969; Misra & Pande, 1974; Lindsay & Norvell, 1978; Tiwari & Mohankumar, 1982), have also been applied to sludges. The term 'available', which has been utilised in micronutrient soil tests to denote the nutrient status of soils with respect to plants, may relate to several different metal species; water-soluble, exchangeable and organically bound forms being of most significance (Cox & Kamprath, 1972). Organically bound metals incorporate those forms which are complexed, chelated, or adsorbed to organic matter in addition to components of living cells, their exudates, and a spectrum of degradation products (Wildung et al., 1979).

Acids used in the extraction of metals from sewage sludge include citric (Jenkins & Cooper, 1964), acetic (Berrow & Webber, 1972; Bloomfield & Pruden, 1975; Neuhauser & Harstein, 1980; Bloomfield & McGrath, 1982; Davis & Carlton-Smith, 1983), hydrochloric (Lagerwerff et al., 1976; Stover et al., 1976; Neuhauser & Harstein, 1980; Logan & Feltz, 1985) and nitric (Bradford et al., 1975), at concentrations ranging from 0.01 to 4.0 M H^+; while ethylenediaminetetraacetic acid (EDTA) is the most frequently applied chelating agent (Bloomfield & Pruden, 1975; Wollan & Beckett, 1979; Bloomfield & McGrath, 1982; Davis & Carlton-Smith, 1983). The validity of equating extractability by such reagents with 'availability' has been questioned, however, since they tend to dissolve forms of metals which are not 'available' and/or do not dissolve forms which are 'available' (Beckett et al., 1983). Nevertheless, such extraction techniques may provide information regarding the relative binding strengths of various metals to sludge solids.

Between 50 and 75% of Cu, Ni and Zn have been extracted by percolating 0.095 M citric acid, buffered at pH 2 to 6, through columns of digested sludge (Jenkins & Cooper, 1964), while Ni and Zn have also been found to be highly soluble in 0.42 M acetic acid, with over 95% of total Zn being present in readily soluble forms in some cases (Berrow & Webber, 1972). Acetic acid-extractable forms of Cu and Pb, however, represented on average only 6.9 and 2.8% of the total respective metal (Berrow & Webber, 1972). A 0.5 M HCl solution extracted 69, 59 and 73% of Cd, Ni and Zn respectively from digested sludges, while only 24 and 18% of Cu and Pb were extracted (Stover et al., 1976). Logan & Feltz (1985) similarly reported >70% of the Cd, Ni and Zn content of a digested sludge but only 26% of the Cu and 4% of the Pb to be HCl-extractable. Such findings suggest that Cu and Pb may be more strongly bound within the sludge matrix than Cd, Ni or Zn.

The effect of incubation on metal extractability in 0.5 M acetic acid and 0.05 M EDTA has been investigated (Bloomfield & Pruden, 1975). Under anaerobic conditions the proportion of the total Cd, Cu and Zn extracted by acetic acid decreased from 60 to 50%, 10 to 0.2% and 60 to 20% respectively, with similar reductions from 75 to 50%, 20 to 0.5% and 70 to 40% being observed for EDTA-extractable metals. However, acetic acid-extractable Pb increased from 1.2 to 5.7%, while EDTA-extractable Pb increased from 8 to 28%. Although EDTA-extractable Ni increased from 29 to 36% during anaerobic incubation, a marked reduction in acetic acid-soluble Ni from 39 to 5% occurred. Subsequent aeration generally increased the acetic acid and EDTA extractability of metals. This change in extractability in specific metals under different redox conditions can be explained by the changes in metal form which occur. For example, the increase in the extractability of Pb by EDTA under anaerobic conditions could result from an increase in $PbCO_3$, which is readily soluble in EDTA (Stover et al., 1976).

A progressive acidification technique based on that developed by Cottenie (1981) for use on soils has been applied to sewage sludges to characterise constituent heavy metals according to their solubilities at different pH values (Adams & Sanders, 1983; 1984; Adams, 1985). The pH value is one of the most influential parameters controlling the transference of metals from immobile solid-phase forms to more mobile, and possibly more bioavailable, solution-phase forms

(Cottenie, 1981; Soon, 1981; Gerritse et al., 1982; Sanders, 1983; Adams & Sanders, 1984). Adsorption equilibria, the stability of organo-mineral complexes and the redox-potential, which all modify solubility relationships, are particularly affected by this parameter (Dhaese & Cottenie, 1979; Cottenie & Verloo, 1984). Progressive acidification of raw sludges, which essentially involved equilibrating sludge-water suspensions at decreasing pH values by addition of HNO_3, resulted in consistent and characteristic solubilisation profiles being observed for each metal studied (Adams & Sanders, 1983; 1984; Adams, 1985). While Cu tended to pass into solution at approximately pH 4.0, Ni and Zn exhibited greater solubility, with thresholds nearer to pH 6.0. To date, the effect of progressive acidification on the solubilities of Cd and Pb present in sewage sludge has not been investigated.

Sequential chemical extraction schemes are considered to be of greater value than single extractants in determining metal distribution in sewage sludge (Stover et al., 1976) and, although more time-consuming, they provide additional information regarding metal bioavailability and mobility (Tessier et al., 1979). Examples of sequential extraction schemes which have been applied to sewage sludges are presented in Table 7, the majority of these being modifications of extraction techniques developed for use on soils (Hodgson, 1963; Lindsay, 1972; McLaren & Crawford, 1973) and sediments (Tessier et al.,1979; Salomons & Förstner, 1980; Meguellati et al., 1983). Reagents utilised were chosen on the basis of their selectivity and specificity towards particular physicochemical forms. It is apparent, however, that much variation exists between schemes in the reagents used to extract specific metal forms.

Stover et al. (1976) proposed a sequential extraction technique for application to sewage sludge which was based on a procedure originally developed for use on soils by McLaren & Crawford (1973). This modified scheme incorporated 1.0 M KNO_3, 0.5 M KF (pH 6.5), 0.1 M $Na_4P_2O_7$, 0.1 M EDTA (pH 6.5) and 1.0 M HNO_3 to fractionate metals into forms designated as exchangeable, adsorbed, organically bound, carbonate, and sulphide respectively. Extraction of exchangeable metals

Table 7 Reagents utilised in the sequential extraction of heavy metals from sewage sludges

Reference	Silviera & Sommers(1977)	Stover *et al.* (1976)	Oake *et al.*(1984)	Emmerich *et al.* (1982b)	Steinhilber & Boswell (1983)	Förstner *et al.* (1981)	Fraser & Lum (1983)	Legret *et al.* (1983)
Sludge type	Digested	Digested	Raw,Activated, Digested	Digested	Digested	Activated	Digested ash	Digested
Metals	Cd,Cu,Pb,Zn	Cd,Cu,Ni,Pb, Zn	Cd,Cr,Cu,Ni, Pb,Zn	Cd,Cu,Ni,Zn	Zn	Cd,Cu,Pb,Zn	Al,Cd,Co,Cr, Cu,Fe,Mn,Ni, Pb,Zn	Cd,Cu,Ni,Pb, Zn
Designated Chemical Form								
Soluble	H_2O			[b]				[b]
Exchangeable	KNO_3	KNO_3	KNO_3	KNO_3	NH_4OAc	[c] NH_4OAc	[c] $MgCl_2$	$BaCl_2$
Adsorbed		KF	KF	Ion-exchange H_2O	EDTA			
Organically bound		$Na_4P_2O_7$	$Na_4P_2O_7$	NaOH	NaOCl NH_4OAc	[d] H_2O_2-HNO_3 NH_4OAc	[d] H_2O_2-HNO_3 NH_4OAc	[d] H_2O_2-HNO_3 NH_4OAc
'Available'	DTPA							
Carbonate		EDTA	EDTA	EDTA		$NH_2OH.HCl$-HNO_3	NaOAc-HOAc	NaOAc-HOAc
Sulphide		HNO_3	HNO_3	HNO_3		[d] H_2O_2-HNO_3 NH_4OAc	[d] H_2O_2-HNO_3 NH_4OAc	[d] H_2O_2-HNO_3 NH_4OAc
Oxide bound[a]					Oxalate[e] Mixture (Dark & UV)	$NH_2OH.HCl$-HNO_3 Oxalate[e]	$NH_2OH.HCl$-HOAc	$NH_2OH.HCl$-HOAc NH_4OAc
Residual	HNO_3			HNO_3		$C.HNO_3$	H_2O_2-HF-HCl	HCl-HF

[a] Co-precipitated with or occluded in Fe and Mn oxides; also designated the 'reducible' phase

[b] Soluble phase separated from solid phase

[c] Correct order of extraction scheme is: exchangeable; carbonate; oxide-bound; organic/sulphide; residual

[d] Organic and sulphide forms classed as a single 'oxidisable' phase

[e] Consists of oxalic acid $(CO_2H)_2$ and oxalate $(CO_2NH_4)_2$

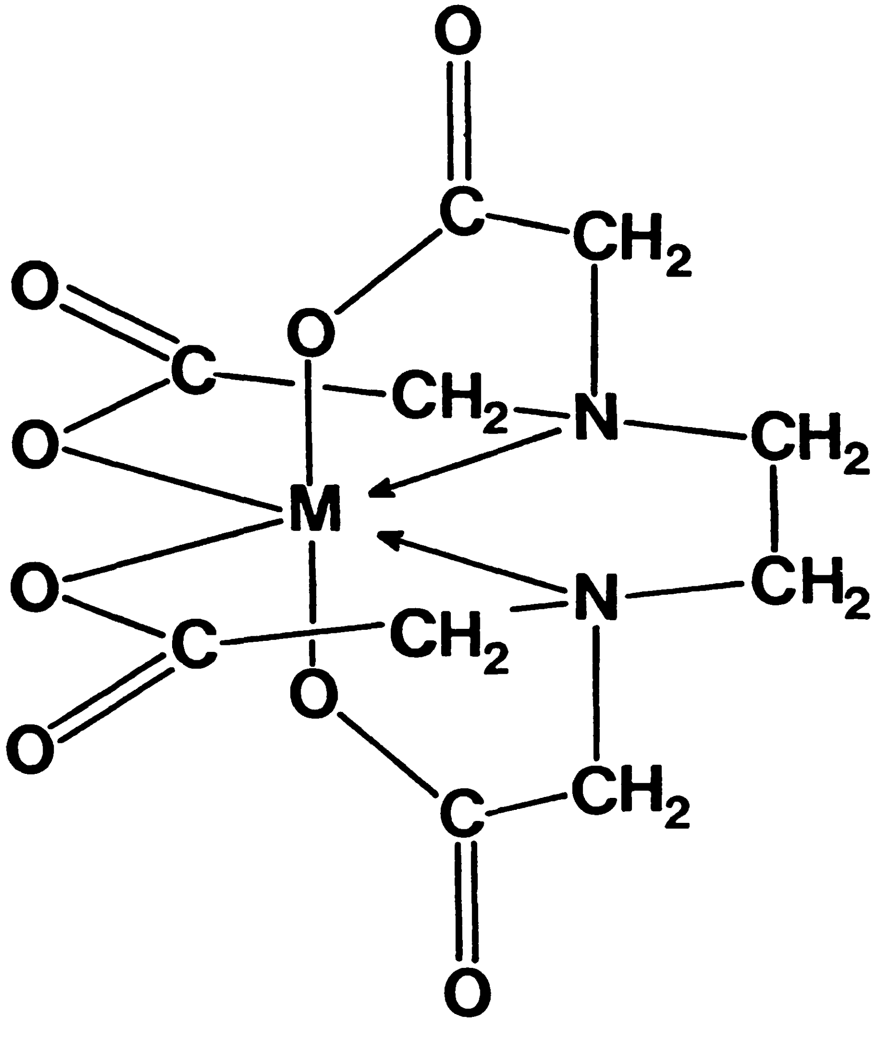
by KNO_3 is based on the ability of K^+ in excess to displace cations from negatively-charged exchange sites located on sludge inorganic and organic components (Stover et al., 1976; Koenig, 1976). The displaced cations form soluble nitrate salts within the extracting solution (Koenig, 1976). Utilisation of KF at the concentration and pH value specified effects the removal of surface adsorbed metals through the formation of soluble metal-fluoride complexes while simultaneously minimising solubilisation of organically bound forms (Stover et al., 1976). Removal of organically bound metals by dilute $Na_4P_2O_7$ has been attributed to the ability of this reagent to solubilise organic matter and to form stable soluble co-ordination complexes with metallic cations (Koenig, 1976; Pickering, 1981). The reaction between soil organic matter and $Na_4P_2O_7$ has been simplified by Schnitzer & Khan (1972) as follows:

$$R\,(COO)_4\,Zn_2 + Na_4P_2O_7 \longrightarrow \underset{\text{soluble}}{R\,(COONa)_4} + \underset{\text{soluble}}{Zn_2P_2O_7} \qquad (1)$$

Selection of EDTA for the extraction of metal carbonates was on the basis of its demonstrated removal efficiency of pure metal precipitates (Stover et al., 1976). Dissolution of metal ions by EDTA is principally through its chelating ability; chelation involving the co-ordination of two or more electron-donating atoms present in the chelating ligand to the same electron-accepting metal ion so as to form a heterocyclic ring termed a chelate (Bell, 1977; Worthington, 1979). EDTA is a sexa-dentate ligand (ie. contains six donor atoms capable of co-ordination to a single metal ion) which forms five chelate rings when all donor atoms are co-ordinated to a metal ion (Bell, 1977), as illustrated (Figure 3). Finally, dissolution of metal sulphides by HNO_3 is based on pH-stability properties (Stover et al., 1976).

In applying this sequential extraction scheme to various digested sewage sludges, Stover et al. (1976) found that while carbonate forms predominated for Cd (49%), Ni (32%) and Pb (61%), Zn and Cu were predominantly present in organically bound (50%) and sulphide (35%) forms respectively. Adsorbed and exchangeable fractions

Figure 3 Chelation of a metal ion (M) by EDTA



together accounted for <10% of the total Cd, Pb and Zn, while approximately 17% of the Cu and 22% of the Ni was present in these two fractions. Using a slightly modified version of this technique, Oake et al. (1984) observed the predominant forms of Cd, Cu, Ni, Pb and Zn present in digested sludges to be identical to those reported by Stover et al. (1976). However, a similar extraction procedure applied by Emmerich et al. (1982b) to digested sludge (Table 7) indicated that while Cd, Ni and Zn occurred predominantly in carbonate form, the major forms of Cu extracted were in the order: organically bound > carbonate > sulphide/residual; more than 60% of the sludge Cu being in the organically bound form. This sequence is the reverse of that reported for Cu by Stover et al. (1976). Also in apparent contradiction to the results of Stover et al. (1976), Legret et al. (1983) stated that Cu in digested sludges was largely associated with the organic phase. However, closer inspection of the form designated as 'organic' by Legret et al. (1983) reveals the inclusion of sulphides as an 'oxidisable' phase (Table 7), suggesting that the rankings of Cu by Stover et al. (1976) and Legret et al. (1983) may not be as dissimilar as they initially appear.

Unfortunately, inter-comparison of the results obtained from sequential extraction schemes is difficult due to the diversity of reagents used to extract particular metal forms (Table 7). Even when the same reagent is employed, the rate and efficiency of leaching will be influenced by the type of sample, the size of particulates and duration of extraction together with pH, temperature, strength of extractant and ratio of solid matter to volume of the extractant (Sterritt & Lester, 1980a; Förstner et al., 1981). Investigation into the effect of varying extraction scheme parameters has been proposed with a view to standardisation of extraction procedures (Förstner et al., 1981) but, to date, has received little attention.

Fractionation on the basis of particle size has been undertaken on digested primary sludge using a scheme of elutriation and filtration through membranes of various mesh sizes (Gould & Genetelli, 1975). All metals studied, including Cd, Cu, Ni, Pb and Zn, were found to be associated with the solid phase, in excess of 90% being in the 'particulate' fraction (having effective Stoke's diameters of >100 µm). Most of the balance was found in the 'supracolloidal' fraction (0.6-100 µm) with the 'colloidal' (0.002 - 0.6 µm) and dissolved (<0.002 µm) fractions accounting for less than 1 to 2% of total metal. Although fractionation of heavy metals based on filtration will not differentiate between physical solid types (Sterritt & Lester, 1984b), Gould & Genetelli (1975) concluded that organometallic interactions were important in digested sludge since heavy metal content correlated to a greater extent with volatile solids than with inert solids.

Using similar methods of filtration and elutriation, Hayes & Theis (1976; 1978) attempted to characterise the distribution of Cd, Cu, Ni, Pb and Zn in anaerobically digested sewage sludge among soluble, precipitated, extracellular and intracellular components. The metals were found to be distributed almost exclusively (>95%) between the inorganic precipitated and organic insoluble fractions; separation of these two components having been achieved by elutriation in a conical upflow clarifier. Although separation was incomplete, 30 to 60% of the total of each heavy metal was found to be predominantly intracellular, extracellular metals (extracted by EDTA) being insignificant (<1%). The authors concluded that microbial uptake actively competed with precipitation in the removal of heavy metals from digester supernatant.

Fractionation by filtration is subject to several experimental errors, including adsorption of soluble metal species onto the filter or filter holder (Hunt, 1979; Gardner, 1982; Sterritt & Lester, 1984b) and clogging of the filter pores under conditions of high solids loading (Wagemann & Brunskill, 1975; Faisst, 1980; Danielsson, 1982). However, provided precautions are taken to avoid such problems, membrane filtration can be used to obtain useful information relating to the solid-phase distribution of heavy metals in complex matrices such as sewage sludge (Sterritt & Lester, 1985).

Baldwin *et al*. (1983) combined a physical solids separation method with various chemical extraction techniques to produce an integrated approach to metal speciation in digested sewage sludge.

Three sludges were fractionated on the basis of both size and density by dispersion and sedimentation in water over a specified time period. The resultant fractions were then treated with reagents in an attempt to determine the location and form of Cu and Zn within the sludges. Both metals were found to be associated with organic colloids coating the mineral fragments, which the authors implied may have been composed of bacterial extracellular material. Some of the Cu was retained on the organic matter in exchangeable or complexed forms (extracted by H_2O_2 treatment). Zinc, however, was displaced more readily by acid $CaCl_2$, indicating the presence of a precipitate or more stable crystalline form. Both metals were thought to be associated with precipitates, both on and in the mineral flakes and grains found in the fractions and also, in a more amorphous form, combined with organic colloidal layers.

Certain studies on metal speciation in sewage sludge have attempted to describe the behaviour of metals in individual fractions and the dynamic nature of certain metal species between the solid and solution phases. Although soluble metal concentrations in sewage sludge are generally low, the potential solubility of some metals in sludge supernatant has been found to be appreciable. Patterson & Hao (1979) reported the potential solubilities of Cd, Cu, Ni, Pb and Zn in anaerobically digested sludge filtrate to be respectively 185, 54, 173, 30 and 8 times greater than their actual determined soluble concentrations, although these authors failed to indicate the form of metal addition.

While precipitation may control the soluble concentration of some metals (Gould & Genetelli, 1975; Mosey, 1976; Hayes & Theis, 1978), organometallic complexation, which may involve simple complex formation and/or chelation, could also be a major mechanism responsible for the association of heavy metals with sludge solids, especially when aerobic conditions exist. Gould & Genetelli (1978a) studied the complexation characteristics of metals in anaerobically digested sludge and, on the basis of Langmuir isotherm results, calculated the stability constant (log K) values for Cd, Cu, Ni and Zn complexation by the sludge solid phase to be 5.19, 4.67, 4.69 and 4.98

respectively at pH 7.0. However, in using an ion-exchange technique developed by Cheng et al. (1975), Patterson & Hao (1979) determined log K values of metal-sludge complexes to be 1.14, 1.44, 1.05, 1.62 and 0.69 for Cd, Cu, Ni, Pb and Zn respectively, which contrast with the values reported by Gould & Genetelli (1978a). Patterson & Hao (1979) also studied metal complexation in the soluble phase of anaerobically digested sludge and found that log K represented -1.18 for Cd, -0.11 for Cu, -2.87 for Ni, 0.001 for Pb and -5.51 for Zn. Such values indicate that sludge solid and soluble phases may have vastly different complexing characteristics, although the data may only be interpreted comparatively since Patterson & Hao (1979) were unable to obtain an accurate value for the ligand concentration and substituted soluble COD and volatile suspended solids for the solution and solid phases respectively (Sterritt & Lester, 1984b).

Several workers have reported increases in organometallic complex stability with increasing pH and solubilisation of metallic ions with decreasing pH (Cheng et al., 1975; Gould & Genetelli, 1978a; 1978b). Such an effect may result from the competition which exists between the metal and hydrogen ions for the weakly acidic complexation sites. Freundlich isotherm results suggest that Cd, Ni and Zn tend to displace one hydrogen ion and Cu two hydrogen ions for each complex formed (Gould & Genetelli, 1978a).

Methylation, using dimethyl sulphate to convert carboxylic and/or phenolic functional groups to corresponding methyl ester or methoxy groups, has also been shown to affect heavy metal binding in sludge, resulting in a decrease in binding capacity for Cd, Cu and Zn (Gould & Genetelli, 1978b). However, methylation had virtually no effect on Ni binding, suggesting that Ni is not predominantly associated with the carboxylic or phenolic functional groups. Tan et al. (1971) investigated complex formation between Zn and alkali-extracted organic matter from anaerobically digested sewage sludge and concluded from infrared spectra that hydroxyl and carboxyl functional groups were involved. However, Boyd et al. (1979) suggested, on the basis of infrared analysis, that the concentration of carboxyl functional groups present in sewage sludge was minimal and that amide groups of peptide bonds in residual proteins were involved in heavy metal binding. The involvement of thiol functional groups has also been demonstrated (Baham et al., 1978). Information relating to the nature of soluble complexing ligands in sewage sludge is scarce. However, Baham & Sposito (1983) identified 55% of the water-soluble organic carbon extracted from digested sludge as being capable of forming soluble complexes with heavy metals, this soluble organic matter being composed principally of proteinaceous material and polysaccharides.

Heavy metals may similarly be present in soils in several different physicochemical forms, namely:

i) in ionic or complexed form in the soil solution;
ii) as readily exchangeable ions in organic and inorganic exchange active material;
iii) as more firmly bound ions in the exchange complexes;
iv) as chelated ions in an organic or organo-mineral complex;
v) incorporated in precipitated sesquioxides or insoluble salts;
vi) incorporated into microorganisms and their biological residues;
vii) held in the crystal lattice structure of primary and secondary minerals (Viets, 1962; Hodgson, 1963; McLaren & Crawford, 1973; Matthews, 1984).

Heavy metals can thus occur in the soil solution phase or may be attenuated in the solid phase through cation-exchange, adsorption, chelation, precipitation or crystallisation (Kuo et al., 1983; Matthews, 1984). The distribution of a metal between the various forms listed is dependent on the chemical properties of the individual metal and a number of soil factors, including the pH value, redox potential, texture, cation-exchange capacity, temperature, moisture content, concentrations of other metals, organic matter status, mineral composition and profile characteristics (Doyle et al., 1978; Davis & Coker, 1980; Sterritt & Lester, 1980a; Matthews, 1984). However, the divisions between these forms are not clear-cut, either in the soil or by the experimental procedure used to isolate them. Distinction between the different forms thus remains largely a matter of definition based on experimental procedures (Koenig, 1976).

Investigation into the solid-phase distribution of heavy metals in soils has generally involved the use of chemical extraction techniques; examples of sequential schemes applied to this matrix being presented in Table 8. Although inter-comparison of the results obtained from sequential extraction procedures is complicated by the diversity of reagents, extraction conditions and soil types used, similarities between schemes with respect to the physicochemical nature of specific metals present in soils are often apparent. Copper, for example, has generally been found to predominate in the residual fraction (McLaren & Crawford, 1973; Emmerich _et_ _al_. 1982b; Sposito _et_ _al_., 1982a; Chang _et_ _al_., 1984; Hickey & Kittrick, 1984); McLaren & Crawford (1973) having reported an average of 50% of the total Cu content of 24 British soils to be present in this fraction. In addition, Cu has frequently been observed to be the only metal significantly associated with the soil organic matter, this fraction generally accounting for up to 30% of the total soil Cu content (McLaren & Crawford, 1973; Emmerich _et_ _al_., 1982b; Hickey & Kittrick, 1984). This is consistent with the known affinity of Cu for organic ligands (Stevenson & Ardakani, 1972; Kerndorff & Schnitzer, 1980). Adsorption to Fe and Mn oxide material has been reported to be of approximately the same significance as organic binding in the retention of Cu in the soil solid phase (McLaren & Crawford, 1973; Hickey & Kittrick, 1984). It would appear, however, that Fe oxides are of relatively greater importance than Mn oxides in this role (Kuo _et_ _al_., 1983). Soluble and exchangeable forms of Cu have consistently been found to represent <1% of the total soil Cu content using the sequential extraction schemes presented in Table 8. Mercer & Richmond (1968) similarly reported that the proportion of the total soil Cu in solution did not differ greatly between eleven agricultural soils of widely differing textures, suggesting that metal form equilibria in soils may shift to maintain a given quantity in solution. According to McLaren & Crawford (1973), the concentration of Cu in soil solution is primarily controlled by equilibria involving Cu adsorbed to organic matter.

Residual forms also tend to predominate for Ni in soils (Emmerich _et_ _al_., 1982b; Chang _et_ _al_., 1984; Hickey & Kittrick, 1984);

Table 8 Reagents utilised in the sequential extraction of heavy metals from soils and sewage sludge-amended soils

	SOILS AND SLUDGE-AMENDED SOILS							SOILS ONLY		
Reference	Silviera & Sommers (1977)	Petruzzelli *et al.*(1981)	Emmerich *et al.*(1982b)	Schalscha *et al.*(1982)	Sposito *et al.*(1982a)	Chang *et al.*(1984)	Hickey & Kittrick (1984)	McLaren & Crawford (1973)	Kuo *et al.*(1983)	Miller *et al.*(1983)
Metals	Cd,Cu,Pb,Zn	Cd,Cu,Ni,Pb Zn	Cd,Cu,Ni,Zn	Cr,Cu,Mn,Ni, Zn	Cd,Cu,Ni,Pb, Zn	Cd,Cr,Cu,Ni, Pb,Zn	Cd,Cu,Ni,Zn	Cu	Cd,Cu,Zn	Cd,Cu,Pb, Zn
Designated Chemical Form										
Soluble	H_2O	H_2O	b	KNO_3						H_2O
Exchangeable	KNO_3	KNO_3	KNO_3	NaF	KNO_3	KNO_3	$MgCl_2$[c]	$CaCl_2$	$MgCl_2$	KNO_3
Adsorbed			Ion-exchange H_2O		Deionised H_2O	Deionised H_2O		HOAc		NH_4F
Organically bound			NaOH	$Na_4P_2O_7$	NaOH	NaOH	H_2O_2-HNO_3 NH_4OAc	$K_4P_2O_7$	NaOCl	$Na_4P_2O_7$
'Available'	DTPA	DPTA								
Carbonate			EDTA	EDTA	EDTA	EDTA	NaOAc			EDTA
Sulphide			HNO_3[d]	HNO_3	HNO_3[d]	HNO_3[d]				HNO_3
Oxide bound[a]							$NH_2OH.HCl$ HOAc	Oxalate[e]	Oxalate[e] CDB[f]	$NH_2OH.HCl$ CDB[f]
Residual	HNO_3		HNO_3[d]		HNO_3[d]	HNO_3[d]	HF-$HClO_4$	HF		C.HNO_3

a Co-precipitated with or occluded in Fe and Mn oxides
b Soluble metals separated from saturation extract cake
c Correct order of extraction scheme is: exchangeable; carbonate; oxide-bound; organically bound; residual
d Sulphide/residual forms classed as one fraction
e Consists of oxalic acid $(CO_2H)_2$ and oxalate $(CO_2NH_4)_2$
f CDB = Citrate-dithionite-bicarbonate

this fraction having been found to represent up to 79% of the total Ni concentration of the soils studied by Hickey & Kittrick (1984). Other workers (Gupta & Chen, 1975; Tessier et al., 1979) have similarly suggested that the majority of the Ni in soils is detrital in nature, and Norrish (1975) has indicated that Ni is commonly occluded by silicates during soil weathering. However, up to 30% of the Ni content of soils may be associated with Fe and Mn oxides (Hickey & Kittrick, 1984). According to Jenne (1968), adsorption to Mn oxides is particularly important in Ni retention, substitution of Ni^{2+} for surface Mn in mixed valence Mn oxides possibly accounting for much of this adsorption (McKenzie, 1972).

Although sulphide/residual forms have frequently been found to predominate for Zn in soils (Emmerich et al., 1982b; Sposito et al., 1982a; Chang et al., 1984), oxide-bound forms also appear to be of importance (Kuo et al., 1983; Hickey & Kittrick, 1984). Hickey and Kittrick (1984), for instance, found an average of 39% of the total Zn content of four soils to be associated with metal oxides. Similar to Cu, the majority of the Zn in this fraction has been reported to be adsorbed to amorphous Fe oxides (Kuo et al., 1983). Such findings support the conclusions of Jenne (1968) and McBride & Blasiak (1979) that specific adsorption on hydrous Fe oxides is a major process of Zn retention in soils.

While Miller et al. (1983) reported Cd and Pb to occur mainly in organically bound forms in soils, Emmerich et al. (1982b) and Chang et al. (1984) found that carbonate forms predominated for these metals. With respect to Cd, however, exchangeable forms have occasionally been found to predominate (Kuo et al., 1983; Hickey & Kittrick, 1984), suggesting that cation-exchange may play a relatively more important role in retaining Cd in soils than it does for metals such as Cu, Ni, Pb and Zn. Hickey & Kittrick (1984) found an average of 37% of the total Cd content of the soils investigated to be exchangeable in $MgCl_2$, while Kuo et al. (1983) reported from 30 to 60% to be extracted using the same reagent. Such high proportions of Cd in exchangeable forms generally indicates that the soils investigated had quite limited specific adsorption capacity or that they contained

Cd in concentrations sufficient to cause saturation of specific adsorption sites, resulting in a large proportion of the metal occupying exchange sites by mass action (Riffaldi & Levi-Minzi, 1975; Singh, 1979; Soon, 1981). According to Miller et al. (1983), soils of low specific adsorption capacity will show higher retention of Cu and Pb compared with Cd and Zn at high metal loadings since competition by Cu and Pb for adsorption sites reduces the amount of organically adsorbed Cd and Zn. Consideration of such factors is likely to be of great importance when highly contaminated sludges are applied to agricultural soils.

Sequential extraction schemes have generally indicated that metals native to soils tend to be more resistant to chemical extraction than those indigenous to sludges. This was emphasised by Emmerich et al. (1982b) who found that while Cu, Ni and Zn were predominantly present in sludges in either organically bound (NaOH-extractable) or carbonate (EDTA-extractable) forms, in excess of 65% of each of these metals occurred in loamy soils in more stable residual forms (extracted by hot HNO_3 digestion). According to Harrison et al. (1981), the mobility and bioavailability of a metal decreases approximately in the order of the extraction sequence. Hence, the findings of Emmerich et al. (1982b) may indicate that Cu, Ni and Zn are present in soils in forms which are potentially less mobile and less bioavailable than those in which they occur in sludges. In a comparative exercise, 0.42 M acetic acid was used to determine 'available' forms of metals in sludges and soils representative of unamended agricultural land (Berrow & Webber, 1972). Although results varied widely, typical extractable levels of all metals in soils, with the exception of Al, were generally found to be lower than average levels in sludges; median values of 'available' Cu, Ni, Pb and Zn in sludge being 140, 25, 16 and 550 times their respective values in soils.

The application of sewage sludge to soils may therefore alter the distribution of a metal cation between the different physicochemical forms and thus affect its availability to plants. Chemical extraction techniques have further been employed in elucidating the transitions in solid-phase metal form which occur when sludge is disposed to land; the major sequential extraction schemes applied being presented in Table 8.

Such techniques have demonstrated that the application of sewage sludge to soils generally causes a shift in solid-phase metal forms in soils away from refractory sulphide/residual forms to those extractable with milder reagents (Emmerich et al., 1982b; Schalscha et al., 1982; Sposito et al., 1982a; Chang et al., 1984). Sulphide/residual forms in three unamended loamy soil profiles constituted the major fractions of Cu (65 to 82%), Ni (80 to 94%) and Zn (92 to 96%) (Emmerich et al., 1982b). Following sludge application, however, large reductions in sulphide/residual Cu, Ni and Zn, to 19 to 24%, 61 to 69% and 28 to 35% respectively, were simultaneously accompanied by significant increases in the less stable and more easily extractable organically bound and carbonate forms. While organically bound forms represented 50 to 52%, 9 to 13% and 19 to 28% of the Cu, Ni and Zn content of the sludge-amended soils respectively, carbonate forms constituted 23 to 30%, 20 to 25% and 41 to 49% respectively (Emmerich et al., 1982b). Enhancement of soluble and exchangeable metal forms in soils following sludge application has also been observed using sequential extraction techniques (Silviera & Sommers, 1977; Petruzzelli et al., 1981; Emmerich et al., 1982b; Schalscha et al., 1982; Sposito et al., 1982a).

Such effects suggest that the application of sewage sludge to soils could provide metals in labile chemical forms that might be potentially more bioavailable than those in non-amended soils. Indeed, marked increases in the amounts of total Cd, Cu, Pb and Zn extractable by diethylenetriaminepentaacetic acid (DTPA) (believed to estimate 'available' metal species) were observed in two silt loam soils 28 days after sewage sludge had been applied (Silviera & Sommers, 1977). A similar extraction scheme resulted in DTPA-extractable forms of Cu, Ni, Pb and Zn in an acidic sandy loam soil reaching maximum values 30 days after the addition of sludge (Petruzzelli et al., 1981). The apparent increase in 'available' metal forms in sludge-soil mixtures with time has been ascribed to dissolution of metal precipitates such as carbonates, hydroxides and phosphates, through changes in pH or gas composition of the soil resulting from microbial activity; oxidation of metal sulphides to sulphates by autotrophic sulphur-oxidising bacteria; and microbial release of metals complexed with sludge organic matter (Huang _et al_., 1977; Silviera & Sommers, 1977; Davis & Coker, 1980; Lewin & Beckett, 1980; Schauer _et al_., 1980; Petruzzelli _et al_., 1981). According to Hinesly _et al_. (1977), even organic matter in apparently stable digested sludge decomposes rapidly after incorporation into the soil. It has been suggested by Terry _et al_. (1979) that part of the native soil organic matter may also biodegrade following sludge addition.

Although the general concensus is that the addition of sludge to soils has the immediate effect of increasing the 'available' metal pool, it has been suggested that over longer time periods there may be a tendency for metal forms to stabilise and to eventually reach the appropriate long-term equilibrium state of the soil (Kelling _et al_., 1977; Beckett _et al_., 1979; Lewin & Beckett, 1980; Williams _et al_., 1980; Emmerich _et al_., 1982b). This phenomenon has been ascribed to the progressive loss of soluble-phase functional groups for metal binding, as demonstrated by infrared spectroscopy (Schaumberg _et al_., 1980; Steinhilber & Boswell, 1983).

Reports in the literature relating to the chemical forms of heavy metals in the solution phase of soils and sludge-amended soils are limited. This is partially due to the large number of possible chemical forms in which metals can exist in solution, together with the analytical problems associated with their determination (Emmerich _et al_., 1982c). In addition, studies on soil solutions are rendered difficult by the very low concentrations of most metals in them (Cox & Kamprath, 1972).

Various chromatographic techniques have been employed in the characterisation of metal species in soil solution, including ion-exchange, adsorbent resin, gel permeation and high performance liquid chromatography (HPLC). An ion-exchange scheme was used to determine the distribution of selected metals present in water-soluble and exchangeable fractions of a sandy soil among free ions, positively and negatively charged complexes, neutral species, and pH-dependent amphoterics (Camerlynck & Kiekens, 1982). Estimation of the stability of complexed metal species was made by observing the passage of solutions through a column of Chelex-100 which retained free ions, highly labile and moderately labile species, while passing slowly labile and inert species (Figura & McDuffie, 1979; 1980). While soluble Cu was largely present as stable complexes, Zn was distributed between free ionic and complexed forms (Camerlynck & Kiekens, 1982). Using an ion-exchange equilibrium method, log K values were calculated for complexes formed at pH 5.0 between divalent metal ions and a fulvic acid preparation from soil to be in the order:

$$Cu > Pb > Fe > Ni > Mn, Co > Ca > Zn > Mg.$$

Log K values were reported to be much higher at pH 5.0 than at pH 3.5; a result that was attributed to proton competition with decreasing pH (Schnitzer & Skinner, 1966; 1967).

Columns packed with 'Amberlite' IR-120 cation exchange resin were used together with 'Amberlite' XAD-2 adsorbent resin to investigate Cd speciation in the solution phase of sewage sludge-amended soils. A considerable amount of Cd in the sludge-amended soil solution occurred in the cationic (Cd^{2+}) form (Butterworth & Alloway, 1981). Utilisation of ion-exchange and chelating resins has permitted discrimination between several soluble metal species, although the choice of exchangeable cation or anion is very important to prevent large pH variations which may greatly modify heavy metal speciation (Astruc *et al.*, 1981). In addition, consideration of the complexing activities of the exchanger is necessary; adsorption of a complex being dependent on the relative strengths, and to a degree, the relative concentrations of the ion-exchanger-metal complexes and the ligand-metal complexes (Laxen & Harrison, 1981).

The use of gel permeation chromatography to study the speciation of heavy metals in the solution phase of soils was first reported by Mercer & Richmond (1968), who investigated the distribution of Cu in the solution phase of a light peat soil and a Lower Greensand soil using 'Sephadex' G-50 columns eluted with 0.02 M ammonium solution, and monitored for UV absorbance at 254 nm. It was tentatively concluded, on the assumption that equilibrium was attained between the Cu added and that already present in the organic fraction, that for the peat solution, about 50% soluble Cu, and for the Greensand solution, about 25 to 30% soluble Cu was associated with the organic fraction of molecular weight exceeding 10000. Inevitably, any physical method of speciation, such as gel permeation chromatography, which involves the separation of free metal ion from complexed metal forms, will result in displacement of the inherent chemical equilibria. In addition, charged species may be retarded or eluted rapidly due to complex interactions with the gel (Sterritt & Lester, 1984b). This may occur as a result of the weak ion-exchange characteristics of 'Sephadex' gels (Sterritt & Lester, 1984b) or may be due to effects of the eluent (Archer et al., 1981). Such limitations may be overcome by selective choice of eluents and gels.

Over the last ten years, the development of HPLC has enabled much improved resolution over gel permeation chromatography. Chromatographic fractionation of sludge-amended soil solutions through a μ-'Bondagel' E500 HPLC column of molecular weight separation range 5000 to 500000 showed that all soluble Cd occurred in a UV absorption peak (λ 280 nm) associated with low molecular weight organic molecules. These low molecular weight Cd species probably included both organic and inorganic complexes, together with free cationic Cd (Cd^{2+}) (Tills & Alloway, 1983). Higher molecular weight complexes of Cd have been separated using 'Sephadex' G-100 gel columns (Butterworth & Alloway, 1981). Metal-fulvic acid solutions extracted directly from three sludge-soil mixtures were fractionated on 'Sephadex' G-10 gel. Neither Cd nor Ni were associated with fulvic acid, although complexation between Cu, Zn and fulvic acid was apparent. Potentiometric titration (Sposito & Holtzclaw, 1977; Sposito et al., 1982b) together with infrared spectra analyses (Schnitzer, 1969; Stevenson & Goh, 1971; Sposito et al., 1976; 1982b) of fulvic acid fractions extracted from soils and sewage sludge-soil mixtures demonstrated the involvement of carboxylic together with phenolic functional groups and possibly N-containing acidic groups associated with protein decomposition in metal-fulvic acid interactions. Involvement of sulphonyl functional groups in metal-fulvic acid interactions in sewage sludge-amended soils has been suggested (Sposito *et al.*, 1976; 1982b), but is not apparent in unamended soils.

Ion-selective electrodes (ISEs), which present the opportunity for direct determination of free (uncomplexed) metal ion concentration *in situ*, have been applied to both soils (Jackson & Bondietti, 1977; Inskeep & Baham, 1983; McBride & Bouldin, 1984) and sludge-amended soils (Street *et al.*, 1977; Sposito *et al.*, 1981; 1982c). Estimates of uncomplexed Cu based on ISE data indicated that approximately 99.5% of the Cu in the solution phase of a calcareous soil (pH 7.0) was in an organically complexed form, this percentage decreasing to approximately 95% at pH 6.0 (McBride & Bouldin, 1984). In contrast, ISE measurements have suggested that the bulk of the Cd present in acidic soil solutions (pH 6.0) exists as the free ionic (Cd^{2+}) form (Jackson & Bondietti, 1977). Similar findings have been reported for Cd present in acidic aqueous extracts of sludge-amended soils on the basis of ISE analyses (Street *et al.*, 1977; Sposito *et al.*, 1982c). However, under alkaline conditions, the Cd^{2+} concentration was observed to decrease approximately 100-fold for each unit increase in pH (Street *et al.*, 1977). ISEs have been utilised, in conjunction with titrations, in the determination of conditional stability constants of complexes formed between Cd^{2+}, Cu^{2+}, Pb^{2+} and fulvic acid extracted from sludge-amended soils (Sposito *et al.*, 1981). Two classes of complexes were found for each of these metals; log K values for the stronger of these complexes being 3.04, 3.88 and 4.22 for Cd^{2+} Cu^{2+} and Pb^{2+} respectively.

From a practical point of view, ISEs are convenient tools for speciation since calibration is simple and an almost direct readout of the free metal ion concentration is obtainable after a short period of time (Sterritt & Lester, 1984b). Provided interference effects are avoided, ISE analysis appears to be fairly reliable for single *in situ* determinations of free metal ions and for titration (Sterritt & Lester, 1984c). One major advantage that ISEs have over many other analytical methods of speciation is that they may be used directly on samples containing suspended solids, thus avoiding prior removal of the solids and consequential alteration of speciation equilibria (Florence, 1982; Sterritt & Lester, 1984b). ISEs may therefore have potential in characterising heavy metals in sewage sludges, although, to date, their application to this matrix has received little attention.

Multipurpose computer programmes, such as 'GEOCHEM', have been useful tools in speciation computations, predicting the metal species likely to occur in the solution phase of soils and sludge-amended soils, under given conditions, on the basis of known chemical equilibria and properties of the soils concerned (Mattigod & Sposito, 1977; Mahler et al., 1980; Mattigod, 1981; Sposito et al., 1982c; Emmerich et al., 1982c). Applying 'GEOCHEM' to sludge-amended soil solution, it was predicted that 60% of soluble Cd would be in the free cationic form, while about 30% would occur as a soluble complex with chloride; the remainder being associated with sulphate. Complexes of Cd with organics were predicted to be insignificant. Free ionic Zn was predicted to be the predominant form, the remainder being complexed with sulphate. Copper occurred almost exclusively in organically complexed forms (Mattigod, 1981). In a similar study, free ionic Cd was estimated to account for 64 to 72% of soluble Cd, while organic complexes of Cd were less than 10% and the inorganic Cd complexes were in the order sulphate > bicarbonate or carbonate > chloride (Mahler et al., 1980).

Computer models are capable of providing predictions of metal speciation in soil solutions under specified conditions, but are dependent on the reliability of the experimental data incorporated into the model. A major limitation of this technique is the lack of reliable experimental data relating to total concentrations of metals and ligands in solution; stability constants of trace metal complexes with organic, inorganic and mixed ligands; solubility product constants for clay soil minerals and thermodynamic exchange constants and exchanger phase activity coefficients (Mattigod & Sposito, 1979). At present, therefore, modelling techniques are useful only for setting limits on speciation (Florence, 1982).

Although analytical approaches to differentiating between free and complexed metal forms in the solution phase of soils and sludge-amended soils are reasonably well established, very little is known regarding the relative availabilities of metals in these forms to plants. According to Halvarson & Lindsay (1977), however, Zn is most readily taken up by plants in the free cationic form (Zn^{2+}), while for Cd, complexes with soluble organic substances of low molecular weight, such as organic acids, in addition to the free cationic form, appear to be the most relevant to plant uptake (Davis & Coker, 1980; Matthews, 1984). Lagerwerff & Milberg (1978) reported that the Cu uptake by soyabeans decreased with the following order of chemical forms: inorganic cationic Cu > complexed cationic Cu > amphoteric Cu > anionic Cu. Mercer & Richmond (1971) found that organo-Cu complexes in peat soils having a molecular weight of less than 1000 were readily available to growing crops, whereas complexes of molecular weight exceeding 5000 were not.

Many studies relating to the uptake of heavy metals from sludge-amended soils by crop and grazing plants have been conducted, a comprehensive review of which has been presented by Sterritt & Lester (1980a). The accumulation of metals by plants would appear to be influenced by a variety of factors, including the pH value (John & Van Laerhoven, 1972; Andersson & Nilsson, 1976; Pinkerton & Simpson, 1977; Mahler _et al._, 1978; Mitchell _et al._, 1978), organic matter status (Haghari, 1974; Gaynore & Halstead, 1976; Maclean, 1976; Andersson, 1977) and phosphate content (Miller _et al._, 1976; Rashid _et al._, 1976; Wallace _et al._, 1978) of the soil; synergistic and antagonistic interactions between heavy metals (Turner, 1973; White _et al._, 1974; Berry, 1976; Miller _et al._, 1977; Chaney & Hornick, 1978); seasonal effects (Haghari, 1974; Giordano _et al._, 1975) and the time span between sludge application and crop planting (Hinesly _et al._, 1977; Kelling _et al._, 1977; Davis & Coker,1980). In addition, metal accumulation is dependent on the plant species, cultivar and organ or part. Cereals, grasses and legumes, for instance, tend to accumulate heavy metals to a lesser extent than faster growing leafy plants such as lettuce and spinach (Bingham et al., 1975; 1976; Dowdy, 1975; McIntyre et al., 1977; Davis & Carlton-Smith, 1980; Bunch, 1982b; Chumbley & Unwin, 1982). In addition, carrots (Le Riche, 1968; Symeonides & McRae, 1977), tomatoes (Thomas et al., 1972; Dowdy & Larson, 1975) and radishes (Doyle et al., 1978; Chumbley & Unwin, 1982) appear to accumulate several metals to a significant degree. However, the edible portions of pea and bean plants concentrate metals to only a limited extent compared to the vines and leaves (Dowdy & Larson, 1975; Bradford et al., 1975; Hinesly et al., 1977), while cereals generally do not accumulate metals to a significant degree in the grain (Kirkham, 1975; Sabey & Hart, 1975; Chumbley & Unwin, 1982; Vlamis et al., 1985). It has generally been found that Cd and Zn are accumulated by plants to a greater extent than Cu and Pb (Sterritt & Lester, 1980a). The behaviour of individual metals in plant tissue after uptake has occurred may also vary, since while Cd and Zn appear to be easily absorbed by roots and subsequently translocated to the aerial parts of plants, Cu and Pb are largely retained in the roots and Ni is generally distributed throughout the plant (Leeper, 1972; Council for Agricultural Science & Technology, 1976).

Contamination of crops to phytotoxic levels, which may reduce yields and cause economic loss, represents one of the potential hazards associated with the application of metal-contaminated sludges to land (Webber, 1972; Cunningham et al., 1975; Sterritt & Lester, 1980a). Of more concern, however, are the metals accumulated from sludge-amended soils into the edible parts of crops at concentrations less than those considered to be phytotoxic since these may have a direct effect on man or an indirect effect whereby they are first accumulated in grazing animals (Matthews, 1984). Additionally, metals which are leached from sludge-amended soils may be mobilised into surface water or groundwater, presenting a potential risk to human health where these waters are used as sources for potable supply (Lester et al., 1983). Although there is much evidence to suggest that no significant movement of heavy metals occurs from the sludge-amended soil layer into the soil profile (Boswell, 1975; Emmerich et al., 1982a; Kuo et al., 1983; Miller et al., 1983), movement of heavy metals may occur through open soil channels or fissures where the soil has had no opportunity to attenuate them (Dowdy & Volk, 1983).

It is evident that heavy metal contaminated sewage sludges applied to land may have potentially adverse effects on public health and the environment. A knowledge of the physicochemical forms in which metals occur in these matrices and of the controlling factors which maintain them as such is necessary in order to be able to predict and thus limit such effects.

2. OBJECTIVES

In view of the importance of metal speciation in assessing the fate of sewage sludge-derived heavy metals in the environment, the work reported here aimed to more fully characterise five metals of major concern, namely Cd, Cu, Ni, Pb and Zn, within these complex matrices using a variety of physical, chemical and electrochemical techniques.

More specifically, the current investigation was undertaken to identify and quantify the different chemical forms in which these metals occur in sewage sludges through the use of a modified version of the sequential extraction procedure proposed by Stover _et_ _al_. (1976). The influence of sludge type on metal distribution was evaluated by comparing the fractionation profiles obtained in both liquid and dried forms of raw, activated and digested sludges originating from major UK sewage treatment works. Application of a modified version of the technique developed by Cottenie (1981) to the same sludge samples was also undertaken in order to make a quantitative determination of metal solubilisation under conditions of increasing acidity. In an attempt to elucidate the nature of metal species affected by such pH changes, residues from the progressive acidification treatment were subjected to the sequential extraction technique.

Sequential chemical extraction was further employed to investigate the transitions in metal form which occur following anaerobic digestion of raw sludge samples in laboratory-scale digesters. The influence of sludge type on metal distribution during anaerobic digestion was evaluated by comparison of the fractionation profiles obtained for both primary and mixed primary (ie. co-settled primary and surplus activated) sludges. In addition, the hypothesis that total metal concentration may be an important parameter influencing metal distribution was investigated through comparison of the fractionation patterns observed as total concentrations of Cd, Cu, Ni, Pb and Zn within the anaerobic digesters were steadily increased.

Evaluation of the influence of both particle size and concentration on heavy metal distributions in raw, activated and digested sludges was undertaken using membrane filtration. Further utilisation of this technique in combination with ion-selective electrode potentiometry and metal titrations aimed to assess the effects of particle size on metal-sludge complexation characteristics.

3. MATERIALS AND METHODS

3.1 Collection, Preparation and Storage of Sludge Samples

3.1.1 Samples for chemical extraction

Sludge samples were collected from five different sewage treatment works within the UK. Raw sludges were collected from Beckton (Thames Water Authority) and Shieldhall (Strathclyde Water Authority), activated from Beckton and Hogsmill (Thames Water Authority) and digested from Beckton, Davyhulme (North West Water Authority) and Perry Oaks (Thames Water Authority). These liquid sludge samples were stored in 10 l polypropylene containers at 4°C. A portion of each sludge was dried at 105°C, ground to pass a 0.5 mm sieve and stored over silica gel.

3.1.2 Samples for particle size filtration

Samples of mixed primary raw, activated and digested sludges were collected from Hogsmill Valley Sewage Treatment Works (Thames Water Authority). The pH and electrical conductivity of each sludge was measured, then each sludge was strained through a 6 mm mesh, to remove large solid matter, and stored in polypropylene containers at 4°C for a maximum of 21 d prior to experimentation.

3.2 Analytical Techniques

3.2.1 Chemical extraction techniques

3.2.1.1 Preconcentration of liquid sludge samples

Preparation of the liquid sludge samples for extraction necessitated obtaining sludge pellets of a specific dry weight for comparability with the dried samples and to maintain the solids:reagent volume ratio. The original 10 l sludge samples were shaken manually for 5 min before sampling, in addition to which the raw sludges were strained through a 6 mm mesh. Volumes of liquid sludges calculated to contain a specific dry weight were centrifuged in a bench centrifuge (Gallenkamp Ltd., UK) in polytetrafluoroethylene (PTFE) bottles (Techmate Ltd., UK) at 2500 xg for 30 min and sufficient supernatant was removed to leave a final known volume. The sludge pellets were resuspended and the samples homogenised by mechanical shaking for 2 h. Volumes of the sludge suspension containing the required solids were then pipetted directly into the centrifuge tubes to be used for the extractions and into Ni crucibles for final determinations of total solids. The samples were subsequently centrifuged at 2500 xg for 30 min, the supernatants being retained and acidified to 1% (v/v) HNO_3 prior to heavy metal analysis while the sludge pellets were subjected to extraction.

3.2.1.2 Sequential extraction

Fractionation of the metals Cd, Cu, Ni, Pb and Zn present in each sludge sample was undertaken using a modified version of the sequential extraction procedure described by Stover et al. (1976), as outlined in Table 9.

Table 9 Conditions for sequential chemical extraction

Designated form extracted	Reagent (AR Grade)	Extraction time (h)	Solids:reagent volume ratio
Soluble/ Exchangeable	1.0 M KNO_3	16	1:50
Adsorbed	0.5 M KF (pH 6.5)	16	1:80
Organically bound	0.1 M $Na_4P_2O_7$	16	1:80
Carbonate	0.1 M EDTA (pH 6.5)	16	1:80
Sulphide	6.0 M HNO_3	16	1:50

Modifications to the original technique included the use of a single 16 h EDTA extraction as opposed to a double 8 h extraction and the replacement of 1.0 M HNO_3 with 6.0 M HNO_3 (Oake et al., 1984), in addition to centrifugation at 2500 xg, which was found to be as effective in solids separation as the 10000 xg used by Stover et al. (1976).

Samples of 0.4 g were extracted by shaking with the appropriate volume of reagent in 50 ml polypropylene centrifuge tubes (MSE Ltd., UK) for 16 h on a reciprocating bench shaker (Gallenkamp Ltd.) set at

60 oscillations per min, followed by centrifugation at 2500 xg for 30 min in a bench centrifuge (Gallenkamp Ltd.). The supernatants were carefully removed and filtered through GF/D (2.7 µm) filters (Whatman Ltd., UK) using a vacuum filtration unit (Amicon Ltd., UK). The filters were pretreated by soaking in 1% (v/v) HNO_3 for 24 h (Laxen & Chandler, 1982). The filtrates were acidified to 1% (v/v) HNO_3 prior to analysis by atomic absorption spectrophotometry, while the remaining sludge pellets were resuspended in 25 ml deionised distilled water and shaken manually prior to centrifugation and addition of the next reagent. This procedure was followed for the sequence of reagents presented in Table 9 and on triplicate samples of each sludge. Metal remaining in the sludge pellets following the 6.0 M HNO_3 extraction was designated as the 'residual' fraction.

3.2.1.3 Progressive acidification

Potentially soluble metal forms were assessed by progressive acidification of sludge samples, based on the method of Cottenie (1981). Samples of 0.5 g of dried sludge were suspended in 15 ml distilled water. Liquid sludges were preconcentrated to give comparable solids concentrations, as previously described (Section 3.2.1.1), total solids determinations being made on triplicate aliquots of each to ascertain the final solids content. Both sample types were prepared directly in 50 ml polypropylene centrifuge tubes (MSE Ltd), to obviate solids loss on transfer, and were stirred by means of magnetic followers for 15 min prior to experimentation to assure complete mixing. Sets of triplicate samples of each sludge type were prepared at either ambient pH or pH 4.0, 2.0 or 0.5. The samples were stirred continuously for a period of 30 min during which time the pH was monitored and adjustments to maintain the pH at 4.0, 2.0 or 0.5 were made by controlled addition of 6.0 M, 4.0 M, 2.0 M or 0.5 M 'Analar' HNO_3 and 6.0 M or 1.0 M 'Analar' KOH. No adjustments were made to the control sets of samples at ambient pH. After 30 min the electrode and magnetic follower were removed from the sample and any adhering solids were rinsed back into the suspension with distilled water. The samples were then made up to 25 ml with distilled water and centrifuged at 3000 xg for 15 min. Supernatants were filtered through GF/D filters (Whatman Ltd.) as in section

3.2.1.2, and the final pH of the filtrates measured prior to acidification to 1% (v/v) HNO_3 and analysis by atomic absorption spectrophotometry.

Each set of triplicate residual pellets remaining from the original dried sludges were dried at 30°C, ground to pass a 0.5 mm sieve and stored over silica gel. Triplicate samples of 0.3 g were then subjected to the sequential extraction procedure described in section 3.2.1.2, in an attempt to elucidate the nature of the metal species extracted by progressive acidification.

3.2.2 Particle size filtration

To study the effect of particle size on the heavy metal binding characteristics of raw, activated and digested sludge solids it was necessary to firstly pass each sludge through a range of filter pore sizes. A microporous cellulosic filter of 0.22 μm pore size (Amicon Ltd.) was used in conjunction with a 3-piece glass filtration holder (Gallenkamp Ltd.), while nylon meshes of pore sizes 10, 100 and 1000 μm (Henry Simon Ltd., UK) were used in conjunction with 3-piece Hartley funnels (Gallenkamp Ltd.). All filters and meshes were pretreated as described previously (Section 3.2.1.2). This range of pore sizes was selected in order to obtain a solids distribution ranging from a simple solubility distinction to inclusion of virtually all particulate matter. The soluble phase was taken to be the filtrate from the 0.22 μm filter; such a pore size has been used previously to separate the soluble fraction (Rossin et al., 1982)

Raw and digested sludges were diluted by 1 in 25 prior to filtration in order to obtain sufficient filtrate sample volume for ISE determinations and to prevent changes in the effective pore size of the filters due to clogging. Dilution was achieved using 0.1 M 'Aristar' KNO_3, so as to maintain an ionic strength equivalent to that of the original sludge samples. The pH value of the diluted samples was adjusted to that of the original undiluted sludges using 'Aristar' KOH and HNO_3.

Each sludge sample was agitated to form a homogeneous suspension and aliquots of sufficient volume to produce 100 ml of filtrate were applied to single 1000 and 100 µm meshes. In order to produce 100 ml of 10 and 0.22 µm filtrates, however, several filter changes were necessary since clogging of these filters occurred even when diluted sludge samples were applied and further dilution of the samples would have provided insufficient solids concentrations for measurement of complexation characteristics in these filtrates. Although the degree of vacuum has been reported not to affect the retention characteristics of the filters (Sheldon, 1972), care was taken in using the minimum vacuum pressure necessary to avoid disrupting particles (Bately & Gardner, 1977). The filtrates from each filter or mesh were stored in polypropylene beakers at 4°C for a maximum of 4 d prior to complexation titration and ISE determinations.

A separate set of filtrations was undertaken on each sludge type to determine the total solids concentration and Cd, Cu and Pb concentrations of each filtrate. The activated sludge was filtered undiluted while the raw and digested sludges were diluted by 1 in 2.5 using 0.1 M KNO_3. Sufficient sample was applied to each filter to produce 15 ml of filtrate; 5 ml of which was used for total solids determination, the remaining 10 ml being acidified to 1% (v/v) HNO_3 and sealed prior to heavy metal determinations. An identical procedure was followed using only 0.1 M KNO_3 or deionised water in the production of blank filtered samples. Blank values indicated that filter contaminants contributed <1% of the concentration of Cd, Cu and Pb present in the sample filtrates. Verification that filter adsorption was negligible was undertaken using standard unacidified metal solutions. Sludge samples were also analysed for total solids and heavy metal concentrations.

3.2.3 Ion-selective electrode determinations

3.2.3.1 Apparatus

Free Cd^{2+}, Cu^{2+} and Pb^{2+} concentrations were determined using Orion model 94-48, 94-29 and 94-82 electrodes respectively in conjunction with an Orion model 90-02 double-junction Ag/AgCl reference electrode (MSE Ltd.). Internal and outer filling solutions of the reference electrode were as recommended by the electrode manufacturer (Anon., 1980). Electrode response was measured with an Orion 701A digital pH/millivolt meter (MSE Ltd.), and an identical meter was used for pH measurements (Section 3.2.6.2). All ISE determinations were conducted in polypropylene containers to minimise adsorption errors. Samples and standards were stirred magnetically at a constant rate using a PTFE-coated follower and were maintained at a constant temperature of 25 ± 1°C using a thermostatically-controlled water-bath (Gallenkamp Ltd.). Constant lighting by fluorescent means was maintained by exclusion of sunlight. The attainment of equilibrium potential values was monitored by means of a Servoscribe IS chart recorder (Labdata Instrument Service, UK).

3.2.3.2 Electrode pretreatment

Daily pretreatment of the ISEs comprised gentle buffing of the shiny surface of the pellet for 30s with Orion 948201 polishing strips (MSE Ltd.), for improved electrode response, followed by vigorous rinsing with deionised distilled water. The electrodes were subsequently cleaned in 0.025 M H_2SO_4 for 10 min (Blaedel & Dinwiddie, 1975; Avdeef et al., 1983) and again rinsed and soaked in deionised water prior to use. Pretreatment of the reference electrode involved replacement of inner and outer filling solutions on a weekly basis, according to the manufacturer's instructions (Anon., 1980). When not in use, the electrodes were stored in deionised water.

3.2.3.3 Electrode calibration

The Cu ISE was calibrated in the range of 10^{-12} to 10^{-3} M Cu^{2+} while the Cd and Pb ISEs were calibrated in the range of 10^{-10} to 10^{-3} M Cd^{2+} and Pb^{2+} respectively. Calibration in the range of 10^{-7} to 10^{-3} M M^{2+} was performed using unacidified metal nitrate solutions prepared by serial dilution of more concentrated stock solutions using deionised distilled water. The pH value of each standard solution was adjusted to and maintained at that of the samples by controlled additions of 'Aristar' HNO_3 and KOH, while ionic strength values were adjusted to those of the samples using 'Aristar' KNO_3.

Extension of a linear Nernstian calibration to below 10^{-7} M M^{2+}

was achieved using metal-ion buffered standards, which provide a controlled source of free metal ions in a manner similar to the regulation of hydrogen ion concentration by pH buffers (Blum & Fog, 1972; Hansen et al., 1972; Perrin & Dempsey, 1974). Regulation of the free metal ion concentration is based on the principles of conditional stability constants (Ringbom, 1963) and side-reaction coefficients (Schwarzenbach, 1957). According to Perrin & Dempsey (1974), at constant ionic strength, the free metal ion concentration, [M], in a solution of metal ion, M, and a ligand, L, forming the 1:1 complex ML can be expressed at equilibrium by the equation:

$$[M] = \frac{[ML]}{\frac{K_{ML}}{\alpha_L} \cdot [L]} \qquad (2)$$

where K_{ML} is the stability constant of the ML complex at the ionic strength used; [L] is the total concentration of uncomplexed ligand; [ML] is the total concentration of the complex formed; and α_L denotes the side-reaction coefficient of the ligand, this term being calculated according to the equation:

$$\alpha_L = 1 + 10^{(pK_1 - pH)} + 10^{(pK_1 + pK_2 - 2pH)} + \ldots \qquad (3)$$

Hence, by maintaining the metal and ligand concentrations at a fixed ratio, the free metal ion concentration may be varied by altering the pH value, since the α_L term is essentially a function of pH.

Buffered standards were therefore prepared by mixing 0.001 M unacidified Cd, Cu or Pb nitrate solutions with 0.01 M of the 'Gold Label' tri-sodium salt of nitrilotriacetic acid (NTA) (Aldrich Chemical Company, UK); the pH value of each standard being adjusted to give the free metal ion concentrations required for calibration, according to equation (2). Stability constant and pKa values used were those of Ringbom (1979), with the exception of the K_{CuNTA} value, which was taken from Sillen & Martell (1971). Adjustment to and maintenance of appropriate pH values and adjustment of ionic strength values to those of the samples was according to the procedures adopted in the preparation of unbuffered standards.

Buffered and unbuffered standards were prepared freshly each day, in 100 ml volumes. Calibration was generally undertaken using four standards over the sample concentration range to be measured, recalibration being performed every 4 h. The logarithmic response of the electrodes was taken into account when plotting analytical response against free metal ion concentration. Between measurements, the electrodes were vigorously rinsed using deionised distilled water.

3.2.4 Determination of complexation parameters

A quantitative assessment of metal-sludge complexation characteristics may be obtained by the determination of the conditional stability constant for the formation of the complex and the concentration of metal binding sites (effectively the complexation capacity) in a given sample.

Heavy metal complexation may be represented by the equilibrium relationship:

$$M + xL \rightleftharpoons ML_x \qquad (4)$$

and the stability constant, K, is given by

$$K = \frac{[ML_x]}{[M]\,[L]^x} \qquad (5)$$

where [M] and [L] are the equilibrium concentrations of the unreacted metal and ligand respectively, [ML] is the equilibrium concentration of the metal-ligand complex and x is the stoichiometric ratio. If a ligand is titrated with increasing amounts of metal using a technique for the differentiation of the free metal, $[M_F]$ (equivalent to [M] in equation (5)), from the bound or complexed metal, $[M_B]$ (equivalent to [ML] in equation (5)), then, for the important case of 1:1 stoichiometry of the complex, values of the conditional stability constant, K', and the total ligand concentration, [L'], may be obtained from the graphical solution of the linear relationship derived by Ruzic (1982):

$$\frac{[M_F]}{[M_B]} = \frac{[M_F]}{[L']} + \frac{1}{K'[L']} \qquad (6)$$

Conditional stability constants and complexation capacities for complexation of Cd, Cu and Pb by sludge ligands were determined experimentally by titrating 100 ml volumes of the filtered and unfiltered samples prepared in section 3.2.2 with increasing concentrations of each of these metals and using ISEs to differentiate between free and bound metal concentrations at each total concentration. Small additions of unacidified 10^{-3}, 10^{-2}, 10^{-1} and 1.0 M metal nitrate solutions were made to the samples using Eppendorf pipettes (Anderman & Co. Ltd., UK) to give total metal concentrations ranging from 10^{-7} to 10^{-2} M. Since filtration induced increased pH values, the filtrate samples were adjusted to and maintained at the pH values of the original undiluted sludge samples using the method described in section 3.2.2. After each metal addition, the samples were allowed to equilibrate; the electrode potential being recorded when the average change in potential was <0.2 mV min^{-1}.

3.2.5 Determination of heavy metals

3.2.5.1 Instrumentation

Heavy metals were determined by atomic absorption spectrophotometry using Perkin-Elmer models 603 and 5000 atomic absorption spectrophotometers (Perkin-Elmer Ltd., UK) fitted with deuterium arc background correction. Flame atomic absorption was undertaken with a single slot air-acetylene burner and flameless atomic absorption was undertaken using Perkin-Elmer HGA 400 and HGA 500 heated graphite atomisers in conjunction with the models 603 and 5000 respectively. Perkin-Elmer single element hollow cathode lamps were used for all five metals studied and absorption signals were measured on Perkin-Elmer model 56 chart records.

3.2.5.2 Glassware

All glassware used was of grade A borosilicate glass. Cleaning involved soaking in a 5% (v/v) solution of 'Decon 90' detergent (BDH chemicals Ltd., UK) for 24 h followed by leaching in 10% (v/v) 'Analar' HNO_3 for 24 h. Thorough rinsing with distilled water was performed after each of these steps. Polypropylene and PTFE containers and apparatus were similarly cleaned.

3.2.5.3 Reagents

All reagents used were of 'Analar' grade, with the exceptions of HNO_3 (S.G. 1.42) and H_2O_2 (100 volumes) used in sample pretreatment methods (Section 3.2.5.5), which were of 'Aristar' quality (BDH Chemicals Ltd.).

3.2.5.4 Standard solutions

Stock solutions of 1 gl^{-1} were prepared from nitrate salts of Cd, Cu, Ni, Pb and Zn. All were stored in 1% (v/v) HNO_3. Working standards were generally prepared by serial dilutions of stock solutions to contain final concentrations of 1% (v/v) HNO_3. However, all standards used for metal determinations in the sequentially extracted fractions (Section 3.2.1.2) were made up in the respective reagents to compensate for any reagent interference effects. Similarly, standards used for metal determinations of filtrate samples obtained from particle sizing filtration (Section 3.2.2) were prepared in 0.1 M KNO_3.

3.2.5.5 Sample pretreatment methods

Pretreatment of sludge samples for determination of total and residual metal concentrations involved either homogenisation or digestion.

3.2.5.5.1 Homogenisation

Preparation of sludges for total metal determinations comprised homogenisation with an Ultra-Turrax Model T45N (Scientific Instruments Co., UK) fitted with a titanium shaft (Stoveland *et al.*, 1978). Liquid sludge samples diluted to 10% (v/v) and dried samples diluted to 0.5% (w/v) with 1% HNO_3 were homogenised in 100 ml volumes at 8000 rev min^{-1} for 10 min. This procedure was followed on triplicate subsamples, blank samples being included.

Sludge pellets remaining after the 6.0 M HNO_3 extractions (Section 3.2.1.2) were similarly homogenised in 25 ml of 1% (v/v) HNO_3 prior to analysis of residual metal content.

3.2.5.5.2 HNO_3-H_2O_2 digestion

A modification of the HNO_3-H_2O_2 method proposed by Krishnamurty *et al.* (1976) was utilised in the digestion of sludge samples; preliminary oxidation with HNO_3 ensuring a controlled reaction. All digestions were performed in 100 ml beakers, fitted with watch-glasses, on a thermostatically controlled hot-plate set at approximately 100°C. Preparation of samples for total metal determinations initially comprised the addition of 20 ml of concentrated HNO_3 to either 10 ml of a liquid or 0.5 g of a dried sludge sample, while the same volume of HNO_3 was also added to the residual sludge pellets. These mixtures were evaporated to near dryness and, after cooling, a further 10 ml of concentrated HNO_3 was added. Successive additions of 10 ml of HNO_3 were made to ensure preliminary oxidation of the more reactive organic matter before adding H_2O_2. Digestion was then continued with repeated additions of 5 ml of H_2O_2 and 5 ml of concentrated HNO_3, evaporating and cooling between each addition, until copious white fumes were evolved and the digestate was a pale straw colour.

After cooling, the digestate and washings resulting from rinsing of the beaker walls and watch-glasses with 1% (v/v) HNO_3 were transferred to a 50 ml volumetric flask and subsequently made up to volume with 1% (v/v) HNO_3.

Digestions were undertaken on at least three replicate samples; blanks being included in each batch of digestions.

3.2.5.6 Analytical procedures

Flame atomic absorption spectrophotometry was used where the concentrations of the metals of interest were above the limits of detection. Otherwise, flameless atomic absorption, which offers improved sensitivity for metals present at low concentrations (Sterritt & Lester, 1980b), was employed.

The operating conditions utilised for flame and flameless atomic absorption were respectively based on those suggested by Perkin-Elmer (1982) and those described by Sterritt & Lester (1980b), summaries of which are presented in Table 10.

For flameless atomic absorption spectrophotometry, both samples and standards were injected into the graphite furnace using Eppendorf pipettes (Anderman & Co Ltd.) fitted with polypropylene 'Standartips' (Anderman & Co Ltd.). The tips were cleaned between injections by rinsing three times in both 10% (v/v) HNO_3 and distilled water and were replaced frequently. Samples and standards were injected in duplicate or until a reproducible signal was obtained. Initially, a method of standard additions was used and compared with the direct calibration method; where appropriate the latter method was adopted for simplicity and speed. Calibration was performed using four standards extending over the sample concentration range to be measured and was normally checked after approximately twenty injections, recalibration being performed if necessary. However, when deterioration of the graphite tubes was rapid, as in the analysis of samples containing aggressive reagents such as KNO_3, KF and 6.0 M HNO_3, calibration was checked more frequently. For samples with high heavy metal concentrations, appropriate dilutions were performed using 1% (v/v) HNO_3 in order to adjust the determinand concentration to within the linear working range.

3.2.6 Routine analyses

3.2.6.1 Total and volatile solids

Total and volatile solids concentrations of sludge samples were determined using standard recommended methods (Department of the Environment, 1972).

3.2.6.2 pH value

An Orion model 701A pH meter fitted with a glass combination

Table 10 Operating conditions for flame and flameless atomic absorption spectrophotometric determination of heavy metals (modified from Perkin-Elmer (1982) and Sterritt & Lester (1980b) respectively)

Metal	Wavelength (nm)	Spectral Band Width (nm)	Flameless AAS Temperature Programme[2] Drying (°C)[3]	Ashing (°C)[3]	Atomising (°C)[4]	Working Linear Range[5] (mgl^{-1})	Flame AAS Working Linear Range (mgl^{-1})
Cd	228.8	0.7	120	250	2100	0.002 - 0.02	0.1 - 2.0
Cu	324.8	0.7	120	800	2700	0.02 - 0.20	0.2 - 5.0
Ni	232.0	0.2	120	900	2700	0.05 - 0.40	0.2 - 2.0
Pb	283.3	0.7	120	350	2300	0.01 - 0.20	0.5 - 20
Zn	307.6	0.7	120	450	2500	0.05 - 1.00	
	213.9[1]	0.7					0.2 - 1.0

[1] High sensitivity wavelength used for determination of Zn by flame atomic absorption spectrophotometry (AAS)

[2] Uncoated graphite tubes used for all metals

[3] Hold times of 30 to 60s used for both drying and ashing steps, dependent on whether 20 or 50 µl sample volumes injected. All programmes used a ramp time of 10s.

[4] Atomising programmes all used a hold time of 5s, except Cu with 4s, and a ramp time of 1s.

[5] Working range for 50 µl injection; limits of useful working range approximately twice these values for 20 µl injections.

electrode (MSE Ltd) was used for pH determinations. Before each pH measurement, the electrode and meter were calibrated against suitable commercial buffer solutions of known pH values (BDH Chemicals Ltd.).

3.2.6.3 Electrical conductivity

A PTl-20 digital water analyser with automatic temperature control (Chemlab Ltd., UK) was used for conductivity measurements. Calibration was performed using standard KNO_3 solutions.

3.2.6.4 Alkalinity

Phenolphthalein and methyl red (total) alkalinity of sludge samples removed from the laboratory–scale anaerobic digesters were determined by titration with HCl according to the recommended method (Department of the Environment, 1981). Sample pretreatment involved centrifugation for 30 min at 27000 xg and 15°C using a Hi-Spin 21 centrifuge (MSE Ltd.) followed by vacuum filtration through GF/C (1.2 μm pore size) filters (Whatman Ltd.); this being necessary for the removal of any turbidity, which may interfere with the visual detection of the titration end-point.

3.2.6.5 Volatile acids

Volatile acid concentrations of sludge samples removed from the digesters were determined spectrophotometrically according to the standard method (Department of the Environment, 1979). Sample pretreatment involved centrifugation and filtration as in section 3.2.6.4.

3.2.6.6 Digester gas composition

A modified thermal conductivity Gas Chromatograph (Gallenkamp Ltd.) containing a column packed with Molecular Sieve 5A (Hewlett-Packard, UK) and equipped with a Servoscribe IS chart recorder (Labdata Instrument Service) was used to determine methane concentrations in the digester gas. The chromatograph was calibrated using pure methane (BOC Special Gases Ltd., UK).

3.3 Anaerobic Digester Simulation

3.3.1 Digester design

The laboratory-scale anaerobic digestion simulation used was essentially that described previously by Kirk et al. (1982). All parts of the apparatus (Figure 4) in contact with the sludge were constructed of borosilicate glass or PTFE. In this description Quickfit (Corning Ltd., UK) reference numbers for each component are appended in brackets.

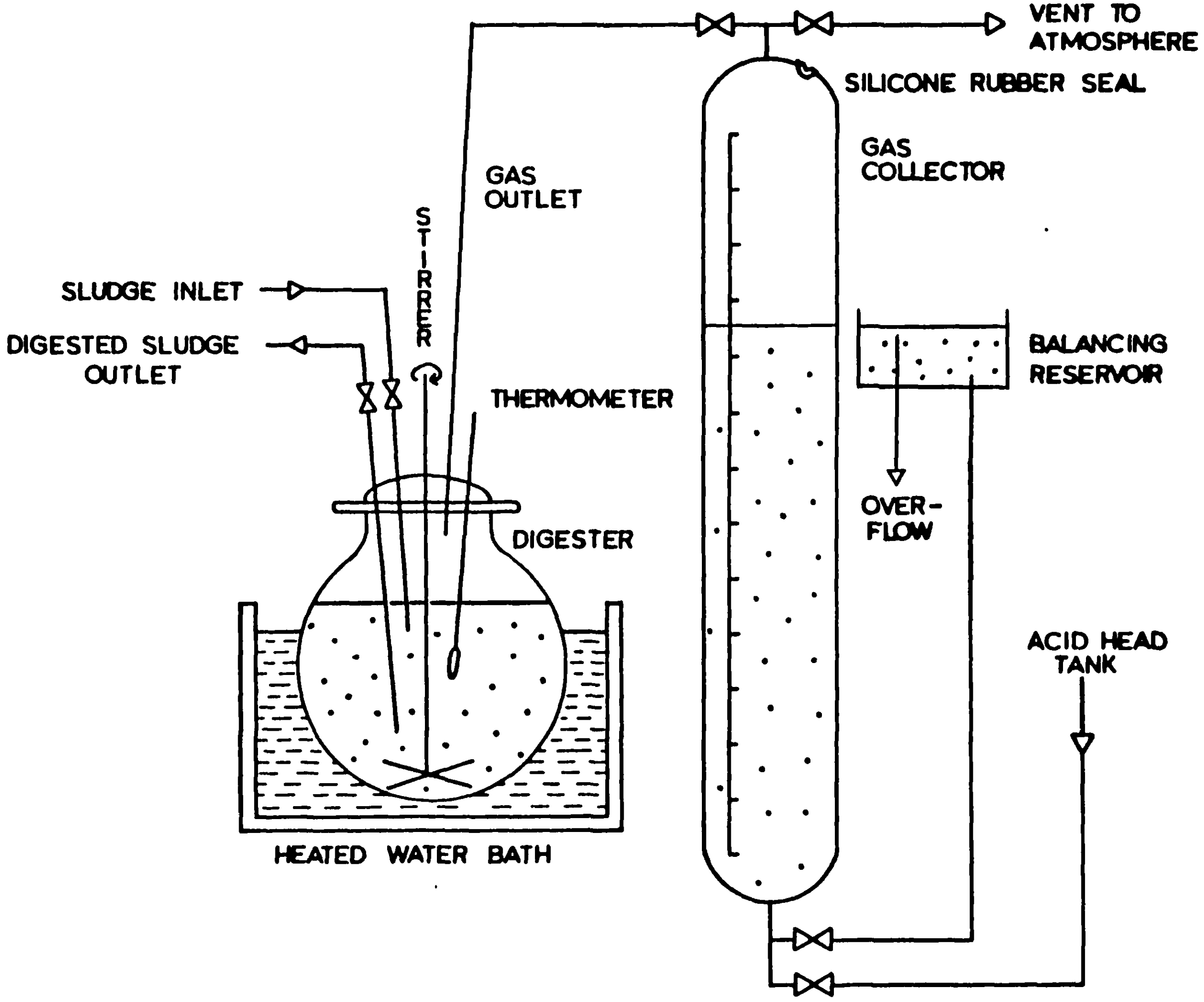
A wide neck reaction vessel (FR2LF) was fitted with a five socket flat flange lid (MAF 2/2), silicone grease being applied to the ground glass joint prior to clamping the two securely together (JC 100F). A PTFE stirrer (ST 7/3) and borosilicate shaft (ST 6/7) were located through a ground sleeve gland (ST 20/2) in the centre neck of the lid using 'Analar' glycerol as lubricant. Sludge inlet and outlet lines, gas outlet and a thermometer were connected through the remaining sockets, each via a screwcap gland (ST5) which included a PTFE seal. Sludge inlets and outlets were attached to short lengths of silicone rubber tubing, clamped near their base during normal operation. Gas was collected by the displacement of water which had been acidified to less than pH 4 to prevent CO_2 absorption (Department of the Environment, 1978), and was measured at atmospheric pressure by the use of a balancing reservoir.

An assembly of four digesters was clamped in a water bath maintained at 35°C; the stirrer shafts connected via flexible couplings to electric motors timed to operate for 15 min in each hour at approximately 100 rev min^{-1}. This prevented the build-up of solids at the base of the digesters and maintained uniform mixing necessary for efficient digestion. Digested sludge was removed under vacuum and fresh sludge was introduced under nitrogen (oxygen free) pressure. Gas samples were taken through a silicone rubber septum attached to the apex of each gas collector.

3.3.2 Feed sludge

Two of the digesters were initially seeded with 1.0 l of freshly digested primary sludge and 0.5 l of raw primary sludge collected from Dartford Sewage Treatment Works (Thames Water Authority) while the remaining two were seeded with the same ratio of freshly digested mixed primary to raw mixed primary sludge collected

Figure 4 Schematic diagram of an anaerobic digester simulation



from Hogsmill Valley Sewage Treatment Works (Thames Water Authority). Sewage sludges from both locations were of mixed industrial domestic origin. The raw mixed primary sludge contained 25% (w/w) surplus activated sludge, in accordance with normal works practice. Subsequent additions to the digesters consisted of raw primary or mixed primary sludges, as appropriate, diluted from indigenous total solids concentrations of 6.4% (w/v) and 5.1% (w/v) respectively to 4.0% (w/v) with distilled water. Additional pretreatment of the raw feed sludges comprised straining through a 6 mm mesh for the removal of fibrous material, homogenisation for 10 min using a T45N Ultra-Turrax disintegrator (Scientific Instruments Co. Ltd.) fitted with a titanium shaft and storage in polypropylene containers at -10°C until required. An appropriate volume of sludge was equilibrated at 35°C immediately prior to feeding.

3.3.3 Operational control

The digesters were operated with a hydraulic retention time of 20 d. Digested sludge (150 ml) was removed every 48 h, following stirring for 30 min, and raw primary or mixed primary sludge was introduced to maintain a total digester volume of 1.5 l. Each digester was operated for a period of three retention times (60 d) prior to metal addition to allow for stabilisation. The establishment of stable digestion was assessed through routine monitoring of total and volatile solids, total alkalinity, pH, volatile acids, percentage methane content of the digester gas (section 3.2.6) and total gas production.

3.3.4 Addition of heavy metals

One digester of each sludge type was designated as 'control' to which no metal feed was dosed while the remaining two digesters were designated 'experimental'.

Total concentrations of the metals Cd, Cu, Ni, Pb and Zn within the 'experimental' digesters were gradually increased from baseline values to concentrations considered to be typical of more contaminated mixed industrial domestic sewage sludges within the UK (Sterritt & Lester, 1981), but below those reported to be inhibitory to the anaerobic digestion process (Hayes & Theis, 1978; Anthony & Breimhurst, 1981). This was achieved by addition of 1.0 ml of an acidified metal solution containing 135, 1125, 285, 2910 and 5700 mgl^{-1} of Cd, Cu, Ni, Pb and Zn nitrates respectively to four feed aliquots followed by cumulative additions of 0.5 ml every four successive feeds, until concentrations of approximately 2.0 mgl^{-1} Cd, 30.0 mgl^{-1} Cu, 5.0 mgl^{-1} Ni, 50 mgl^{-1} Pb and 100 mgl^{-1} Zn were obtained. At these concentrations, the maximum total metal load of each sludge (K), as calculated using the formula developed by Mosey (1976) (Appendix 1), would be approximately 175 meq kg^{-1}, assuming a digested sludge solids concentration of 0.025 kgl^{-1} and a pH value between 6.9 and 7.3. This figure is significantly less than the K-value of 400 meq kg^{-1} indicative of potential digester failure (Mosey, 1976). Thus, the maximum metal concentrations selected would not be expected to inhibit digestion either individually or on an additive basis.

3.3.5 Sampling

Sludge samples required for monitoring of digester performance, in terms of total and volatile solids, alkalinity and volatile acids content in addition to pH value, were removed from each digester at intervals of 2 d during the initial stabilisation period but every 4 d following the initiation of heavy metal addition. Gas samples were taken over identical time periods for analysis of the percentage methane content. Raw influent sludges were sampled intermittently to monitor total and volatile solids concentrations. Samples for total and fractionated metal determinations were taken every 4 d from 'experimental' digesters during the period of metal addition and every 8 d from the 'control' digesters, but on feed days which alternated with those selected for routine monitoring. These samples, in addition to raw sludge aliquots, were dried at 30°C, ground to pass a 0.5 mm sieve and stored over silica gel for up to 14 d prior to total (section 3.2.5) and fractionated (section 3.2.1.2) metal determinations.

4. RESULTS

4.1 Characterisation of Heavy Metals in Sewage Sludges Using Chemical Extraction Techniques

4.1.1 Properties of the sewage sludge samples

The seven sewage sludges selected for investigation were considered to be representative of the major types produced in the UK. The total and volatile solids concentrations together with the Cd, Cu, Ni, Pb and Zn contents of each of these sludges are presented in Table 11. The total metal concentrations given represent means of the values determined in triplicate samples of both the liquid and dried forms of each sludge using digestion followed by atomic absorption spectrophotometry (section 3.2.5). Total concentrations of each metal varied widely between sludges, reflecting primarily the nature of the influent received by the respective sewage treatment works.

4.1.2 Fractionation of metals in sewage sludges by sequential chemical extraction

An assessment of the distribution of Cd, Cu, Ni, Pb and Zn among various chemical forms in the raw, activated and digested sludges, which were either maintained in the original liquid state or dried, was made through utilisation of a sequential chemical extraction procedure (section 3.2.1.2).

4.1.2.1 Investigation of the sequential chemical extraction technique

Preliminary investigation of the sequential extraction technique originally devised by Stover *et al*. (1976) was undertaken using sludge sample D7, in its dried state. Five replicates of each extraction were conducted in order to validate statistical analysis.

4.1.2.1.1 The effect of centrifugation speed on metal extraction

A comparison of low and high speed centrifugation was incorporated into a preliminary application of the sequential extraction technique to sludge sample D7 in order to determine whether possible solids loss suffered during low speed centrifugation affected metal recovery. Following each extraction step, samples were centrifuged at both 1000 xg and 27000 xg (the maximum speed recommended for the

Table 11 Solids and heavy metal contents of the sewage sludge samples

Source	Sludge Type	Designation	Total solids (% w/v)	Volatile solids (% w/w)	Total metal concentration (mgkg^{-1} dry solids)				
					Cd	Cu	Ni	Pb	Zn
Beckton (TWA)	Raw	R1	3.10	67.6	17.3	416	61	431	2460
Shieldhall (Strathclyde)	Raw	R2	1.70	57.4	6.36	806	51.8	362	963
Beckton (TWA)	Activated	A3	1.50	74.8	22.0	593	98.6	385	2370
Hogsmill (TWA)	Activated	A4	0.60	66.1	3.25	432	36.2	223	510
Beckton (TWA)	Digested	D5	0.80	59.5	24.5	614	93.9	723	2880
Davyhulme (NWWA)	Digested	D6	3.00	57.5	10.6	563	114	941	1430
Perry Oaks (TWA)	Digested (Dewatered)	D7	8.80	61.7	26.3	708	213	527	1300

type of centrifuge tube used) in an MSE Hi Spin centrifuge. Analysis of the filtered supernatants by atomic absorption spectrophotometry (Table 12) indicated that centrifugation speed generally had minimal effect on the concentrations of metal extracted by a particular reagent and on the metal recovered by summation of the five fractions. Therefore, for reasons of practicality and safety, a centrifugal speed of 2500 xg, consistent with that used by Oake et al. (1984), was adopted in preference to the 10000 xg originally used by Stover et al. (1976).

4.1.2.1.2 The effect of HNO_3 concentration on metal extraction

Oake et al. (1984) further modified the technique of Stover et al. (1976) by replacing the 1.0 M HNO_3 used in the extraction of 'sulphide' metal forms with 6.0 M HNO_3. Substitution of the latter reagent for the former in the extraction sequence resulted in 15.7 $mgkg^{-1}$ Cd, 592 $mgkg^{-1}$ Cu, 23.7 $mgkg^{-1}$ Ni, 50.7 $mgkg^{-1}$ Pb and 403 $mgkg^{-1}$ Zn being extracted in the 'sulphide' form from sludge sample D7. A comparison of these values with the concentrations of each metal extracted by 1.0 M HNO_3 from the same sludge (Table 12) indicated that 6.0 M HNO_3 increased the percentage of the total metal extracted by 49.5, 57.9, 2.46, 5.49 and 20.1% for Cd, Cu, Ni, Pb and Zn respectively.

4.1.2.1.3 A comparison of flame and flameless atomic absorption spectrophotometry for analysis of the extracts

Samples produced during the preliminary extraction of sludge D7 were analysed for heavy metals by flameless atomic absorption spectrophotometry. However, the length of time required to complete the analysis combined with the rapid deterioration of the graphite tubes caused by the presence of the extractants (6 M HNO_3, KNO_3 and KF in particular) prompted an evaluation of the use of flame atomic absorption for all or part of the analysis. Samples subjected to centrifugation at 27000 xg were therefore analysed by both methods to assess whether significant interference occurred during flame analysis. While results produced by flameless atomic absorption have been presented in Table 12, those obtained using the flame mode are given in Table 13. Application of a *t*-Test (Bowker & Lieberman, 1972) to

Table 12 Effect of centrifugation speed on the sequential extraction of metals from sludge sample D7

Fraction	Cd		Cu		Ni		Pb		Zn	
	1000xg	27000xg	1000xg	27000xg	1000xg	27000xg	1000xg	27000xg	1000xg	27000xg
KNO_3 ($mgkg^{-1}$)	0	0	19.6	15.6	17.4	18.5	0	0	40.5	65.0
(% RSD)	(0)	(0)	(4.90)	(1.50)	(2.60)	(3.70)	(0)	(0)	(44.9)	(35.0)
KF	0	0	53.0	58.2	24.2	22.8	0	0	80.0	75.0
	(0)	(0)	(1.90)	(5.90)	(3.10)	(5.30)	(0)	(0)	(13.3)	(11.2)
$Na_4P_2O_7$	8.31	8.10	58.0	59.0	48.9	42.5	344	330	825	805
	(2.40)	(1.40)	(2.20)	(4.80)	(14.4)	(7.90)	(3.10)	(4.10)	(1.20)	(0.70)
EDTA	5.62	5.63	136	140	96.5	96.2	190	181	151	141
	(9.20)	(11.6)	(0.80)	(2.00)	(5.90)	(1.30)	(1.70)	(2.10)	(26.4)	(5.60)
HNO_3 (1.0 M)	2.79	2.57	172	192	18.2	18.7	23.1	20.4	141	133
	(4.00)	(5.20)	(2.70)	(4.80)	(3.50)	(4.50)	(15.4)	(9.10)	(5.40)	(8.00)
Sum Total	16.7	16.3	439	465	205	199	557	531	1240	1220
Recovery	63%	62%	62%	66%	96%	93%	106%	101%	95%	94%

the data indicated that there was no significant difference between the two sets of analyses at the 0.05 level of significance. Therefore, where metal concentrations were above the practical limit of determination, flame atomic absorption was used.

Table 13 Metal content of samples produced by sequential extraction of sludge sample D7, as determined by flame atomic absorption spectrophotometry

Fraction	Cd	Cu	Ni	Pb	Zn
KNO_3 ($mgkg^{-1}$)	0	16.4	18.5	0	54.5
(% RSD)	(0)	(5.00)	(1.30)	(0)	(40.3)
KF	0	59.0	24.2	0	74.3
	(0)	(4.80)	(3.00)	(0)	(11.5)
$Na_4P_2O_7$	8.16	64.3	44.5	325	766
	(1.00)	(4.20)	(8.40)	(27.1)	(8.50)
EDTA	5.92	140	97.3	193	143
	(0.70)	(1.65)	(6.00)	(2.90)	(7.00)
HNO_3 (1.0 M)	2.62	201	21.7	21.1	137
	(4.50)	(9.80)	(6.20)	(3.90)	(8.40)

4.1.2.2 Statistical evaluation of the sequential chemical extraction procedure

The reproducibility of the sequential extraction technique applied to the sewage sludge samples was statistically evaluated using fractionation data obtained from five replicate samples of both liquid and dried forms of sludge sample D5 (Table 14). The degree of reproducibility was found to vary greatly between different fractions and was dependent on the concentrations of metals present. Thus, while relative standard deviation (RSD) values of <10% were observed consistently for the $Na_4P_2O_7$, EDTA and HNO_3 fractions, which contained

Table 14 Metal content of fractions derived by sequential extraction from liquid and dried forms of sludge sample D5

Fraction	Cd		Cu		Ni		Pb		Zn	
	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)
KNO_3 (mgkg^{-1})	0.04	0.55	16.1	4.96	8.70	8.65	1.37	15.3	11.4	112
(% RSD)	(37.7)	(19.3)	(1.10)	(15.4)	(8.10)	(20.3)	(11.1)	(10.5)	(19.3)	(23.4)
KF	0.27	0.64	30.0	0.91	6.80	6.81	2.36	0	79.8	103
	(10.9)	(19.1)	(3.60)	(17.5)	(11.7)	(13.2)	(21.8)	(0)	(9.70)	(6.50)
$Na_4P_2O_7$	0.94	1.69	34.6	36.5	13.1	12.9	291	157	1470	1150
	(3.80)	(8.70)	(5.40)	(10.5)	(3.50)	(6.80)	(8.40)	(1.80)	(2.10)	(1.20)
EDTA	14.9	9.81	88.4	67.9	49.6	44.2	426	344	653	782
	(1.20)	(1.40)	(3.60)	(1.20)	(2.90)	(5.70)	(1.30)	(2.50)	(0.80)	(0.70)
HNO_3	8.46	5.76	459	387	12.1	11.6	78.6	108	671	598
	(0.60)	(2.00)	(2.00)	(1.70)	(7.30)	(8.40)	(4.00)	(2.40)	(2.20)	(2.20)
Residual	0.14	0.14	25.0	14.7	6.52	4.00	7.25	7.38	46.0	37.9
	(67.3)	(55.9)	(4.30)	(14.5)	(10.3)	(13.4)	(13.5)	(17.4)	(14.8)	(3.80)

the higher metal concentrations, much greater variation between replicates was apparent for the KNO_3, KF and residual fractions, particularly with respect to Cd, where concentrations often approached the detection limit. It was generally found, however, that liquid samples displayed more variation betwen replicates than corresponding dried forms. This was particularly apparent from fractionation data obtained for Cu, which resulted in RSD values of <5% in dried sludge but frequently >10% in liquid samples. The percentages of metal recovered by summation of the extracted fractions (including metal extracted from the residual pellets for both the liquid and dried sludges and that present in the original supernatants decanted from the liquid sludges (section 3.2.1.1)) are presented in Table 15. Recoveries were reasonably consistent for each metal across the range of sludges studied, averaging 96, 101, 100, 109 and 101% for Cd, Cu, Ni, Pb and Zn respectively.

4.1.2.3 Metal distributions resulting from sequential chemical extraction

Metal fractionation profiles resulting from sequential extraction of both liquid and dried forms of each sludge sample are illustrated in Figures 5-9. Metal concentrations determined in the individual fractions are presented as percentages of the total metal concentrations determined for each sludge to facilitate comparison between the different metals and between the different sludges. With respect to the liquid sludges, values representing the percentage of the total metal present in the KNO_3 fraction include the metal content of the supernatants produced during pre-concentration of these samples; these latter values are also presented separately in Appendix II.

It is apparent from Figures 5-9 that fractionation profiles tended to exhibit greater variation between individual metals than between sludge type.

4.1.2.3.1 Copper

The distribution pattern of Cu was dominated in all seven sludges by the HNO_3 - extractable fraction (Figure 5), which was particularly prominent in the dried digested sludges, representing >50%

Table 15 Percentage metal recoveries from samples of raw (R), activated (A) and digested (D) sludges following sequential extraction

	Metal Recovery (%)													
	R1		R2		A3		A4		D5		D6		D7	
	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)
Cd	102	72	77	97	103	95	117	129	101	* 78	93	87	110	88
Cu	100	83	108	136	94	97	94	93	106	85	103	101	125	85
Ni	108	120	106	99	100	92	92	97	114	98	86	89	105	87
Pb	105	108	120	154	96	115	108	122	112	88	97	102	108	87
Zn	92	85	106	125	102	103	102	117	102	98	87	103	112	86

* means of five replicates, data for other sludges being means of three replicates

Figure 5 Percentage total Cu content of fractions derived by sequential chemical extraction of dried (▨) and liquid (☐) forms of sewage sludge

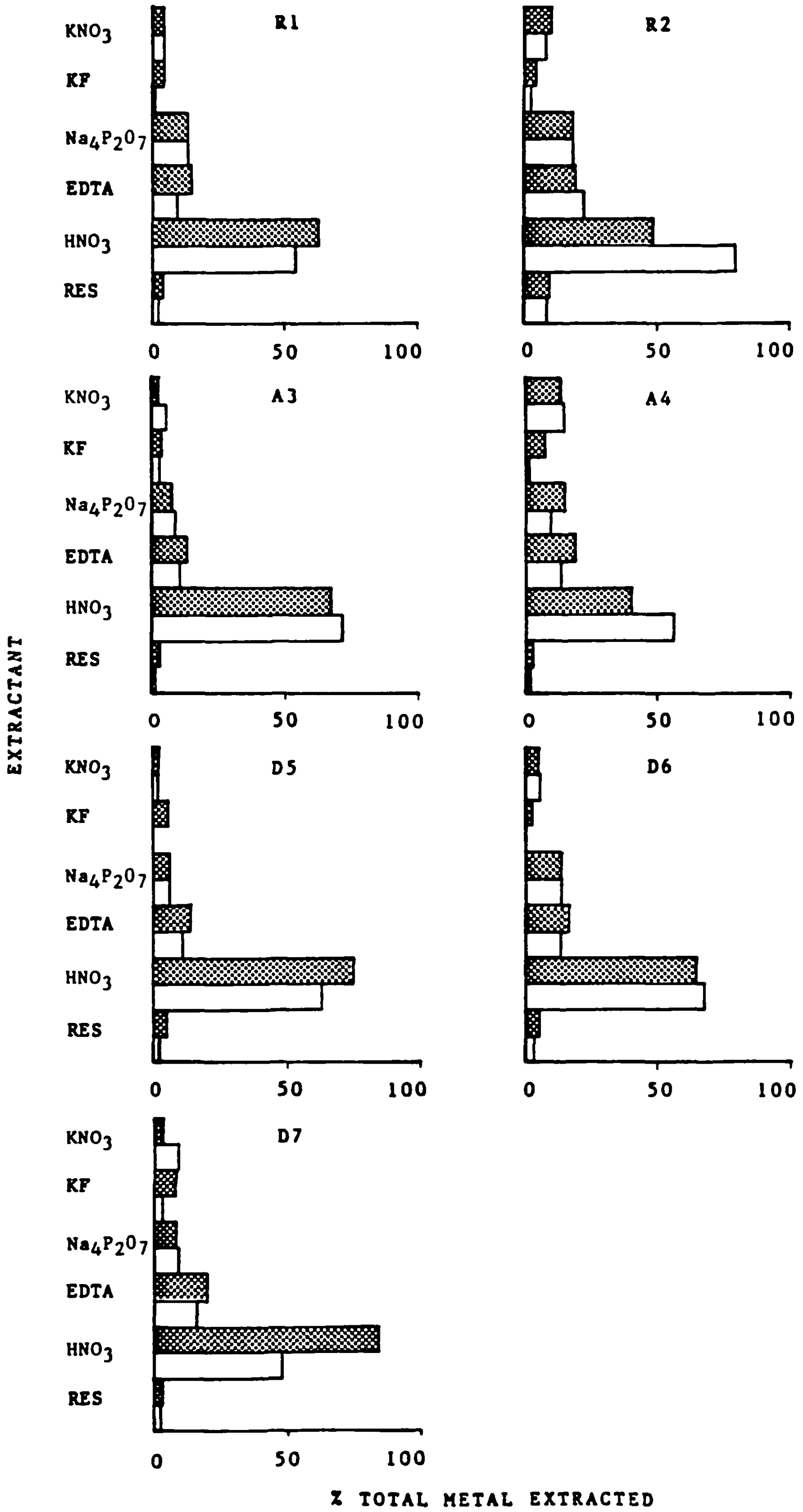


Figure 6 Percentage total Cd content of fractions derived by sequential chemical extraction of dried (▨) and liquid (☐) forms of sewage sludge

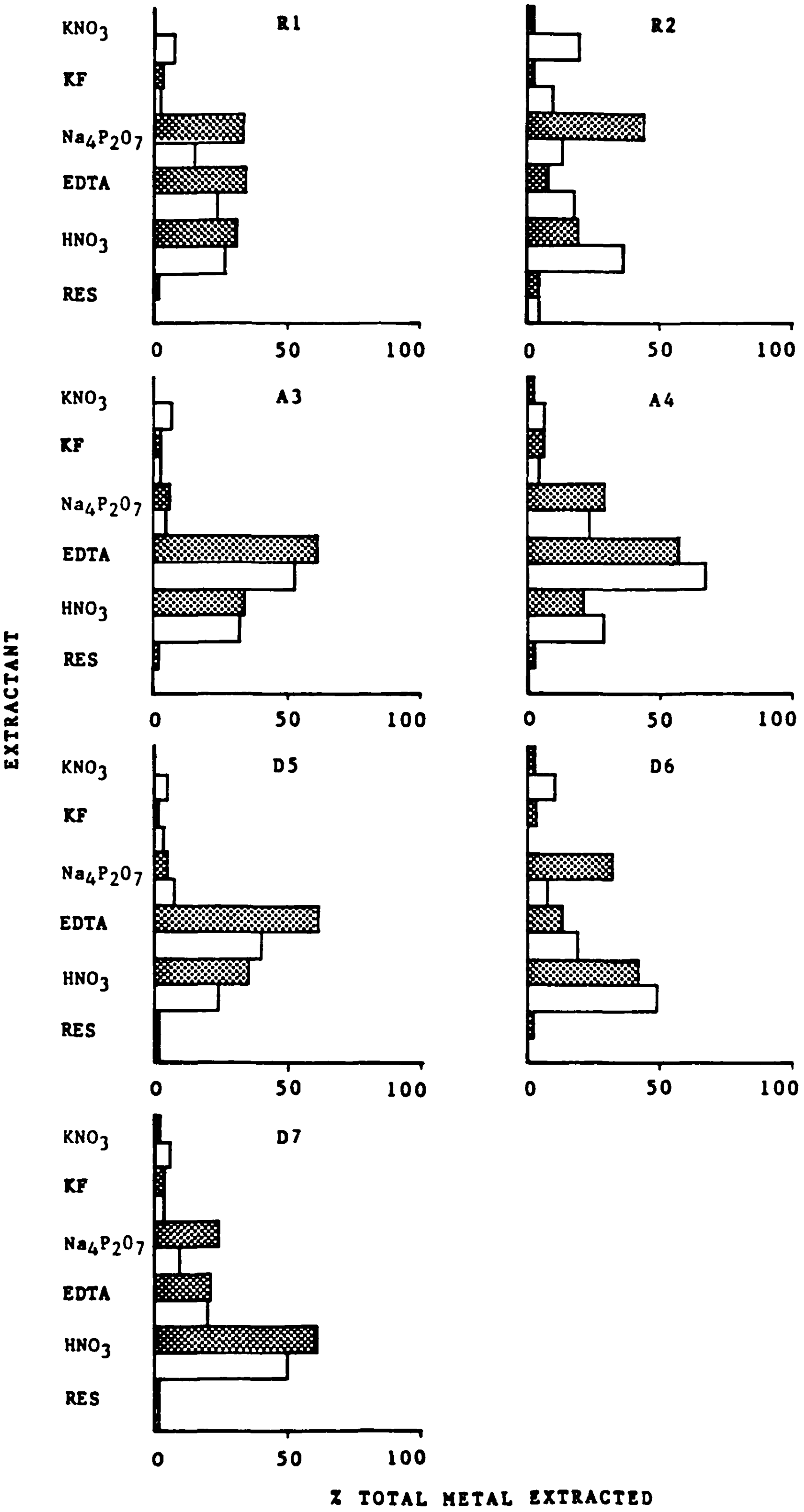


Figure 7 Percentage total Ni content of fractions derived by sequential chemical extraction of dried (▨) and liquid (☐) forms of sewage sludge

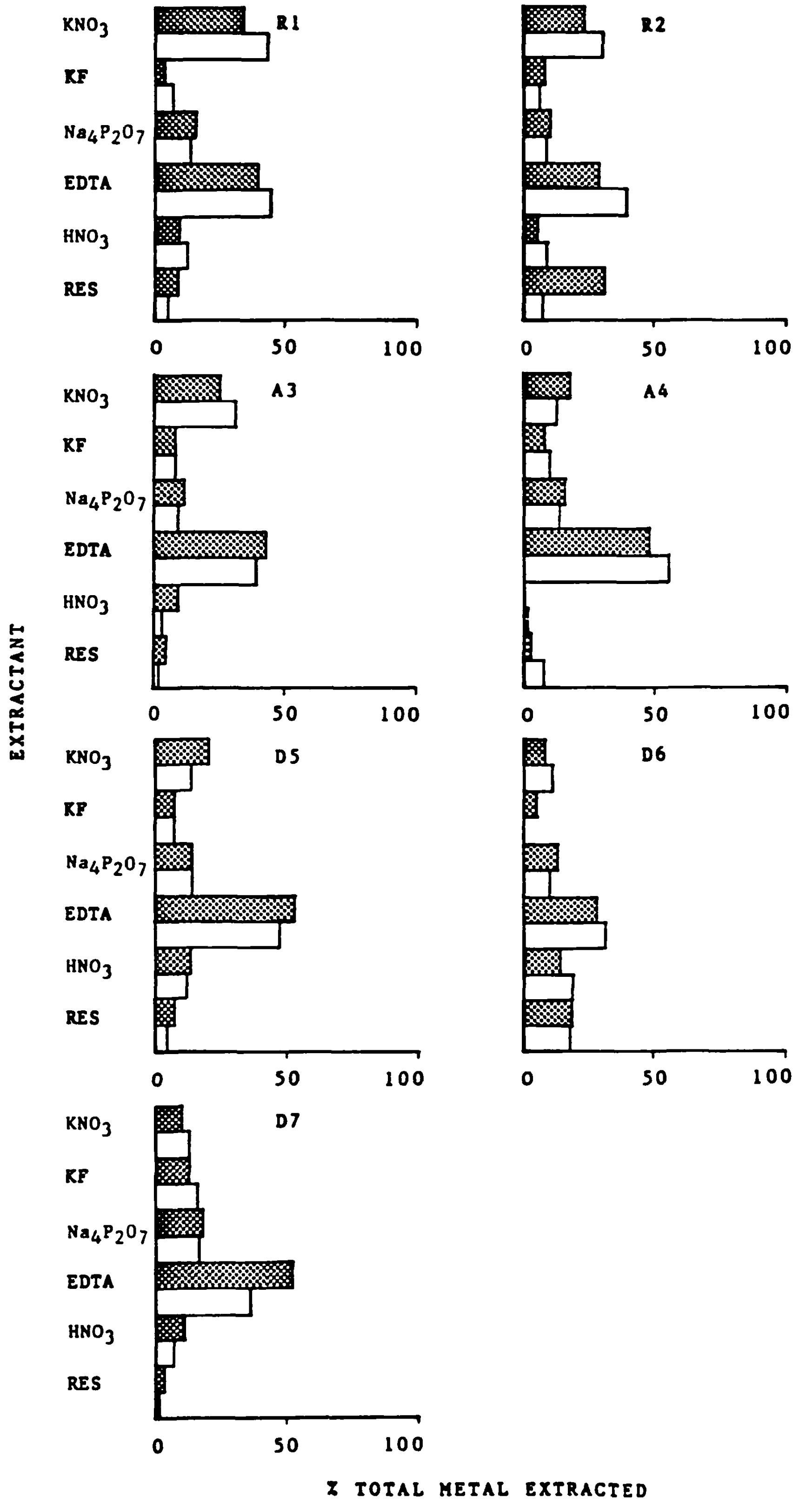


Figure 8 Percentage total Pb content of fractions derived by sequential chemical extraction of dried (▨) and liquid (☐) forms of sewage sludge

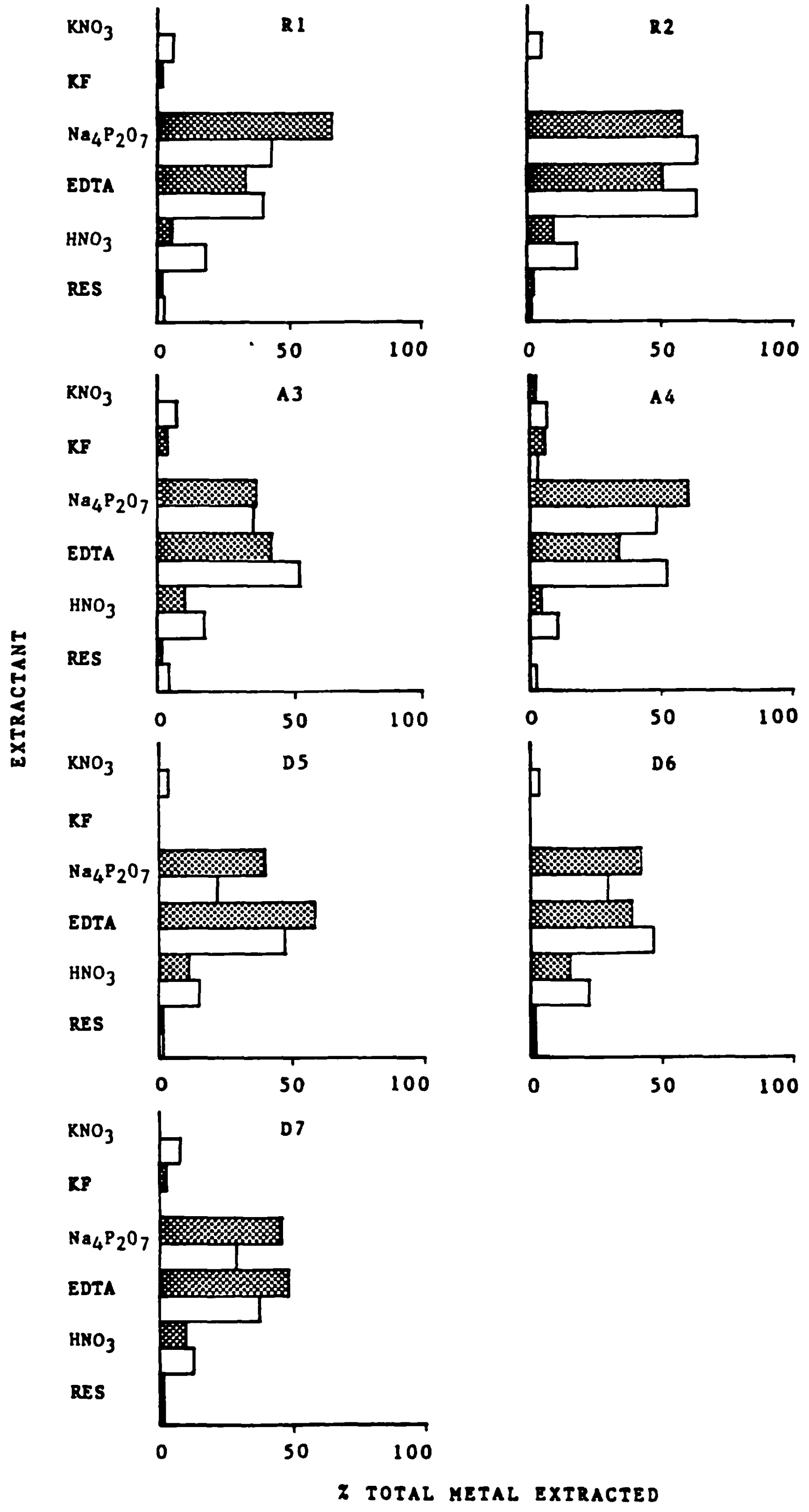
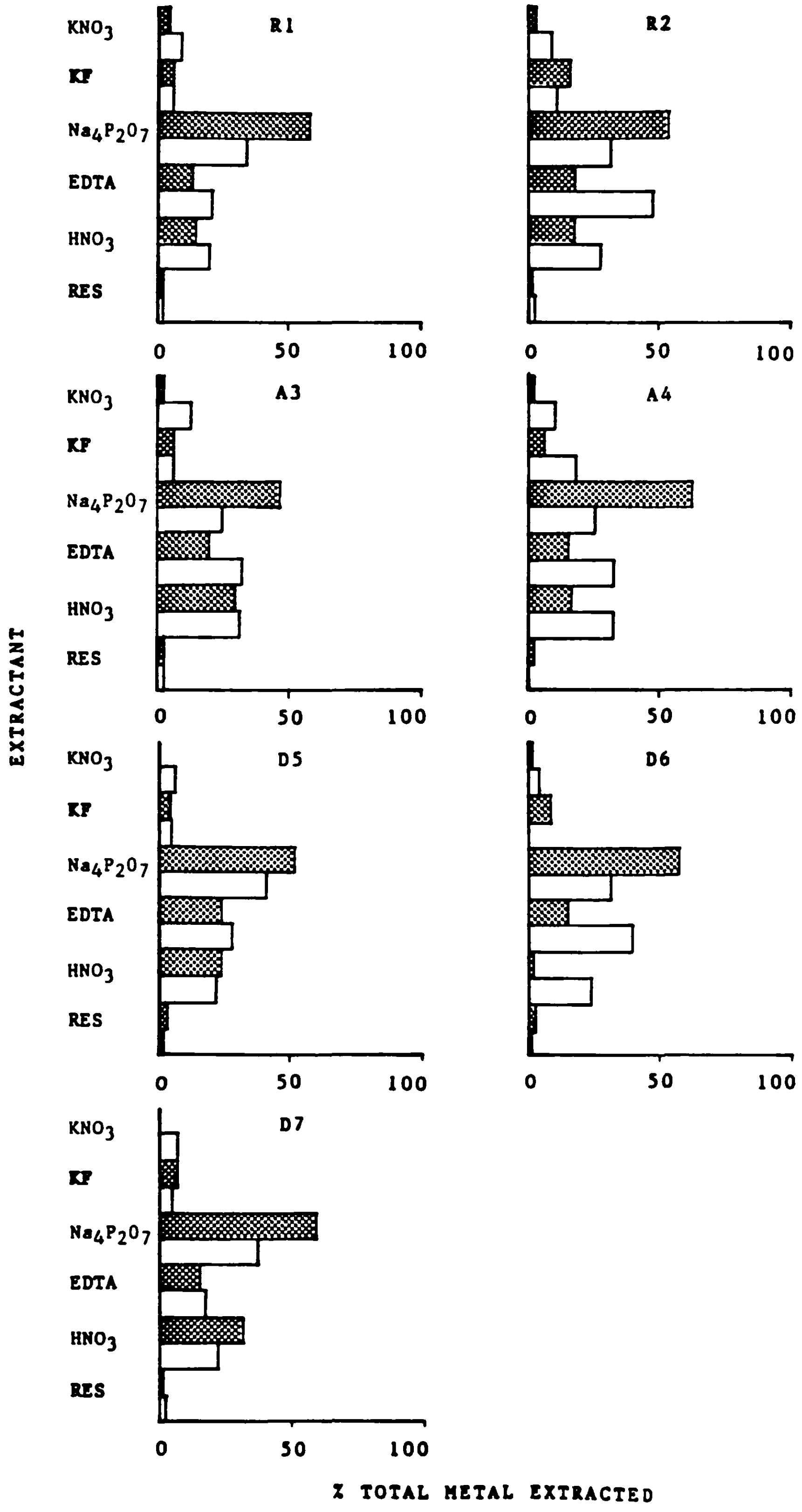


Figure 9 Percentage total Zn content of fractions derived by sequential chemical extraction of dried (▨) and liquid (☐) forms of sewage sludge



of the total Cu content of each sample. The ranking of the Cu fractions was typically:

HNO_3 > EDTA > $Na_4P_2O_7$ > KNO_3 > KF, residual,

which according to the technique designation represented:

sulphide > carbonate > organically bound > soluble/exchangeable > adsorbed, residual.

The distribution pattern of Cu in liquid sludges was almost identical to that of corresponding dried sludges, although the magnitude of the EDTA and KF fractions tended to be greater in the dried forms.

4.1.2.3.2 Cadmium

According to Figure 6, the rankings of the forms of Cd observed in the dried forms of the raw, activated and digested sludges respectively were:

$Na_4P_2O_7$ > EDTA, HNO_3 > KF, residual > KNO_3,
EDTA > $Na_4P_2O_7$, HNO_3 > KF > residual > KNO_3,
HNO_3 > $Na_4P_2O_7$ > EDTA > KF > residual > KNO_3,

respectively representing:

organically bound > carbonate, sulphide > adsorbed, residual > soluble/exchangeable,
carbonate > organically bound, sulphide > adsorbed > residual > soluble/exchangeable,
sulphide > organically bound > carbonate > adsorbed > residual > soluble/exchangeable.

Such changes in the juxtaposition of the three largest fractions with sludge type indicates an increased stability of Cd following sludge digestion. The same fractions appeared to dominate in both liquid and dried forms of the activated and digested sludges although fractionation profiles of Cd in liquid and dried forms of the raw sludges were different. The KNO_3 fraction was of greater significance for the liquid sludges than for the corresponding dried samples; concentrations of Cd extracted by KNO_3 from liquid forms ranging from 4.0 to 19%, whereas <2.0% of the Cd content of the dried forms was present in this fraction. In contrast, however, drying appeared to enhance the percentage Cd content of the $Na_4P_2O_7$ fraction.

4.1.2.3.3 Nickel

Nickel was the only metal extracted in significant proportions by KNO_3 (Figure 7), this fraction being of greater importance for the raw sludges (averaging 32.0% of the total Ni) than for either the activated or digested sludges (averaging 21.4 and 12.5% of the total Ni respectively). However, the largest single fraction in which Ni occurred was the EDTA- extract, which contained from 28.2 to 55.0% of total Ni. The most frequent ranking of the metal forms into which Ni was partitioned in the raw and activated sludges was:

$$\text{EDTA} > KNO_3 > Na_4P_2O_7 > HNO_3 > \text{KF} > \text{residual},$$

representing:

carbonate > soluble/exchangeable > organically bound > sulphide > adsorbed > residual.

With respect to the digested sludges, however, Ni appeared to be distributed more evenly among all but the predominant EDTA fraction. Sludge sample R2 was unique in that the drying process appeared to greatly enhance the residual fraction, but this was not observed to the same extent in any of the other sludge samples, where the quantities of Ni extracted from the two forms of sludge were almost identical.

4.1.2.3.4 Lead

In contrast to Ni, <2.0% of the total Pb content of the dried sludges investigated was extracted by KNO_3. In addition, significant concentrations of KF-extractable Pb, averaging 5.4% of the total, were found only in the activated sludges. The majority of the Pb present was distributed between the $Na_4P_2O_7$ and EDTA fractions, the predominance of either form being influenced by sludge type (Figure 8). Thus, the Pb fractionation sequences observed for the dried forms of raw, activated and digested sludges respectively were:

$Na_4P_2O_7$ > EDTA > HNO_3 > residual > KF > KNO_3,
$Na_4P_2O_7$, EDTA > HNO_3 > KF > residual > KNO_3,
EDTA > $Na_4P_2O_7$ > HNO_3 > residual > KF > KNO_3,

respectively representing:

organically bound > carbonate > sulphide > residual > adsorbed > soluble/exchangeable,
organically bound, carbonate > sulphide > adsorbed > residual > soluble/exchangeable,
carbonate > organically bound > sulphide > residual > adsorbed > soluble/exchangeable.

The rankings of the three most significant fractions were repeated in the liquid forms of the raw and digested sludges, while the EDTA fraction predominated in the liquid activated sludges. In all but one liquid sludge (sample A4), the ranking of the minor fractions was altered to KNO_3 > residual > KF. Overall, drying appeared to have the effect of reducing the magnitude of the KNO_3, EDTA and HNO_3 fractions but enhancing the KF and $Na_4P_2O_7$ fractions.

4.1.2.3.5 Zinc

From Figure 9 it is evident that Zn was predominantly present in the $Na_4P_2O_7$ fraction in all seven of the dried sludge samples; this fraction comprising from 46 to 62% of the total Zn content. The majority of the remaining Zn was fairly evenly distributed between the EDTA and HNO_3 fractions. Concentrations extracted by KF were higher for Zn than for any other metal investigated, representing from 2.8 to 15% of the total Zn content of the dried sludges. Based on the frequency of occurrence, the ranking of Zn fractions in the dried sludges was:

$Na_4P_2O_7$ > EDTA, HNO_3 > KF > KNO_3, residual, representing:

organically bound > carbonate, sulphide > adsorbed > soluble/ exchangeable, residual.

In the liquid forms of the sludges, however, Zn was distributed more evenly between the $Na_4P_2O_7$, EDTA and HNO_3 fractions, suggesting that the drying process may have enhanced the $Na_4P_2O_7$ fraction but reduced the other two fractions. Similar to observations made for Cd and Pb, concentrations of Zn present in the KNO_3 fraction of all liquid samples, which ranged from 3.9 to 12% of the total, were greater than those in corresponding dried samples, in which this fraction generally represented <3.0% of the total Zn content.

4.1.3 Metal solubilisation from sewage sludges following progressive acidification

Characterisation of Cd, Cu, Ni, Pb and Zn according to their solubilities at different pH values was undertaken in both liquid and dried forms of each sludge sample using a progressive acidification technique (section 3.2.1.3).

4.1.3.1 Investigation of the progressive acidification technique

The method of Cottenie (1981) was modified slightly in that the ambient pH samples were not maintained at their original pH values by addition of acid or alkali, but were allowed to drift. The rationale for this was to make this first step of the progressive acidification technique more readily comparable with the first stage of the sequential extraction technique, as both are effectively an evaluation of the easily extractable forms of metals present. Having already been mixed for 15 min prior to experimentation, the pH of the ambient dry samples was found to vary by only ± 0.2 pH units and that of the liquid samples by ± 0.1 pH unit over the 30 min experimental period. There was, however, some difference in pH between the liquid sludge samples and the dried samples resuspended in distilled water, which itself has a pH value of approximately 5.0. A comparison of the concentrations of metals solubilised from a sample of resuspended dried sludge maintained at the pH value of the origin liquid sample with those solubilised from an unadjusted dried sample indicated differences of <2.0% of the total metal present. Differences of this magnitude were considered negligible, therefore no pH adjustments to the resuspended dried samples were made.

A further observation made during preliminary extractions was that although the samples could be maintained at pH 4.0, 2.0 or 0.5 for 30 min with only minor variations (± 0.02) occurring in pH, the subsequent addition of distilled water (to make up the volume) and centrifugation and filtration of the sample tended to give final pH values within the ranges 3.67 to 4.24, 1.74 to 2.52 and 0.54 to 1.33. In an attempt to reduce the final pH, a repeat pH 0.5 extraction was made on sludge sample R2 (which had previously shown a final pH of 1.33) using distilled water which had been adjusted to pH 0.5 using HNO_3. Following addition of the acidified water and centrifugation and filtration of the sample, the final pH was 0.67. Analysis of the filtrate gave metal concentrations that differed from those of the initial pH 0.5 extract by -33% of total Cu, -11.6% of total Cd, +2.64% of total Ni, +23.2% of total Pb and -23.5% of total Zn. Since these results were inconsistent, pH adjustment of the distilled water was discontinued.

4.1.3.2 Statistical evaluation of the progressive acidification technique

The mean concentrations and RSDs of metals solubilised from 5 replicates of both liquid and dried forms of sludge sample D7 following progressive acidification are given in Table 16. The method was found to be reproducible for dried samples, with RSD values of <10%, except where Cd and Pb concentrations approached the limits of detection. Metal solubilisation from the liquid samples, however, exhibited a lower degree of reproducibility, frequently producing RSD values of >10%, even where metal concentrations were high.

4.1.3.3 Metal solubilisation patterns resulting from progressive acidification

The solubilisation patterns obtained for each metal were generally independent of sludge type (Figures 10-14). A definite

Table 16 Metal solubilised by progressive acidification of liquid and dried forms of sludge sample D7

pH	Cd		Cu		Ni		Pb		Zn	
	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)	(dried)	(liquid)
Ambient (mgkg^{-1})	0.12	2.37	6.48	77.3	21.1	25.8	1.10	32.2	0.50	122
(% RSD)	(24.0)	(6.60)	(5.50)	(4.50)	(3.40)	(7.70)	(12.7)	(5.60)	(5.70)	(11.1)
4.0	2.22	0.29	2.12	4.54	82.7	108	1.26	4.43	217	72.1
	(16.0)	(25.0)	(7.70)	(0.80)	(8.80)	(7.20)	(11.7)	(10.1)	(8.70)	(16.5)
2.0	23.4	0.76	83.6	1.41	172	164	239	74.9	763	359
	(1.40)	(17.6)	(3.80)	(13.6)	(2.10)	(11.9)	(5.00)	(11.1)	(4.00)	(6.30)
0.5	19.3	3.24	307	1.56	202	212	558	220	761	771
	(5.80)	(12.0)	(4.30)	(35.5)	(4.00)	(14.9)	(4.50)	(11.6)	(6.50)	(7.40)

Figure 10 Percentage total Cu content solubilised by progressive acidification of dried (○) and liquid (●) forms of sewage sludge

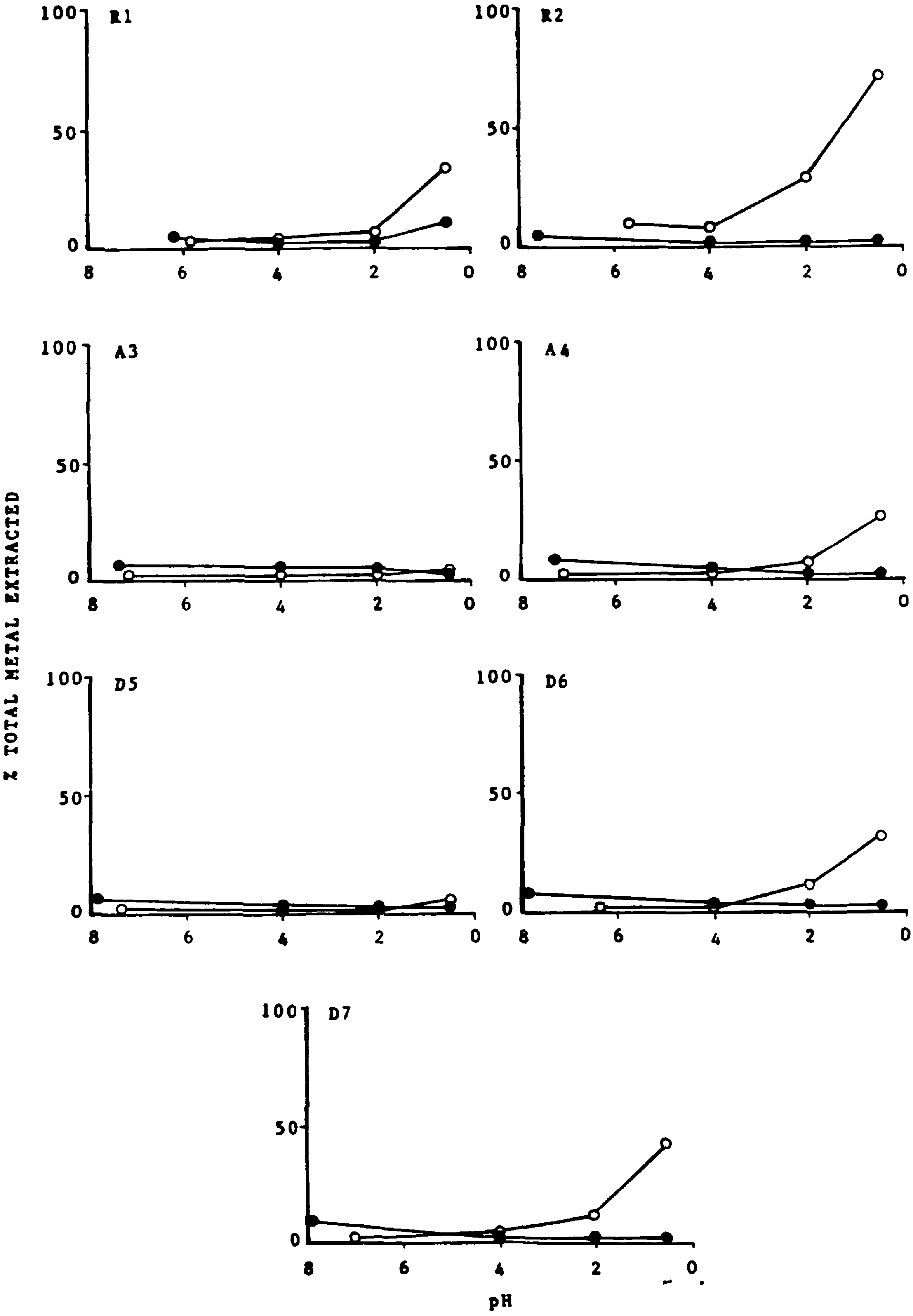


Figure 11 Percentage total Cd content solubilised by progressive acidification of dried (○) and liquid (●) forms of sewage sludge

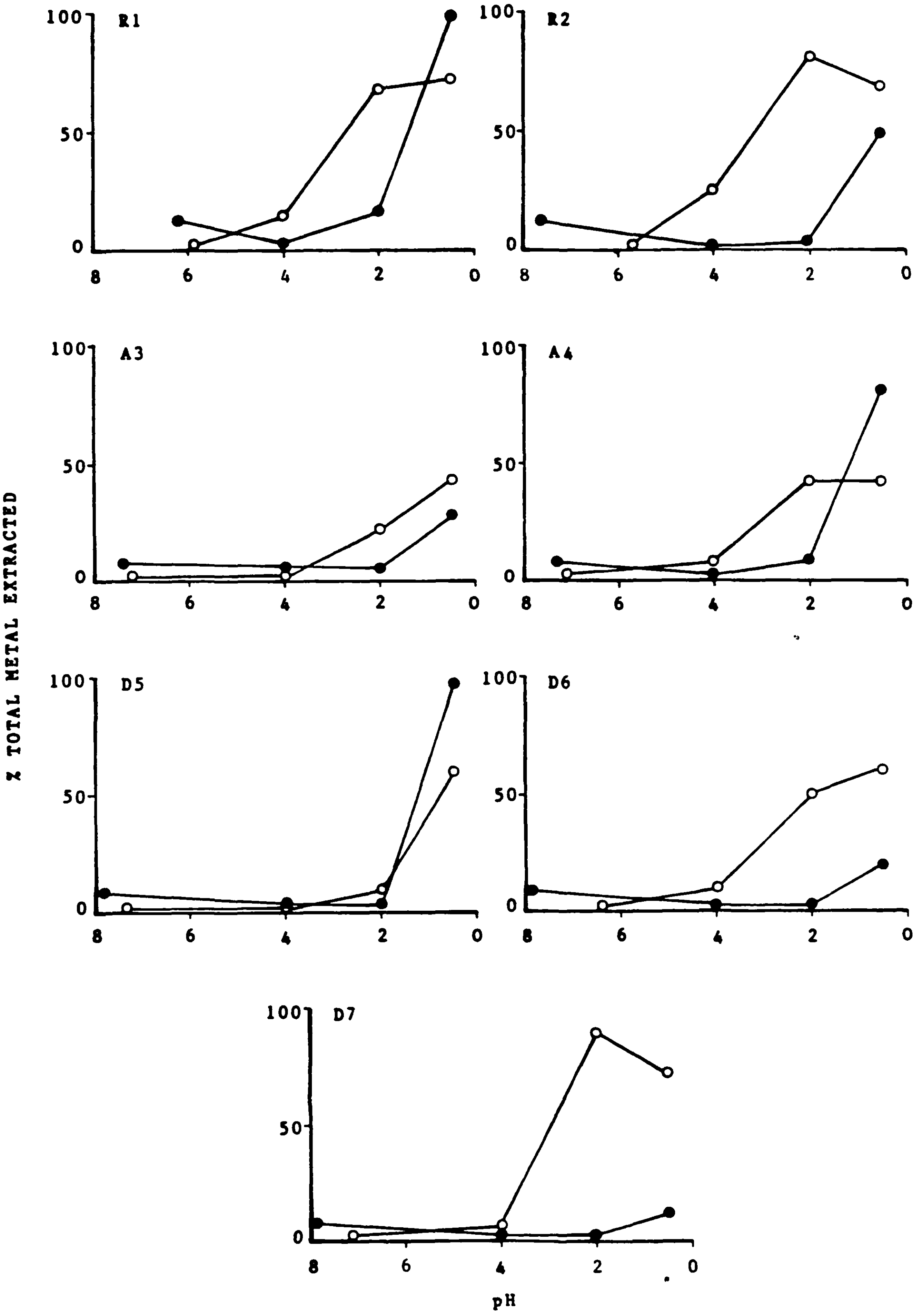


Figure 12 Percentage total Pb content solubilised by progressive acidification of dried (o) and liquid (●) forms of sewage sludge

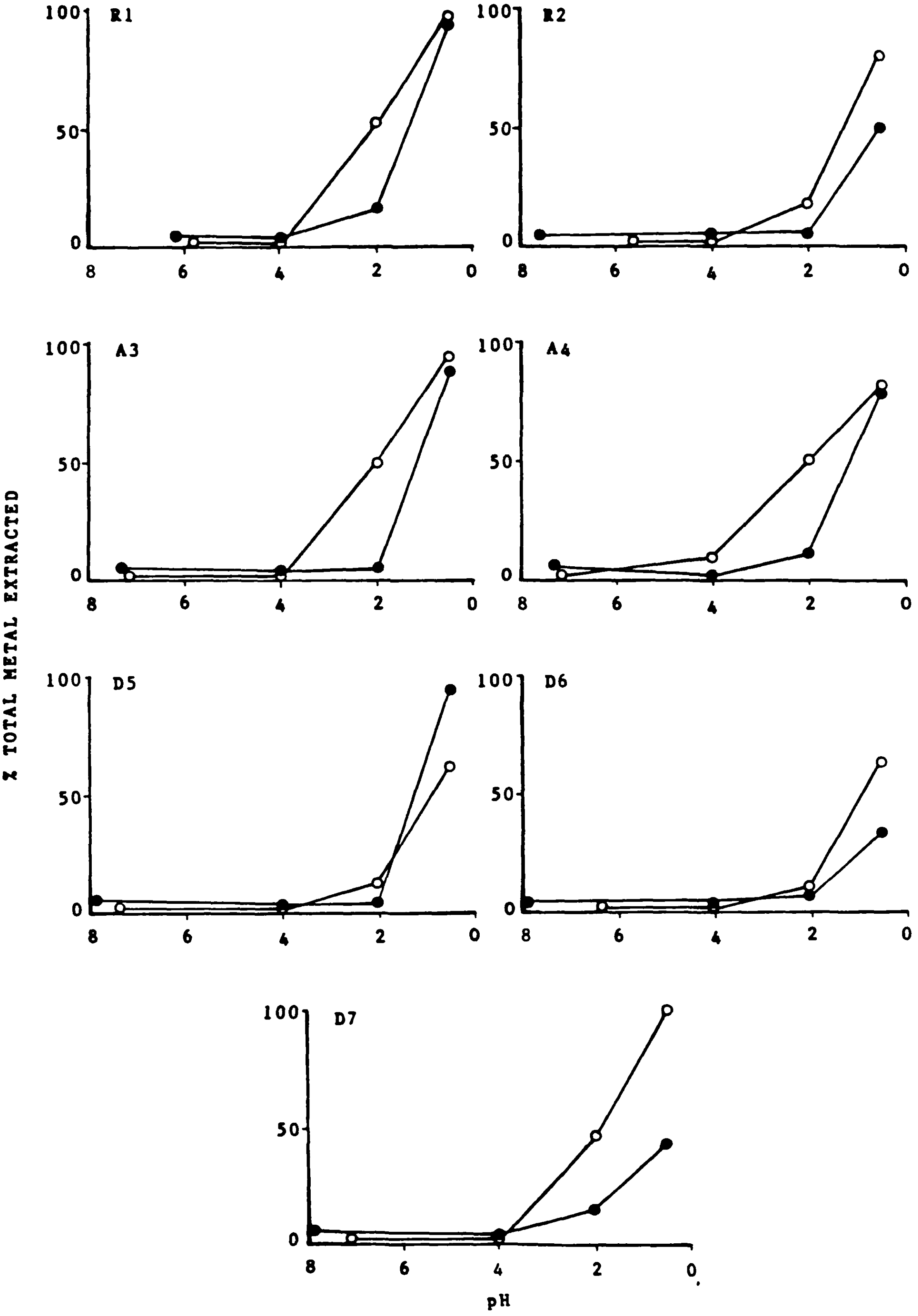


Figure 13 Percentage total Zn content solubilised by progressive acidification of dried (○) and liquid (●) forms of sewage sludge

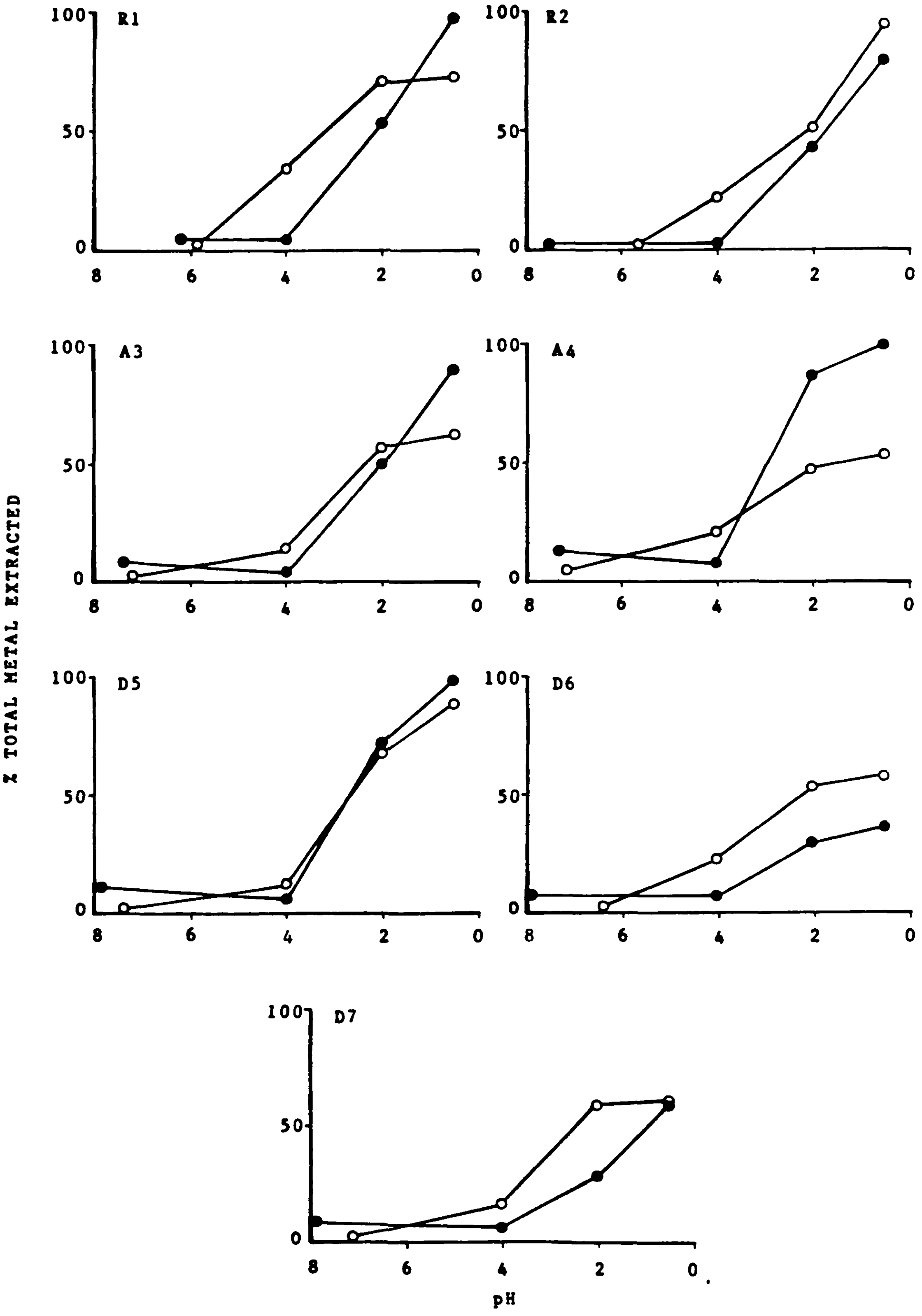
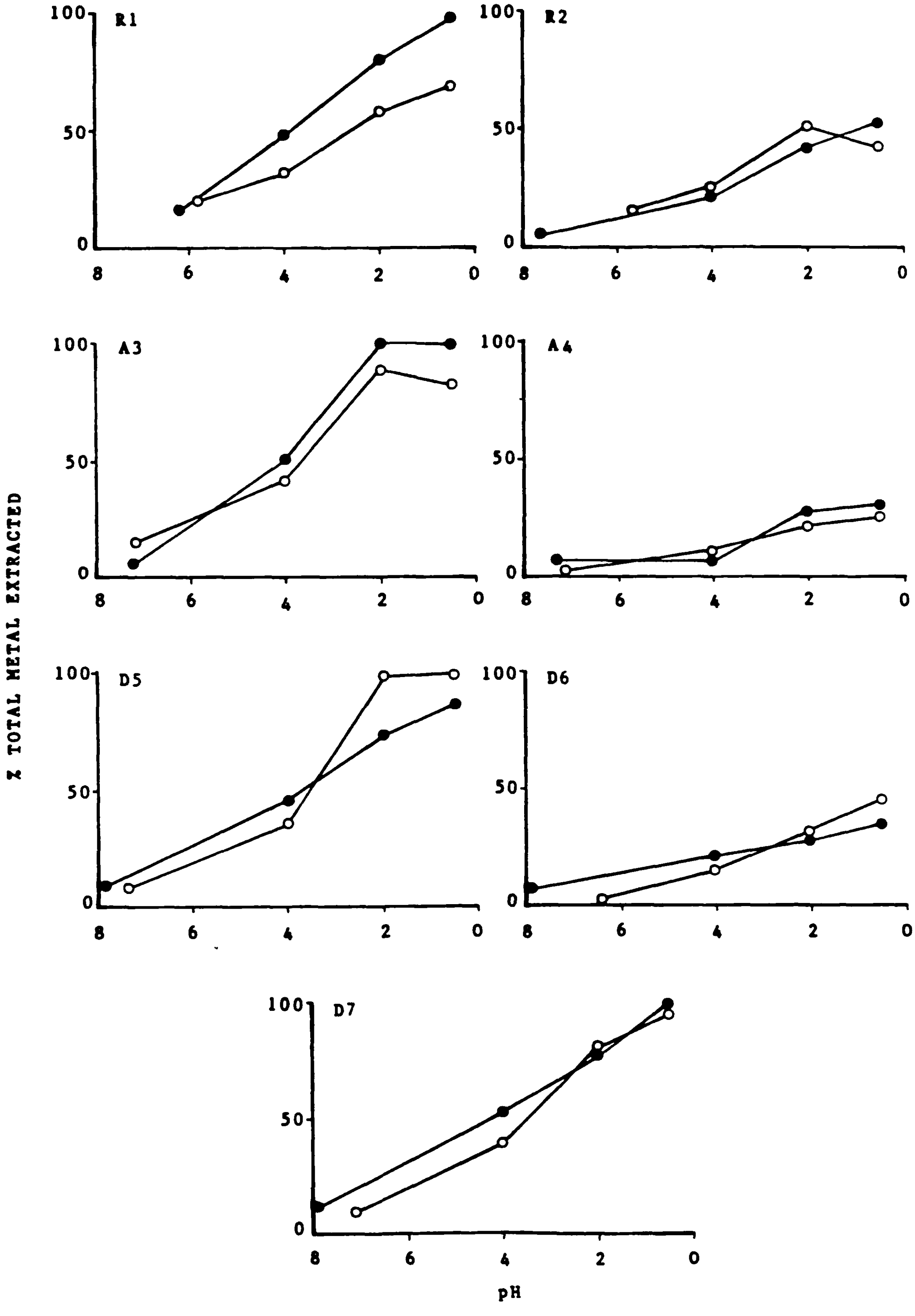


Figure 14 Percentage total Ni content solubilised by progressive acidification of dried (○) and liquid (●) forms of sewage sludge



break-point or threshold pH was identified for four of the metals, above which the percentage solubilisation was relatively low and stable and below which solubilisation tended to increase exponentially with decreasing pH.

4.1.3.3.1 Copper

For Cu, the threshold of solubilisation was between pH 4.0 and pH 2.0 for dried sludges but no break-point could be identified for corresponding liquid forms as the percentage of metal extracted tended to decrease with increasing acidity from all but sample R1 (Figure 10). Even at pH 0.5, the concentration of Cu extracted from the liquid sludges was generally <1% of the total metal content, while concentrations solubilised at ambient pH values were from 4- to 100-fold greater. It became apparent that following acidification of the filtrates of ambient pH samples, humic material was being precipitated. A comparison of the Cu content of the settled supernatant with that of the shaken sample indicated that approximately 90% of the Cu extracted from the liquid samples was contained in this precipitated organic fraction, and this may explain the reduced solubilisation apparent at lower pH. Solubilisation was greater in corresponding dried sludges, an average 31% of the total Cu being extracted at pH 0.5. Significant variations in the concentrations extracted from dried samples at this pH were apparent, however, particularly between sludges of similar type, as demonstrated by sludge samples A4 and A3, from which 27 and 4.3% respectively of the total Cu content of each was solubilised at pH 0.5.

4.1.3.3.2 Cadmium

The threshold value for Cd was pH 4.0 for the dried samples but nearer to pH 2.0 for the liquid forms (Figure 11). The majority of the potentially soluble Cd in the dried sludges appeared to be extracted at pH 2.0 as significant increases at pH 0.5 were only observed for sludge samples A3 and D5. With respect to the liquid samples, however, most of the potentially soluble Cd was released at pH 0.5. A phenomenon similar to that occurring for Cu was observed in that less Cd was extracted from liquid sludges at pH 4.0 than at the ambient pH values, but no comparable reduction was observed for dried forms.

4.1.3.3.3 Lead

Lead exhibited a clear break-point at pH 4.0 in the dried sludges, below which solubilisation increased rapidly, to between 62 and 100% at pH 0.5 (Figure 12). It was apparent that Pb was less easily extracted from dried digested sludges than from raw or activated types, and some evidence for this occurring in the liquid sludges was also apparent. The solubilisation pattern displayed by the liquid sludges differed from that of the dried forms in that the majority of Pb was released at below pH 2.0. However, with the exception of sludge sample D5, the percentage of Pb extracted from the liquid sludges at pH 0.5 was lower than that extracted from corresponding dried forms. Again, Pb solubilised from liquid sludges at their ambient pH values was up to 8 times higher than that released at pH 4.0.

4.1.3.3.4 Zinc

The threshold of solubilisation for Zn in dried sludges approached pH 6.0 (Figure 13). The majority of the potentially soluble Zn was extracted at approximately pH 2.0, with only slight increases in the concentration released being observed at pH 0.5, at which point values ranged from 53 to 94%. Zinc appeared to be more easily extracted from raw sludges than from dried forms of activated and digested sludges. The threshold of solubilisation for Zn in liquid samples was typically pH 4.0. Despite the initial resistance to acidification displayed by Zn in liquid sludges, the solubilisation that occurred at pH 0.5 was in several cases greater than that apparent for corresponding dried samples. A degree of resistance to solubilisation from both liquid and dried forms appeared to be conferred by the digestion process, and was particularly apparent in samples D6 and D7. As for Cd, Cu and Pb, concentrations of Zn released at the ambient pH values of the liquid sludges were slightly higher than those released at pH 4.0.

4.1.3.3.5 Nickel

In contrast to Cu, Cd, Pb and Zn, no definite threshold at which Ni solubilisation markedly increased could be identified for liquid or dried sludges (Figure 14). In addition, concentrations of Ni released at ambient pH values from both forms were lower than those released at the other three pH values and the percentage of Ni solubilised thus increased gradually with increasing acidity to produce near-linear plots. The concentration of Ni released at pH 0.5 ranged from 25 to 100% of the total sludge content, the higher values being exhibited by the three sludges originating from the same works, namely samples R1, A3 and D5, in addition to the dewatered digested sludge sample D7. In contrast to the behaviour of the other metals studied, Ni solubilised from the dried sludges followed a very similar pattern to that exhibited by analogous liquid forms, with only slight variations in the concentrations extracted at a particular pH being apparent.

4.1.4 Cross-comparison of the progressive acidification and sequential extraction techniques

In an attempt to elucidate some of the reactions occurring during progressive acidification, residues of the dried forms of sludges which had been adjusted to pH 4.0, 2.0 and 0.5 were subjected to sequential extraction. Residues of the liquid sludges were not treated in this way, however, as the reproducibility of the progressive acidification and sequential extraction techniques (Tables 16 and 14 respectively) was found to be consistently lower for liquid than for corresponding dried samples. The resultant fractionation patterns, together with data from acidification and sequential extraction of the original dried samples at ambient pH have been presented for each of the five metals using samples R1, A3 and D7, which were considered to be most representative of each sludge type (Figures 15-19). Fractionation data for the remaining sludges are tabulated in Appendix III.

The relatively low concentrations of metals solubilised at ambient pH represented the readily soluble species of each and were comparable to those extracted by KNO_3 in the sequential extraction of the original dried samples, except where a significant proportion of the metal existed in an 'exchangeable' form. Quantification of

Figure 15 Extraction of Zn from three sludge types by progressive acidification and by sequential extraction of both the original sample at ambient pH and the residues produced following each pH reduction

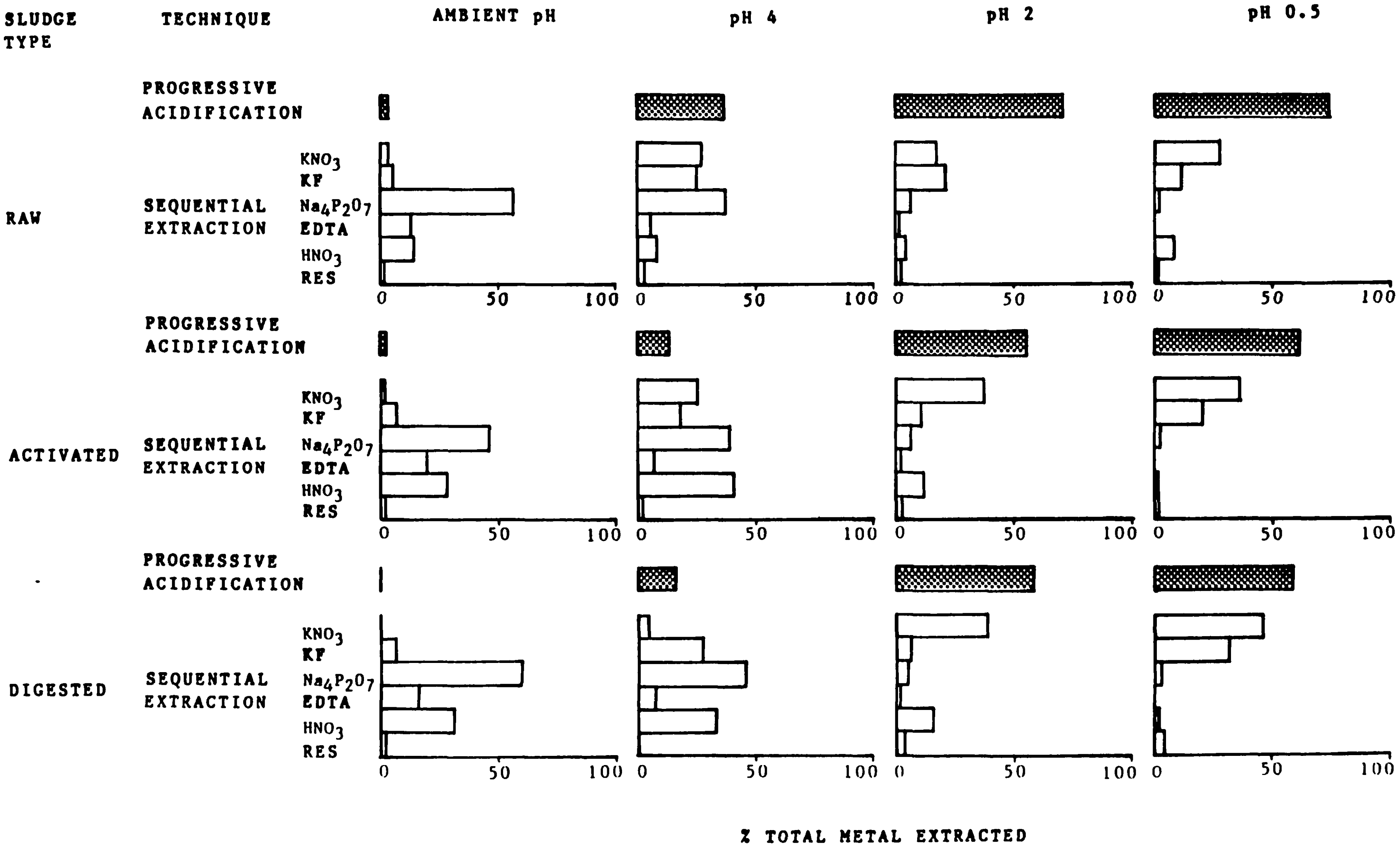


Figure 16 Extraction of Ni from three sludge types by progressive acidification and by sequential extraction of both the original sample at ambient pH and the residues produced following each pH reduction

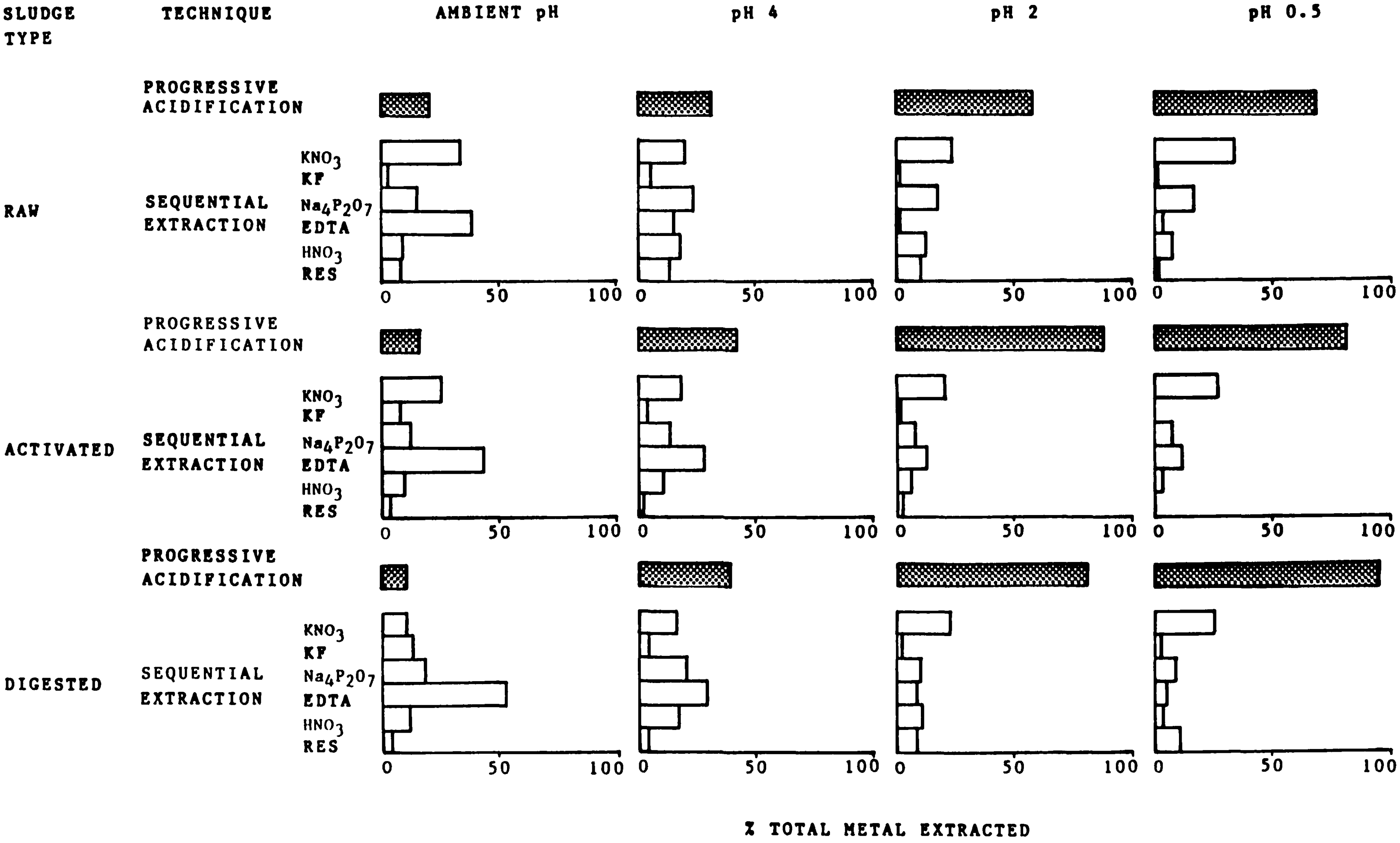


Figure 17 Extraction of Cd from three sludge types by progressive acidification and by sequential extraction of both the original sample at ambient pH and the residues produced following each pH reduction

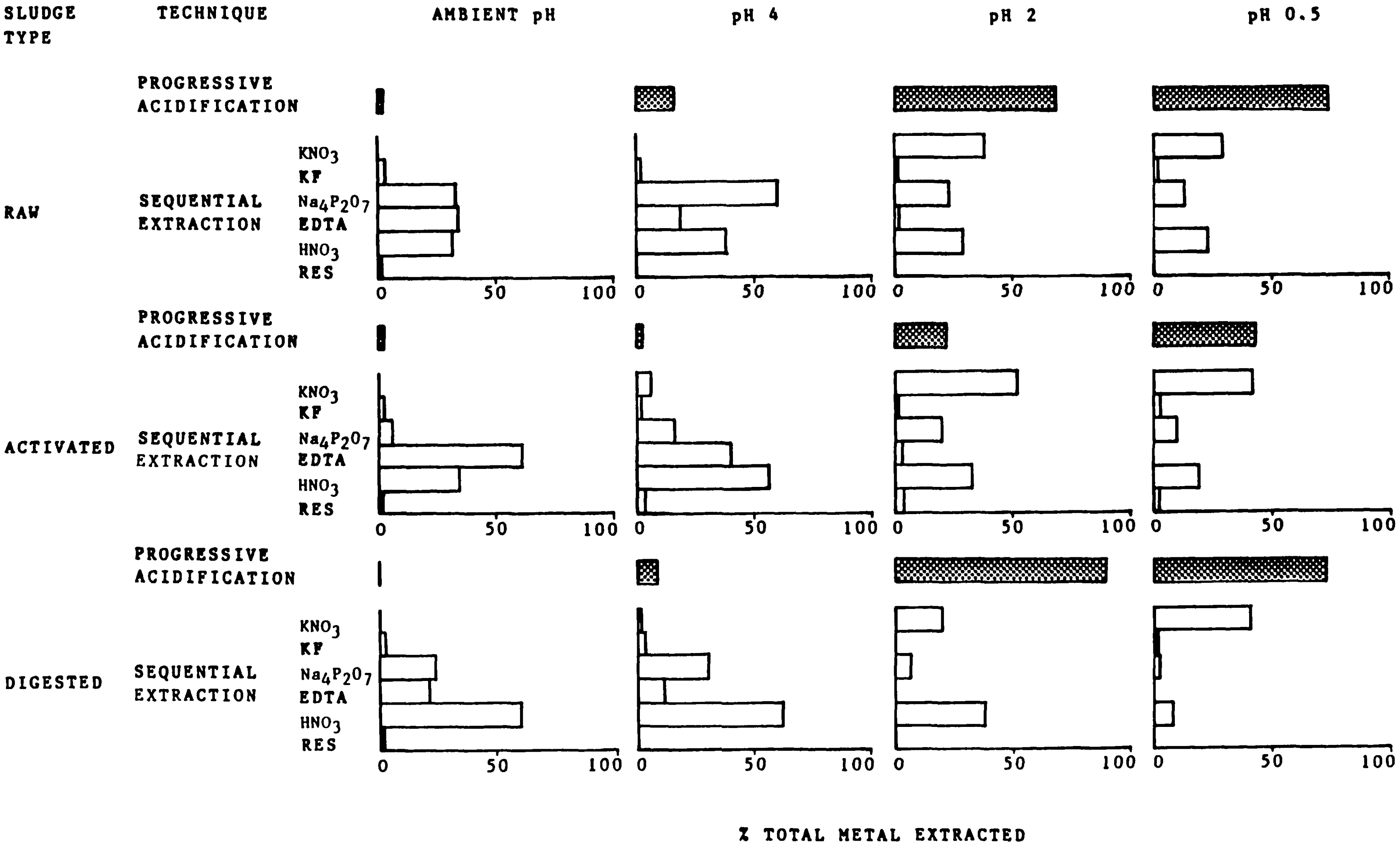


Figure 18 Extraction of Pb from three sludge types by progressive acidification and by sequential extraction of both the original sample at ambient pH and the residues produced following each pH reduction

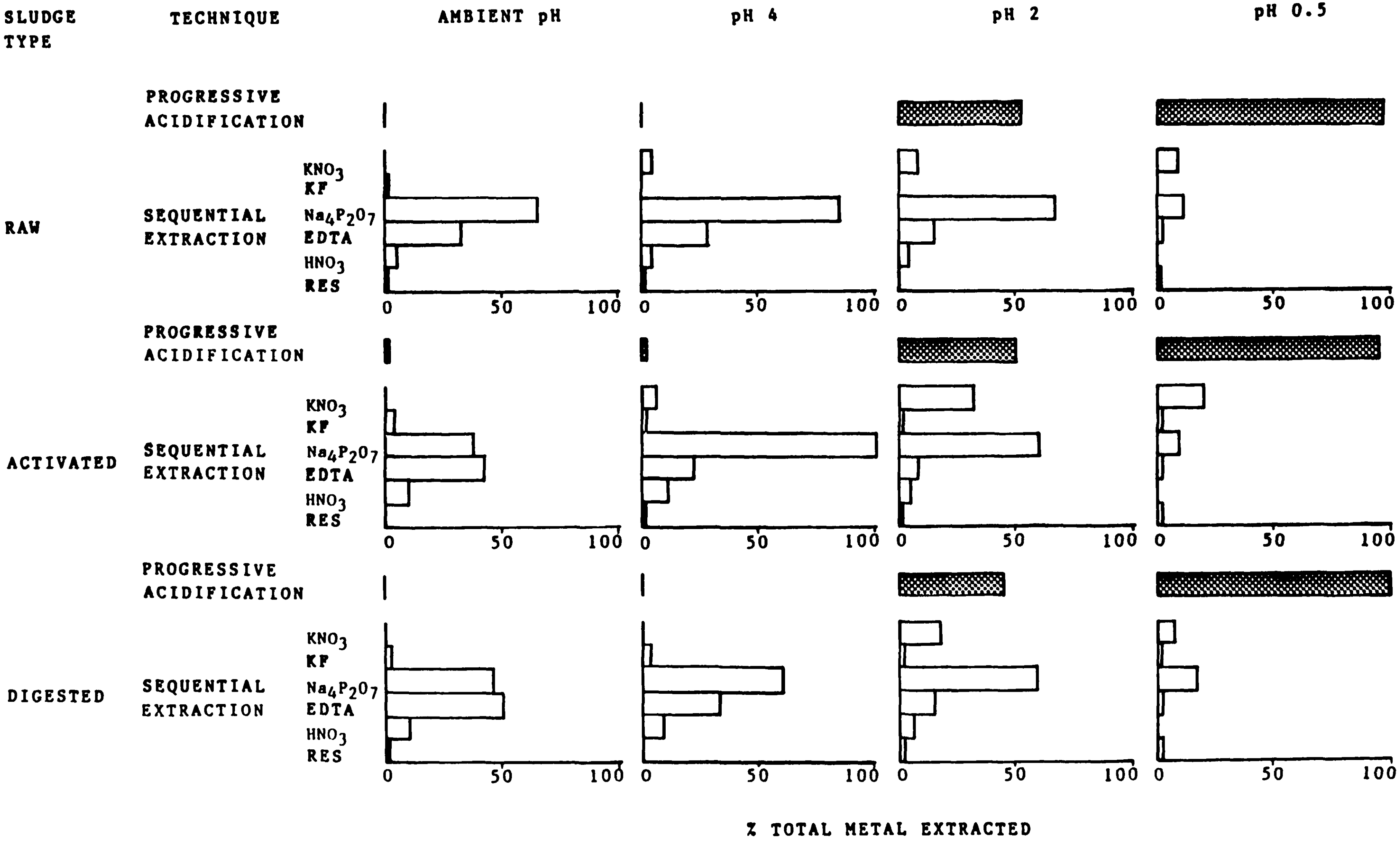
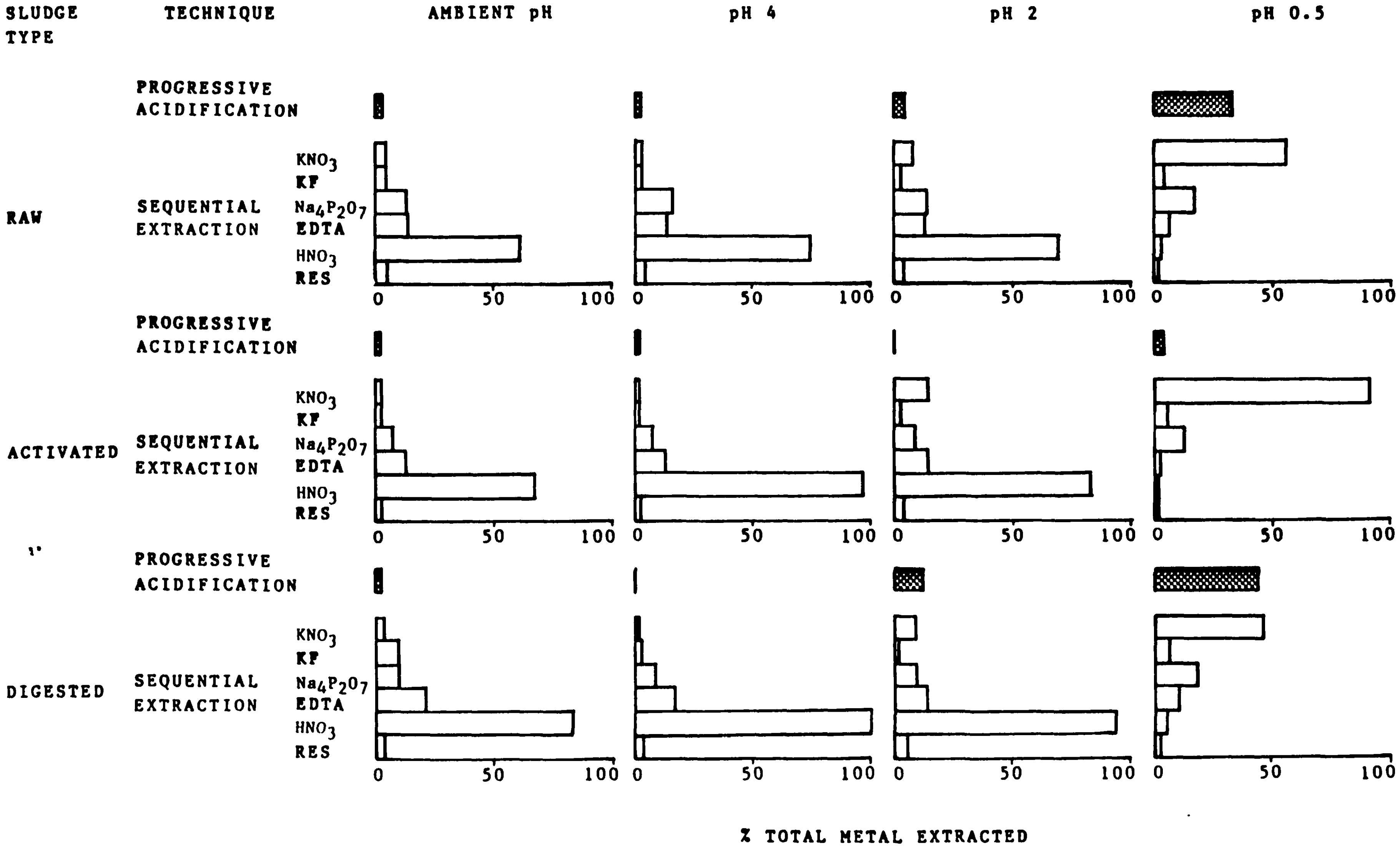


Figure 19 Extraction of Cu from three sludge types by progressive acidification and by sequential extraction of both the original sample at ambient pH and the residues produced following each pH reduction



'exchangeable' metal by subtraction indicated that this fraction was only of significance for Ni and Cu, comprising up to 15 and 10% of respective total concentrations. The 'exchangeable' forms of Cu were more prominent in the activated sludges than in the raw or digested samples.

The most noticeable effect of reducing the pH by progressive acidification was the redistribution of metal species present in the residues towards more easily extractable forms. This was particularly apparent for Zn, which, as illustrated in Figure 15, gradually shifted from a predominantly $Na_4P_2O_7$-extractable form at ambient pH to KNO_3-extractable and KF-extractable forms at pH 0.5. The demonstrated increase in KF-extractable Zn following acidification, which was particularly significant for sludge sample A4, was not observed for any other metal studied.

Nickel fractionation at ambient pH was poorly defined but as successive proportions of metal were removed by acidification, the profiles became skewed towards the more easily extractable forms (Figure 16). The percentage of the total Ni in all the fractions subsequent to KNO_3 decreased with increasing acidity, the most marked diminution being that of the EDTA fraction, which represented less than 5.0% of total Ni at pH 0.5 in all but sludge sample A3.

The transitions which occurred in the distribution of Cd following acidification were similar for all sludge types despite variation in the fractionation profiles at ambient pH (Figure 17). Dissolution of the EDTA-extractable fraction, which was initially one of the most dominant, commenced between ambient pH and pH 4.0. However, considerable changes in the fractionation profile of Cd occurred between pH 4.0 and pH 2.0, whereby the EDTA fraction was severely reduced and a significant proportion of Cd, averaging 33%, became extractable by KNO_3. Gradual reductions in the percentages of Cd removed by $Na_4P_2O_7$ and HNO_3 were also observed, although the latter fraction appeared to be more resistant at the lower pH values.

Lead was unique in its response to acidification in that it was almost entirely solubilised, as was evident from the residual Pb concentration at pH 0.5 (Figure 18). Although no significant increase in Pb solubilisation was apparent between ambient pH and pH 4.0, a reduction in the EDTA-extractable fraction coincided with an accentuation of the $Na_4P_2O_7$-extract. The EDTA and HNO_3 fractions decreased between pH 4.0 and pH 2.0 but in contrast to what was observed for Zn, the fractionation profile of Pb did not show a progressive shift through each of the fractions towards the soluble state, as the $Na_4P_2O_7$-extractable fraction predominated even at pH 2.0. An approximate 6-fold reduction in this latter fraction between pH 2.0 and pH 0.5 with no accompanying increase in the KNO_3-extractable fraction would seem to suggest that the increased solubilisation observed at pH 0.5 was primarily the result of the direct dissolution of the $Na_4P_2O_7$ fraction.

Copper remained predominantly in the HNO_3-extractable fraction in the acidified residues until pH 0.5, when it was transferred to KNO_3-extractable forms (Figure 19). Virtually no transitions in the fractionation profiles were apparent between ambient pH and pH 2.0, apart from a slight increase in KNO_3-extractable forms at the latter pH value. However, subsequent dissolution of HNO_3-extractable forms resulted in a reduction of this fraction from an average of 70% of total Cu at pH 2.0 to 4.0% at pH 0.5, and a simultaneous increase in the KNO_3 fraction. This transition was particularly evident for the activated sludges. While slight reductions in EDTA-extractable Cu in raw and activated sludges were also observed between pH 2.0 and pH 0.5, the $Na_4P_2O_7$ fraction remained constant. In digested sludges, however, concentrations of Cu extracted by EDTA remained uniform but those extracted by $Na_4P_2O_7$ typically increased 2-fold, perhaps indicating that Cu associated with organic material exhibited a certain degree of resistance to acidification. Despite the demonstrated occurrence of Cu in relatively unstable forms, in excess of 50% of the total remained within the sludge residues following acidification to pH 0.5.

4.2 Evaluation of Heavy Metal Distributions During the Anaerobic Digestion of Sewage Sludge

4.2.1 Equilibration and performance of the anaerobic digesters

Prior to investigating metal distributions it was necessary to establish that the anaerobic digesters had achieved equilibrium and were performing efficiently.

Through routine monitoring of total and volatile solids, total alkalinity, pH, volatile acids, percentage methane content of the digester gas and total gas production it was apparent that each of the four digesters had reached stable conditions within 40 d. However, an additional retention period (20 d) was allowed prior to experimentation to ensure optimum digestion. Mean values of digestion performance parameters obtained for each sludge type upon stabilisation are presented in Table 17. It is apparent that the digesters fed on primary sludge achieved greater reductions in volatile solids, displayed lower volatile acids concentrations and produced greater volumes of digester gas than those treating mixed primary sludge, despite virtually identical volatile solids loadings. Such behaviour indicates that the digesters operating on a feed of primary sludge were performing more efficiently than those operating on a feed of mixed primary sludge.

4.2.2 The influence of anaerobic digestion on metal distribution

A comparison of metal distributions in raw and digested primary and mixed primary sludges was undertaken to assess the effects of anaerobic digestion on sludge metal forms. Aliquots of digested sludge removed from the primary and mixed primary 'experimental' digesters in addition to the respective raw sludge samples were subjected to sequential extraction. Total concentrations of Cd, Cu, Ni, Pb and Zn (in terms of mgl^{-1}) were therefore constant in raw and digested samples of the same sludge type (Table 18). To date, no other comparison has been reported between raw sludge fractionation and the same sludge fractionated following anaerobic digestion.

The resultant metal distributions observed in raw and digested

Table 17 Mean values of stabilised digestion parameters prior to metal addition

Sludge	pH	Loading Rate ($gVSl^{-1}d^{-1}$)	Total Solids (gl^{-1})	Volatile Solids Reduction VSR(%)	Volatile Acids (mgl^{-1})	Alkalinity (mgl^{-1})	Gas Production ($mlgVSR^{-1}d^{-1}$)	Gas Composition $\%CH_4$
Hogsmill Mixed Primary	7.52	1.66	25.4	46	155	4177	501	60
Dartford Primary	7.35	1.60	23.9	55	51	2954	549	58

Table 18 Comparison of initial and final total metal concentrations observed in the 'experimental' digesters

	Concentration (mgl^{-1})				
	Initial Observed		Final Observed		Final Expected
Metal	Mixed Primary	Primary	Mixed Primary	Primary	Both Sludges
Cd	0.14	0.16	1.88	1.61	2.00
Cu	14.1	15.2	29.0	28.5	30.0
Ni	1.32	1.39	4.60	4.10	5.00
Pb	10.2	6.30	51.0	44.9	50.0
Zn	20.6	30.2	93.3	84.7	100

forms of the mixed primary and primary sludges are illustrated in Figures 20 and 21 respectively. It is apparent that Cd extracted by $Na_4P_2O_7$ predominated in both types of raw sludge. However, anaerobic digestion had the effect of reducing this fraction while simultaneously increasing the EDTA-extractable and HNO_3-extractable forms, with the EDTA-extractable fraction predominating in both types of digested sludge. In addition, anaerobic digestion increased the proportion of the total Cd content extracted by KNO_3 from the primary sludge, although such an effect was not apparent with respect to the mixed primary sludge.

The changes which occurred in the distribution of Pb following anaerobic digestion were very similar to those exhibited by Cd, except that slight increases in KNO_3-extractable forms of Pb were observed in both types of digested sludge.

The ranking of the most dominant forms between which Cu was distributed in both raw sludges was:

$$\text{EDTA} > HNO_3 > Na_4P_2O_7,$$

representing:

carbonate > sulphide > organically bound

However, the HNO_3 fraction was markedly increased as a consequence of anaerobic digestion and became predominant in both types of digested sludge, although more so in the mixed primary digested sludge. While the KNO_3, $Na_4P_2O_7$, EDTA and residual fractions were reduced following digestion, this process had a variable effect on the proportion of total Cu extracted by KF, depending on the sludge type.

Nickel extracted by KNO_3 was of particular significance with respect to the raw sludges, averaging 30% of the total Ni content of each. In addition, residual species were greater for Ni than for any other metal studied, this being particularly evident with respect to the mixed primary sludge in which these forms constituted 48% of the

Figure 20 Comparison of metal distributions in mixed primary raw (▨) and digested (☐) sludges

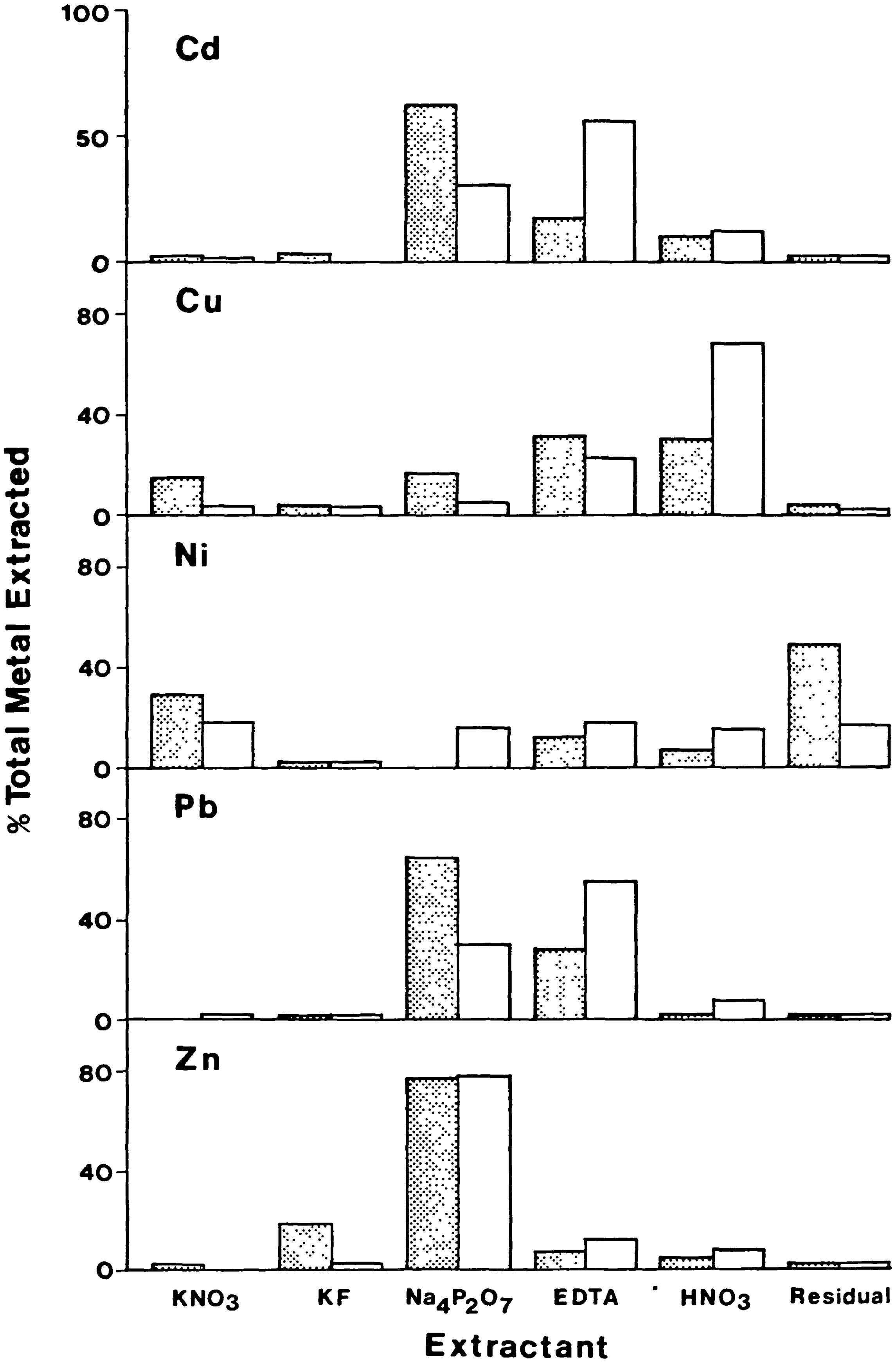
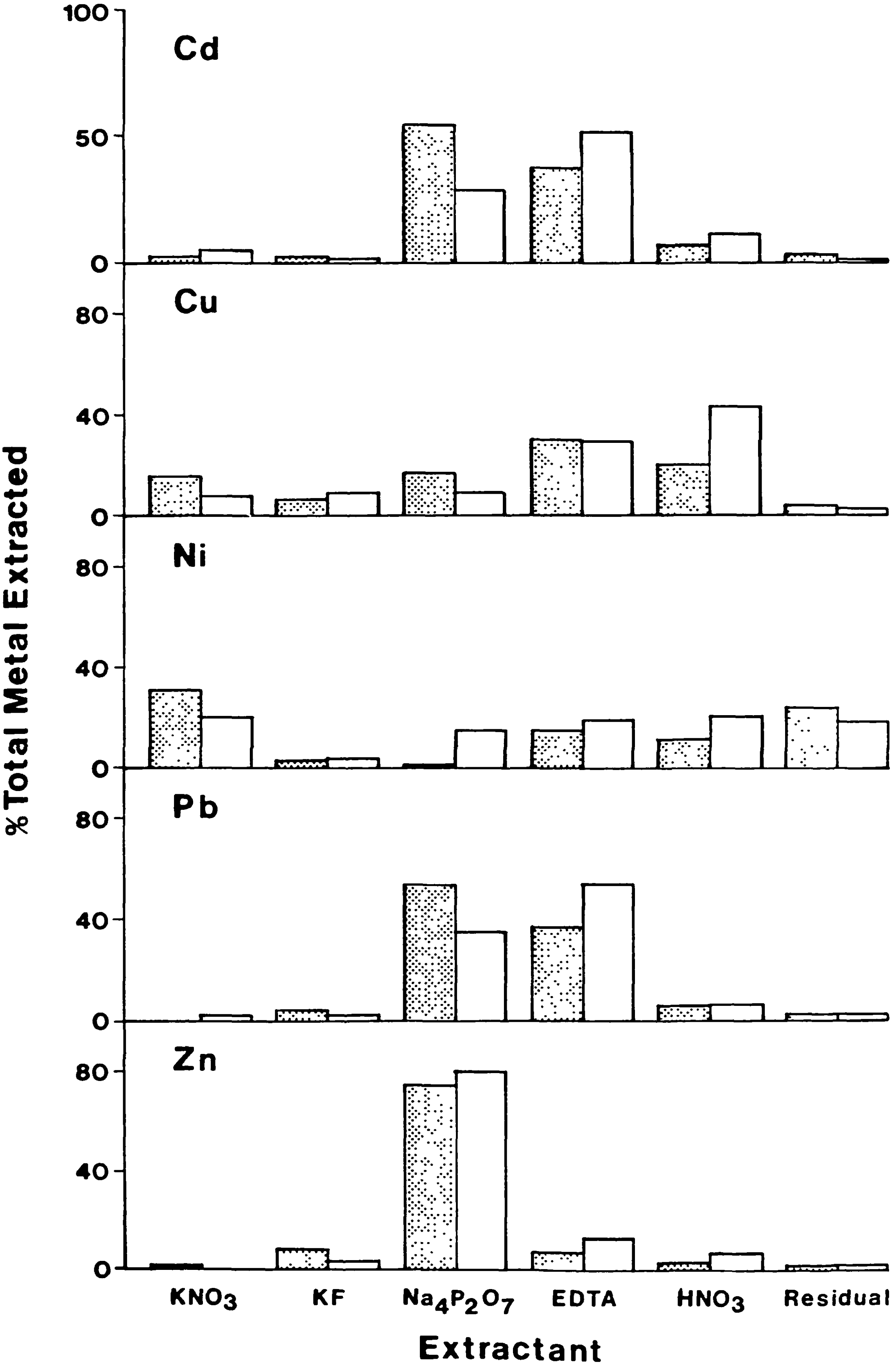


Figure 21 Comparison of metal distributions in primary raw (▨) and digested (☐) sludges



total Ni content. Anaerobic digestion caused a marked reduction in KNO_3-extractable and residual forms of Ni in both sludges, although these forms remained of greater significance for this metal than for Cd, Cu, Pb or Zn in the digested sludges. In addition, anaerobic digestion had the effect of increasing the $Na_4P_2O_7$-, EDTA- and HNO_3-extractable forms of Ni. Thus, overall, Ni appeared to redistribute evenly between all but the KF fraction in both the primary and mixed primary sludges as a result of digestion.

The KF fraction was of greater significance for Zn in the raw sludges than for any other metal studied, constituting approximately 18% of the total Zn content of the mixed primary raw sludge. Following digestion, however, KF-extractable and KNO_3-extractable forms of Zn were reduced, corresponding to slight increases in the EDTA and HNO_3 fractions. In contrast to the other metals studied, Zn remained predominantly in the $Na_4P_2O_7$ fraction in both the raw and digested sludges.

4.2.3 The effect of increasing total metal concentration on metal distribution

The 'experimental' digesters were spiked with metal in each mixed primary and primary feed for 40d and 32d respectively. Since the raw sludges from the two works possessed dissimilar indigenous total metal concentrations, these periods of metal dosing were selected to achieve comparable total metal concentrations (in terms of mgl^{-1}) at the end of the respective experimental periods (Table 18).

Total metal concentrations in the 'experimental' digesters increased steadily over the period of experimentation, while the 'control' digesters were maintained at inherent metal concentrations. Sum total metal concentrations recovered by sequential extraction analysis of digested sludge samples removed from the 'control' and 'experimental' digesters throughout the period of metal addition were consistently within ± 10% of the total metal concentrations determined. Mean recoveries for individual metals are presented in Table 19. In contrast to the other metals studied, the sum total Zn recovered by sequential extraction consistently exceeded respective deter-

Table 19 Percentage of total metal recovered by sequential extraction over the period of metal addition (mean ± SD)

	Mean Recovery (%)			
	'Experimental' Digesters		'Control' Digesters	
Metal	Mixed Primary Sludge (n = 11)	Primary Sludge (n = 9)	Mixed Primary Sludge (n = 6)	Primary Sludge (n = 5)
Cd	98.7 ± 6.57	101.4 ± 1.94	95.5 ± 4.68	93.6 ± 3.58
Cu	100.4 ± 2.42	98.0 ± 2.29	99.5 ± 2.66	99.6 ± 4.62
Ni	94.1 ± 4.80	97.4 ± 4.10	88.8 ± 4.49	97.0 ± 1.87
Pb	97.8 ± 1.72	100.1 ± 2.37	96.5 ± 1.38	97.8 ± 2.68
Zn	105.0 ± 2.69	107.4 ± 3.17	104.2 ± 5.12	101.6 ± 1.82

mined totals.

To elucidate the effects of increasing total metal concentration on metal distribution between individual fractions, Figures 22-29 present the fractions as percentages of the total metal determined on the respective effluent sludges. In addition, the overall effects of increasing total metal concentration on the actual concentrations in individual fractions are presented in Appendix IV. In all cases, an assessment of the relationship between total metal concentration and concentrations in individual fractions (both in terms of $mgkg^{-1}$ dry solids) was undertaken by linear regression analysis (Bowker & Lieberman, 1972).

The resulting correlation coefficients and analysis of variance (Bowker & Lieberman, 1972) for each regression are presented in Tables 20 and 21. Linear regression analyses were also performed to statistically evaluate the relationship between the different fractions among which the metals distributed throughout the period of metal addition, the resulting correlation coefficients and analyses of variance being presented in Tables 22 and 23.

Metal distribution values obtained for the 'control' digesters were consistent (Appendix V), generally exhibiting RSDs of <10%.

4.2.3.1 Cadmium.

Increasing the total Cd concentration in the 'experimental' digesters had markedly different effects on the distribution of certain fractions in the two digested sludge types (Figures 22 and 23). Most notably, the considerable increase in the proportion of total Cd present in the KNO_3-extractable form in the mixed primary digested sludge was in complete contrast to the trend exhibited by this fraction in the primary digested sludge. In addition, the reduction in the $Na_4P_2O_7$ fraction at a total Cd concentration of approximately 50 $mgkg^{-1}$ in the former sludge was not exhibited by the latter. However, trends exhibited by the KF, EDTA, HNO_3 and residual fractions as a result of increasing total Cd concentration were similar in both sludges. Thus, increases in the percentage of total Cd present in the

Figure 22 Effect of increasing total Cd concentration on Cd distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in mixed primary digested sludge

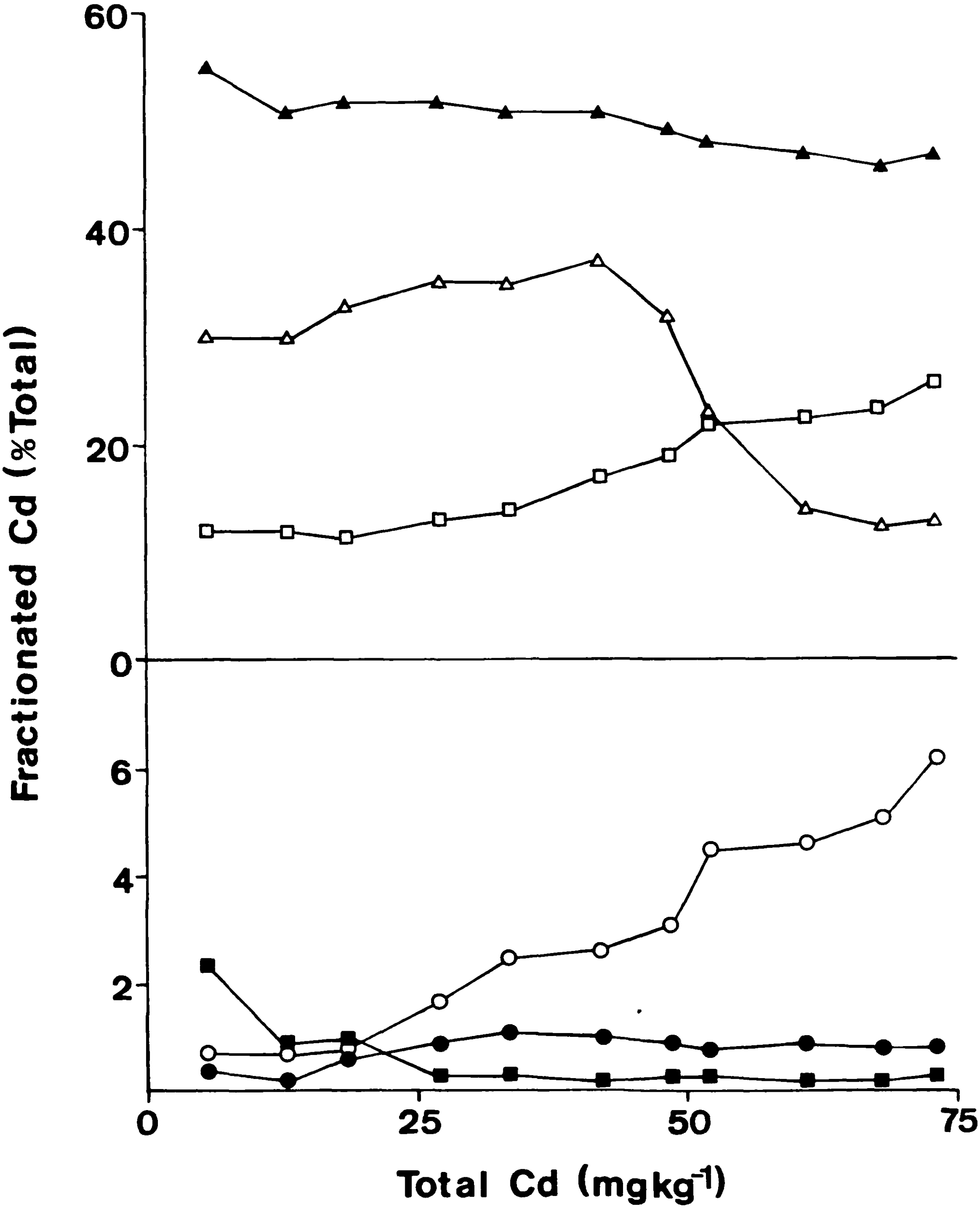


Figure 23 Effect of increasing total Cd concentration on Cd distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in primary digested sludge

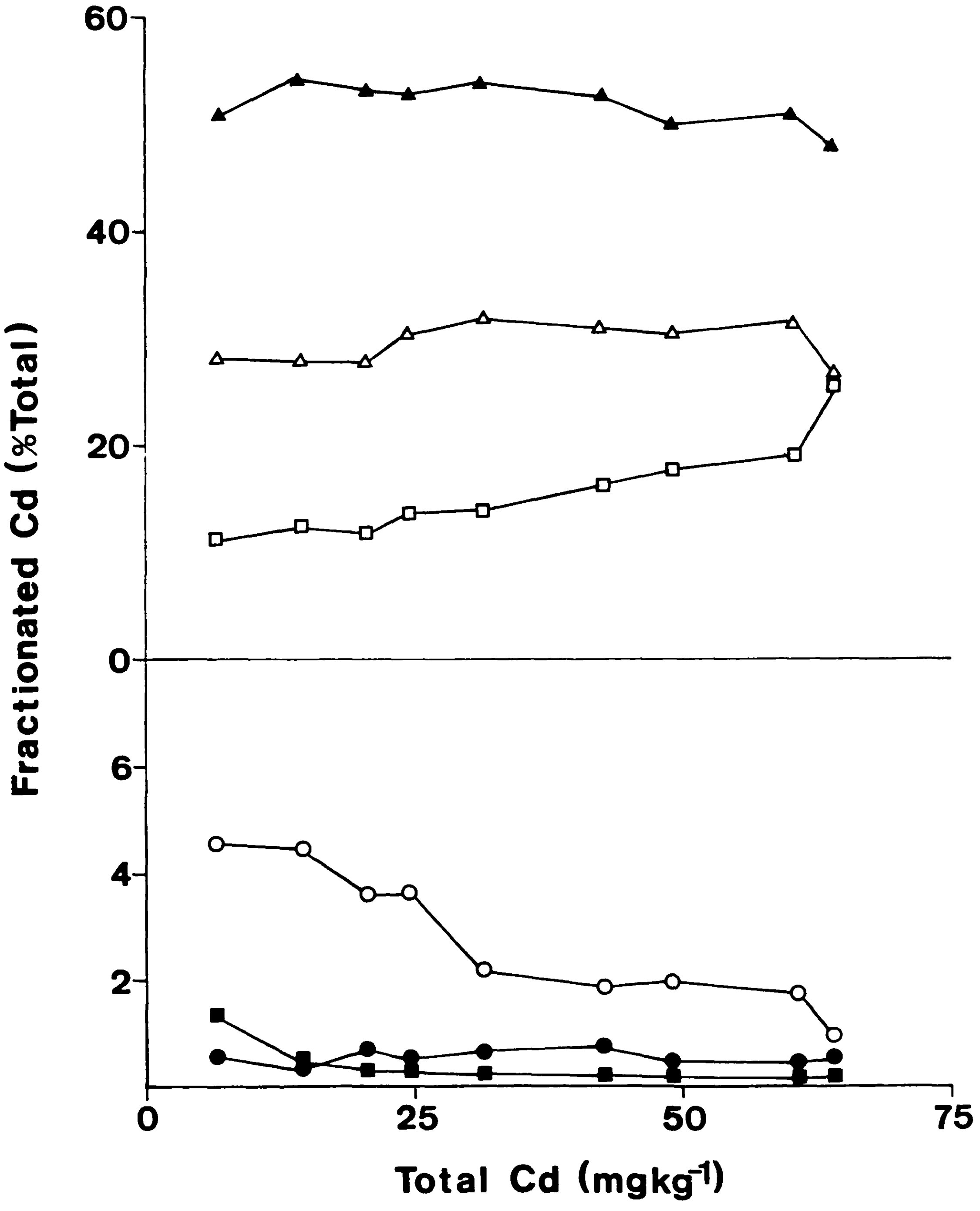


Figure 24 Effect of increasing total Ni concentration on Ni distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in mixed primary digested sludge

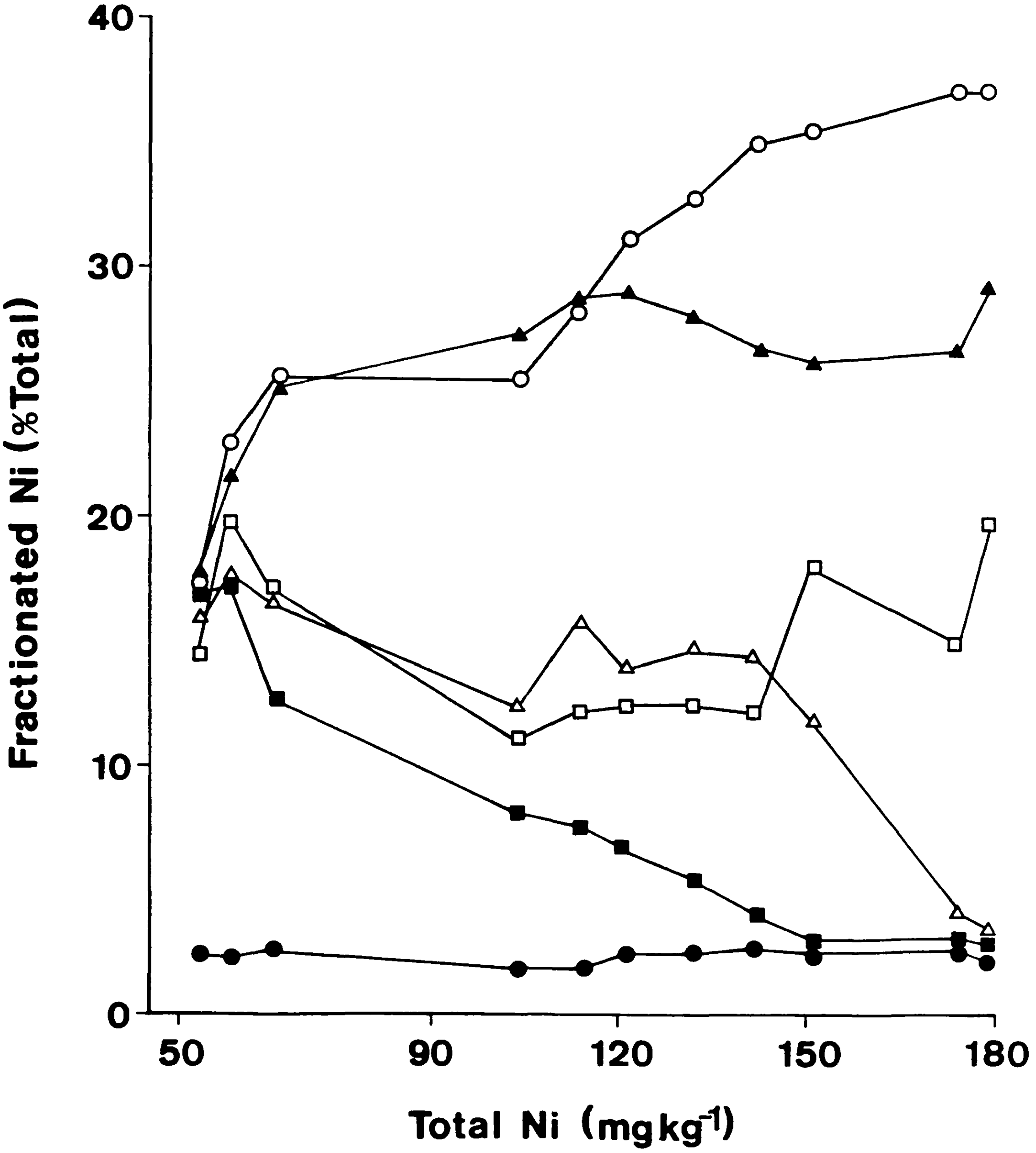


Figure 25 Effect of increasing total Ni concentration on Ni distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in primary digested sludge

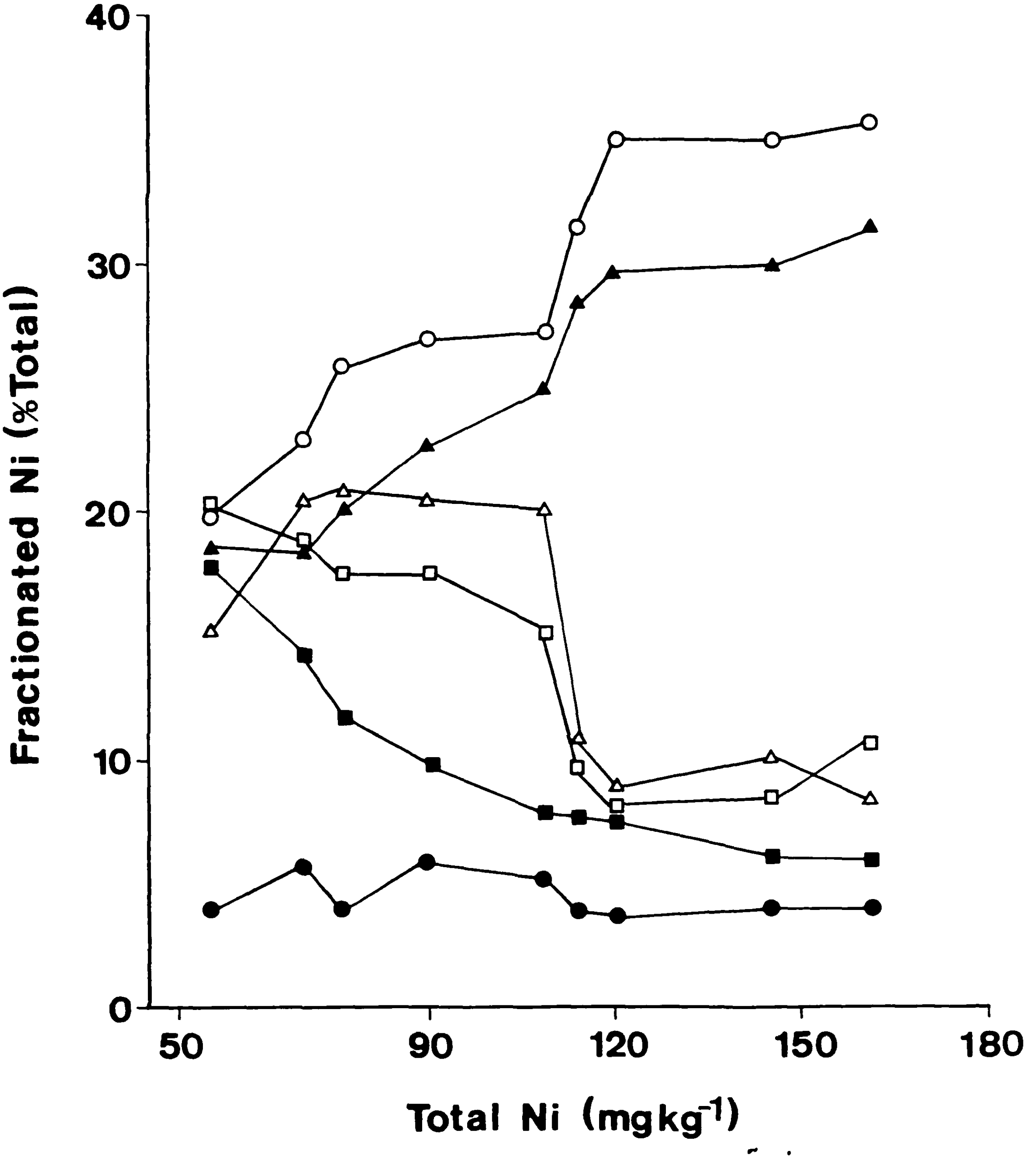


Figure 26 Effect of increasing total Pb concentration on Pb distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in mixed primary digested sludge

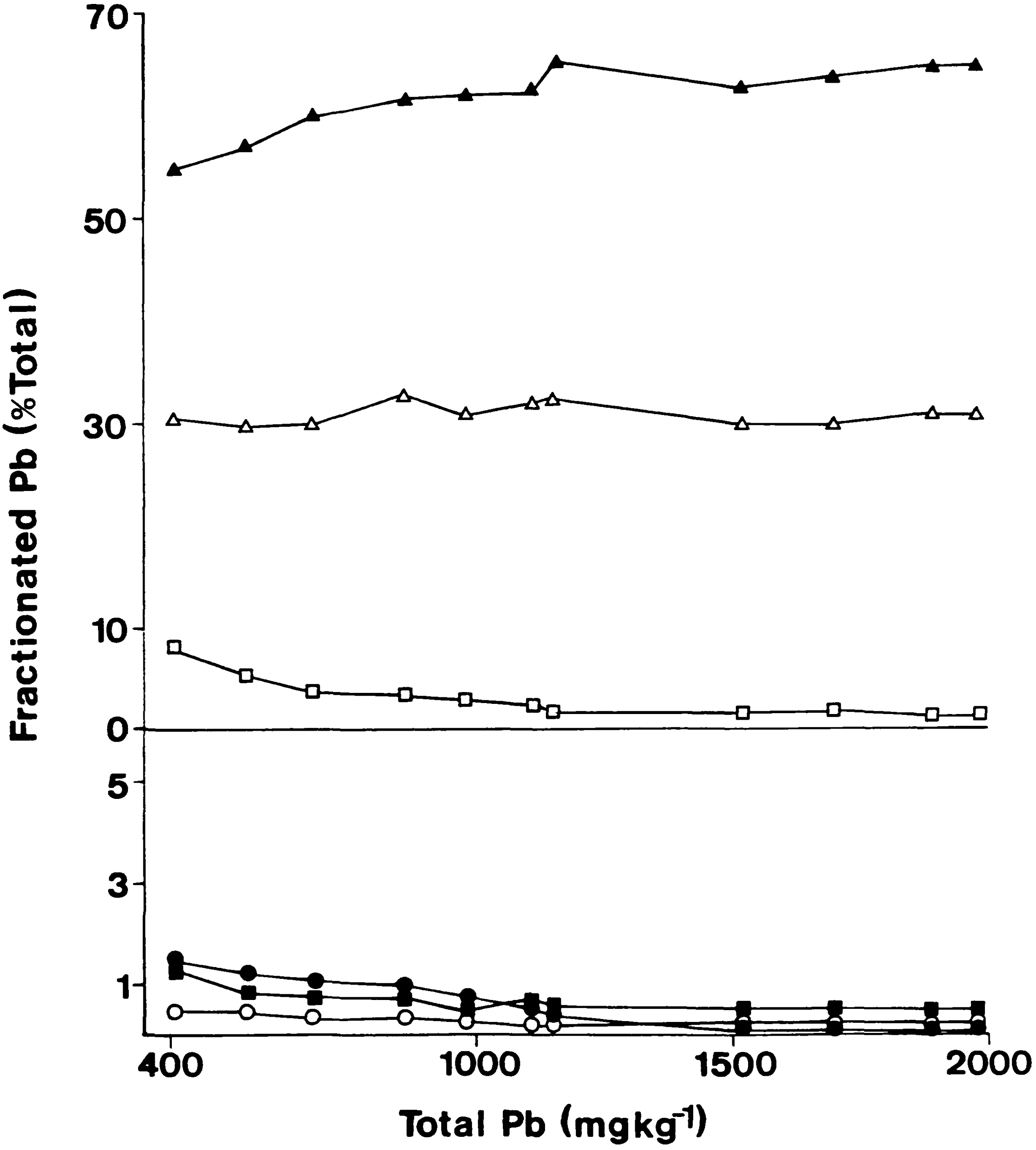


Figure 27 Effect of increasing total Pb concentration on Pb distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in primary digested sludge

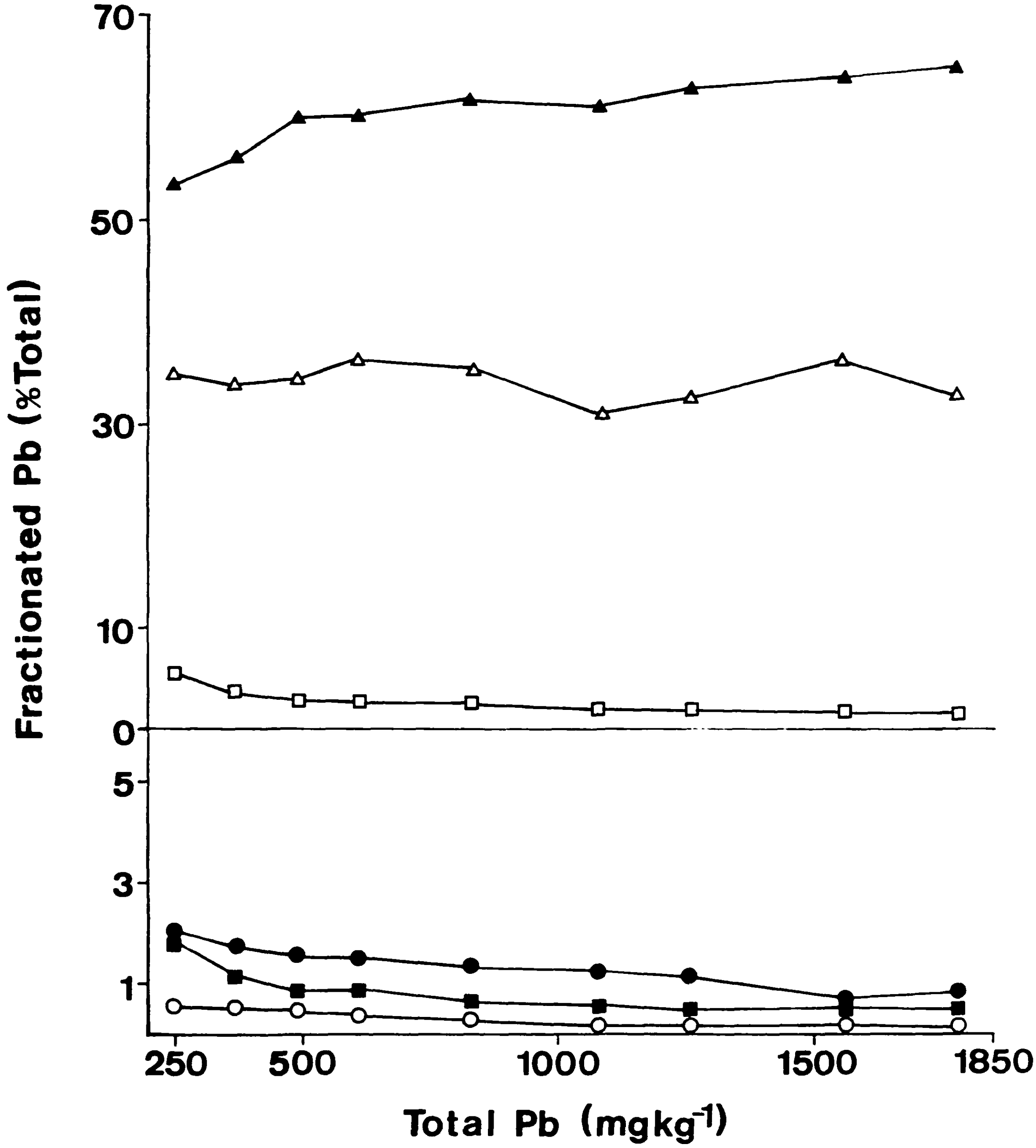


Figure 28 Effect of increasing total Zn concentration on Zn distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in mixed primary digested sludge

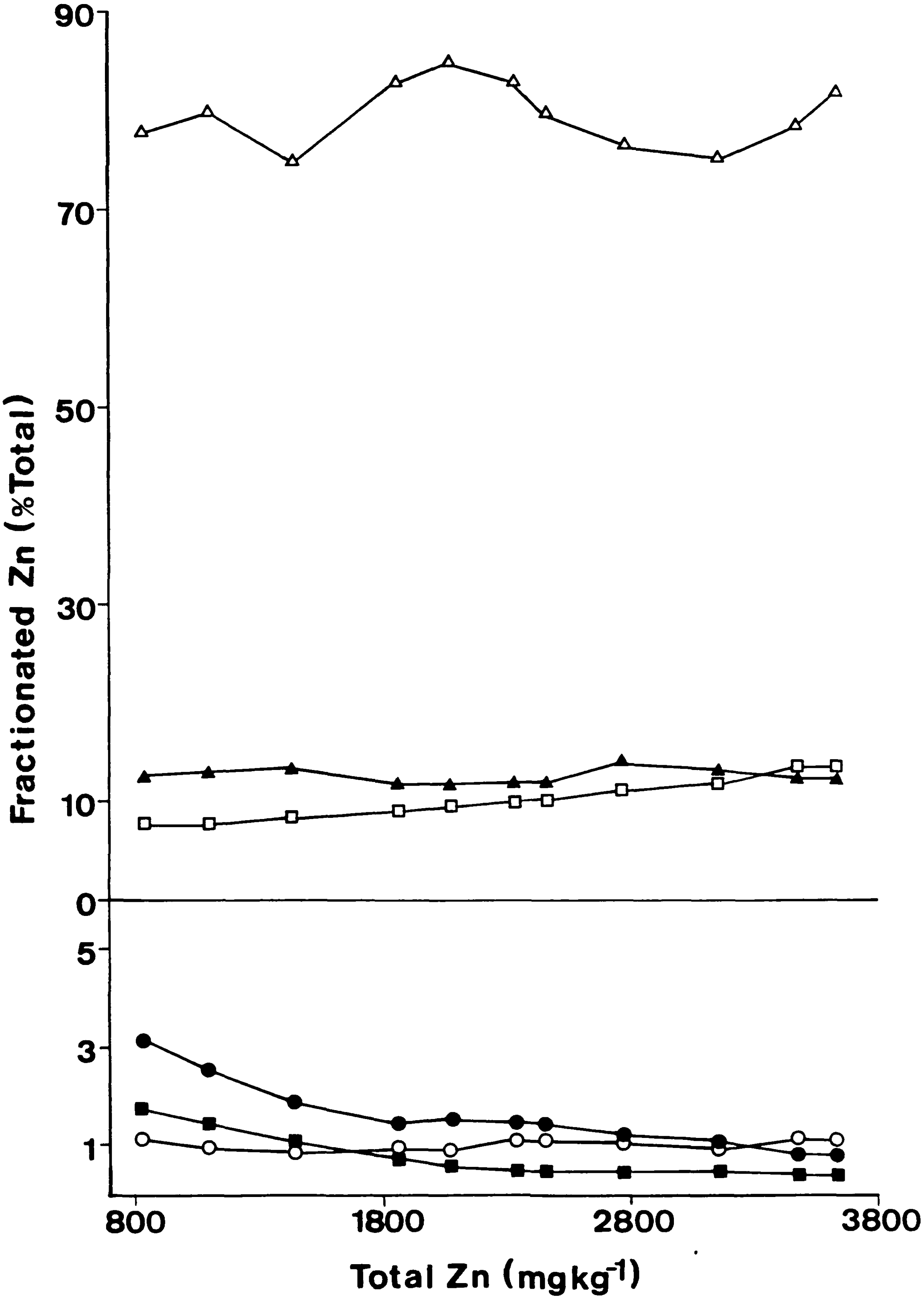


Figure 29 Effect of increasing total Zn concentration on Zn distribution between KNO_3 - (○), KF - (●), $Na_4P_2O_7$ - (△), EDTA - (▲) and HNO_3 - (□) extractable and residual (■) forms in primary digested sludge

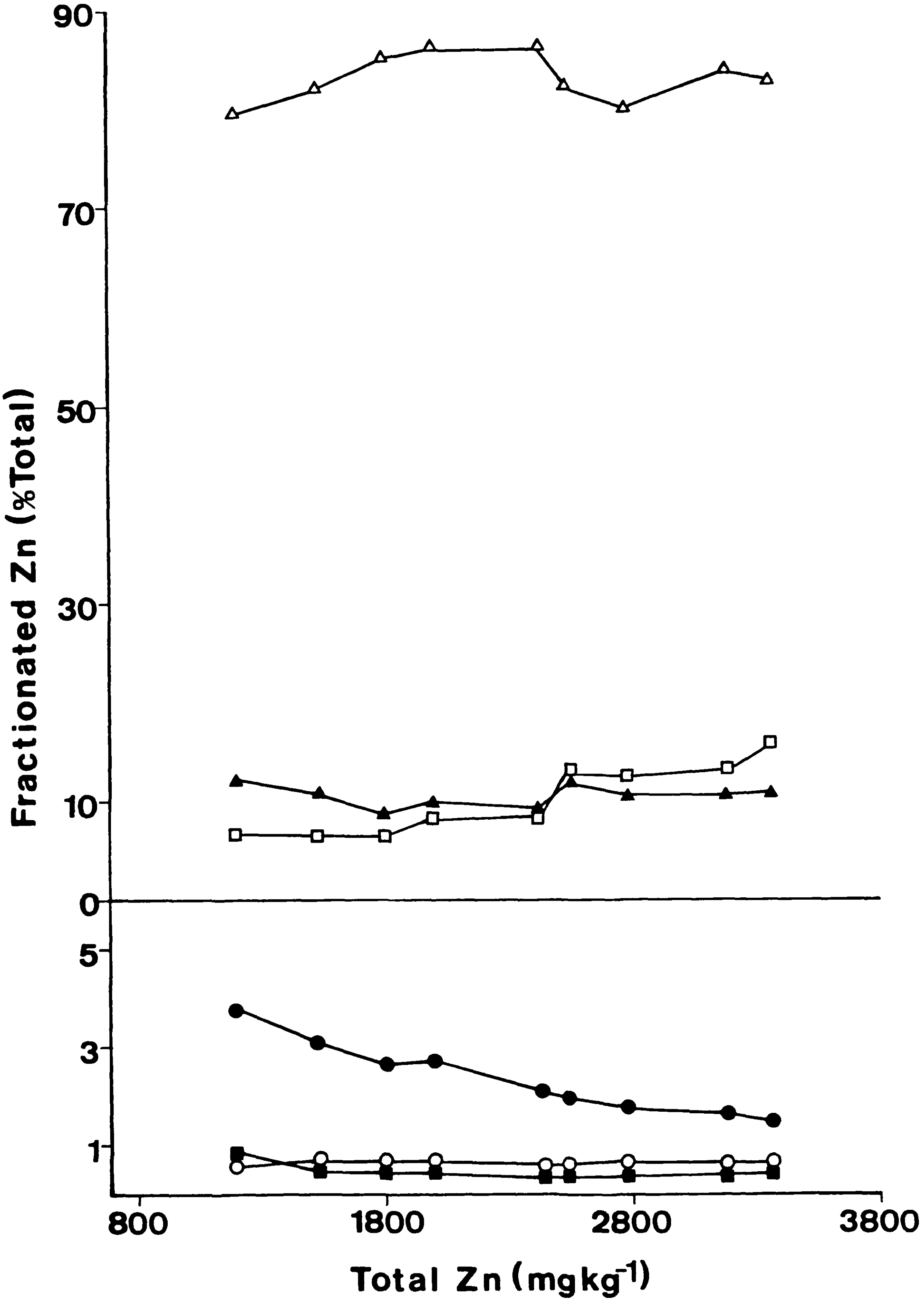


Table 20 Linear regression analysis of total versus fractionated metal in mixed primary digested sludge

	Total Metal				
Fraction	Cd	Cu	Ni	Pb	Zn
KNO_3	0.950**	0.300	0.993**	0.570	0.986**
KF	0.975**	0.371	0.930**	-0.868**	0.338
$Na_4P_2O_7$	0.537	0.544	0.137	0.997**	0.995**
EDTA	0.998**	0.685*	0.991**	1.000**	0.988**
HNO_3	0.979**	0.788**	0.884**	-0.034	0.988**
Residual	0.313	0.559	-0.858**	0.576	-0.423

Table 21 Linear regression analysis of total versus fractionated metal in primary digested sludge

	Total Metal				
Fraction	Cd	Cu	Ni	Pb	Zn
KNO_3	0.836**	-0.369	0.993**	0.710*	0.920**
KF	0.952**	-0.255	0.844**	0.890**	0.459
$Na_4P_2O_7$	0.989**	-0.143	0.114	0.994**	0.997**
EDTA	0.997**	0.536	0.994**	1.000**	0.963**
HNO_3	0.962**	0.886**	0.282	0.954**	0.971**
Residual	0.880**	0.819**	-0.405	0.977**	0.709*

*,** = Correlation coefficients (r values) significant at the 0.05 and 0.01 levels respectively

Table 22 Linear regression analysis between different metal fractions in mixed primary digested sludge

	<u>r</u> values for metals			
	Cd	Ni	Pb	Zn
Fractions Regressed	KNO_3			
KF	0.884**	0.951**	-0.262	0.266
$Na_4P_2O_7$	0.288	0.095	0.581	0.979**
EDTA	0.934**	0.977**	0.570	0.975**
HNO_3	0.991**	0.910**	-0.155	0.977**
Residual	0.512	-0.889**	0.443	-0.461
	KF			
$Na_4P_2O_7$	0.644*	0.175	-0.851**	0.348
EDTA	0.983**	0.892**	-0.865**	0.311
HNO_3	0.923**	0.788**	-0.122	0.220
Residual	0.191	-0.872**	-0.398	-0.724*
	$Na_4P_2O_7$			
EDTA	0.581	0.161	0.998**	0.969**
HNO_3	0.384	-0.173	-0.080	0.980**
Residual	-0.190	-0.089	0.582	-0.463
	EDTA			
HNO_3	0.968**	0.867**	-0.043	0.977**
Residual	0.293	-0.811**	0.579	-0.332
	HNO_3			
Residual	0.451	-0.871**	-0.038	-0.353

*,** = correlation coefficients (<u>r</u> values) significant at the 0.05 and 0.01 levels respectively

Table 23 Linear regression analysis between different metal fractions in primary digested sludge

Fractions Regressed	*r* values for metals			
	Cd	Ni	Pb	Zn
	KNO_3			
KF	0.744*	0.791*	0.574	0.333
$Na_4P_2O_7$	0.852**	0.150	0.756*	0.894**
EDTA	0.850**	0.997**	0.708*	0.922**
HNO_3	0.729*	0.193	0.657	0.927*
Residual	0.630	-0.372	0.644	0.701*
	KF			
$Na_4P_2O_7$	0.933**	0.508	0.848**	0.506
EDTA	0.953**	0.796*	0.885**	0.357
HNO_3	0.918**	0.624	0.932**	0.323
Residual	0.800**	-0.418	0.825**	0.102
	$Na_4P_2O_7$			
EDTA	0.996**	0.012	0.994**	0.946**
HNO_3	0.914**	0.717*	0.936**	0.955**
Residual	0.862**	-0.529	0.976**	0.679*
	EDTA			
HNO_3	0.940**	0.208	0.947**	0.993**
Residual	0.859**	-0.368	0.977**	0.762*
	HNO_3			
Residual	0.904**	-0.115	0.906**	0.772*

*,** = correlation coefficients (*r* values) significant at the 0.05 and 0.01 levels respectively

HNO_3 fraction were simultaneously accompanied by reductions in the EDTA and residual fractions, while the KF fraction remained reasonably consistent throughout the experimental period. With the exceptions of the $Na_4P_2O_7$ and residual fractions in the mixed primary sludge, all of the individual fractions were linearly correlated to the total Cd concentrations in both sludge types (Tables 20 and 21). Linear correlations were similarly demonstrated between the KNO_3, KF, EDTA and HNO_3 fractions and between the KF and $Na_4P_2O_7$ fractions with respect to the mixed primary sludge (Table 22), while all but the KNO_3 and residual fractions were correlated in the primary sludge (Table 23).

4.2.3.2 Copper

The distribution patterns observed for Cu in the 'experimental' digesters throughout the period of metal addition are not illustrated due to the lack of significant correlations between total Cu concentrations and concentrations in the individual fractions (Tables 20 and 21). This is likely to be due to the random variation in concentration data obtained for specific fractions and the limited increase in total Cu concentration which could be undertaken due to the high inherent sludge Cu content. Overall, however, the ranking of Cu fractions appeared to be little affected by increasing total Cu concentrations in either sludge, with the HNO_3 fraction predominating throughout.

4.2.3.3 Nickel

From an appraisal of Figures 24 and 25 it is evident that increasing the total Ni concentration in the 'experimental' digesters had the effect of increasing the percentage of this metal present in KNO_3 and EDTA fractions in both sludges, the percentage of Ni present in the former fraction being essentially doubled over the experimental period. In addition, both sludges exhibited overall reductions in the percentage of Ni present in the $Na_4P_2O_7$ and residual fractions, while the KF fraction remained reasonably constant. It is apparent, however, that conflicting trends were observed between the two sludges with respect to the HNO_3 fraction. The progressive increase in the concentration of Ni present in the HNO_3 fraction in the mixed primary digested sludge was demonstrated to be linearly correlated to the total Ni concentration (Table 20) and to the concentration of Ni present in all but the $Na_4P_2O_7$ fraction (Table 22). In contrast, however, the concentration of Ni present in the HNO_3 fraction in the primary digested sludge was extremely variable throughout the experimental period. Hence, this fraction was correlated only to the $Na_4P_2O_7$ fraction (Tables 21 and 23).

4.2.3.4 Lead

The distribution of Pb among the individual fractions was not markedly affected by increasing total concentrations in either sludge (Figures 26 and 27). Overall, however, there was a redistribution of Pb away from the KNO_3, KF, HNO_3 and residual forms towards $Na_4P_2O_7$ and EDTA forms as the total concentration in each sludge increased, the latter fraction increasing in predominance throughout. Unfortunately, no significant linear correlations were observed between total Pb and KNO_3, HNO_3 and residual fractions of the mixed primary digested sludge (Table 20), the concentrations of Pb in these fractions remaining reasonably constant despite increasing total concentrations (Appendix IV). Consequently, only three significantly linear correlations were observed between the fractions, with the $Na_4P_2O_7$ and EDTA fractions being negatively correlated to the KF fraction but positively correlated to one another (Table 22). In contrast, however, all of the individual fractions were positively correlated to total Pb with respect to the primary digested sludge (Table 21). In addition, all of the individual fractions were linearly related to one another, with the exception of the KNO_3 fraction, which was significantly correlated to neither the KF, HNO_3 nor residual fractions (Table 23).

4.2.3.5 Zinc

Similar to Pb, the distribution patterns of Zn in the two digested sludges were little affected by increasing total concentrations (Figures 28 and 29). Zinc extracted by $Na_4P_2O_7$ remained pre-eminent throughout the experimental period, this fraction being of greater significance with respect to Zn than for any of the other metals studies. It was apparent, however, that the rank order of the other fractions was slightly altered in both sludge types from EDTA > HNO_3 > KF > residual > KNO_3, at the beginning of the experimental period, to HNO_3 > EDTA > KF > KNO_3 > residual (primary sludge) and HNO_3 > EDTA > KNO_3 > KF > residual (mixed primary sludge) at the end of the experimental period. Thus overall, a redistribution away from the KNO_3, KF, EDTA and residual forms towards HNO_3 and $Na_4P_2O_7$ fractions was evident. Trends exhibited by the majority of these fractions, with the exception of the KF fractions in both sludges in addition to the residual fraction in the mixed primary sludge, were, in terms of concentration, significantly correlated to the total Zn (Tables 20 and 21). In addition, significant positive correlations were observed between the KNO_3, $Na_4P_2O_7$, EDTA, HNO_3 and residual fractions in the primary sludge as the total Zn concentration increased (Table 23). With respect to the mixed primary sludge, however, the KNO_3, $Na_4P_2O_7$, EDTA and HNO_3 fractions were positively correlated to one another while the KF and residual fractions were negatively correlated (Table 22).

4.2.4 The effects of metal concentration on digester performance

The effects of increasing total concentrations of Cd, Cu, Ni, Pb and Zn on digester performance were assessed through routine monitoring of process parameters (Section 3.3.3) in both the 'control' and 'experimental' digesters of each sludge type throughout the period of metal addition. Results obtained for each digester with respect to pH value, total solids concentration, volatile solids reduction, alkalinity and gas composition are presented in Appendix VI. Since inhibition of anaerobic digestion by toxic substances is generally exemplified by a reduction in gas production and an increase in volatile acids concentration (Kirk et al., 1982), values obtained for these two process parameters in the digesters receiving mixed primary and primary sludges are illustrated in Figures 30 and 31 respectively.

It is evident from Table 39 (Appendix VI) and Figure 31 that the 'experimental' digester receiving primary sludge was not adversely affected by increasing metal concentrations. Trends exhibited in terms of gas production and composition, pH, total solids con-

Figure 30 Gas production (▲) and volatile acids concentration (■) in 'control' (---) and 'experimental' (—) anaerobic digesters receiving mixed primary sludge throughout the period of metal addition

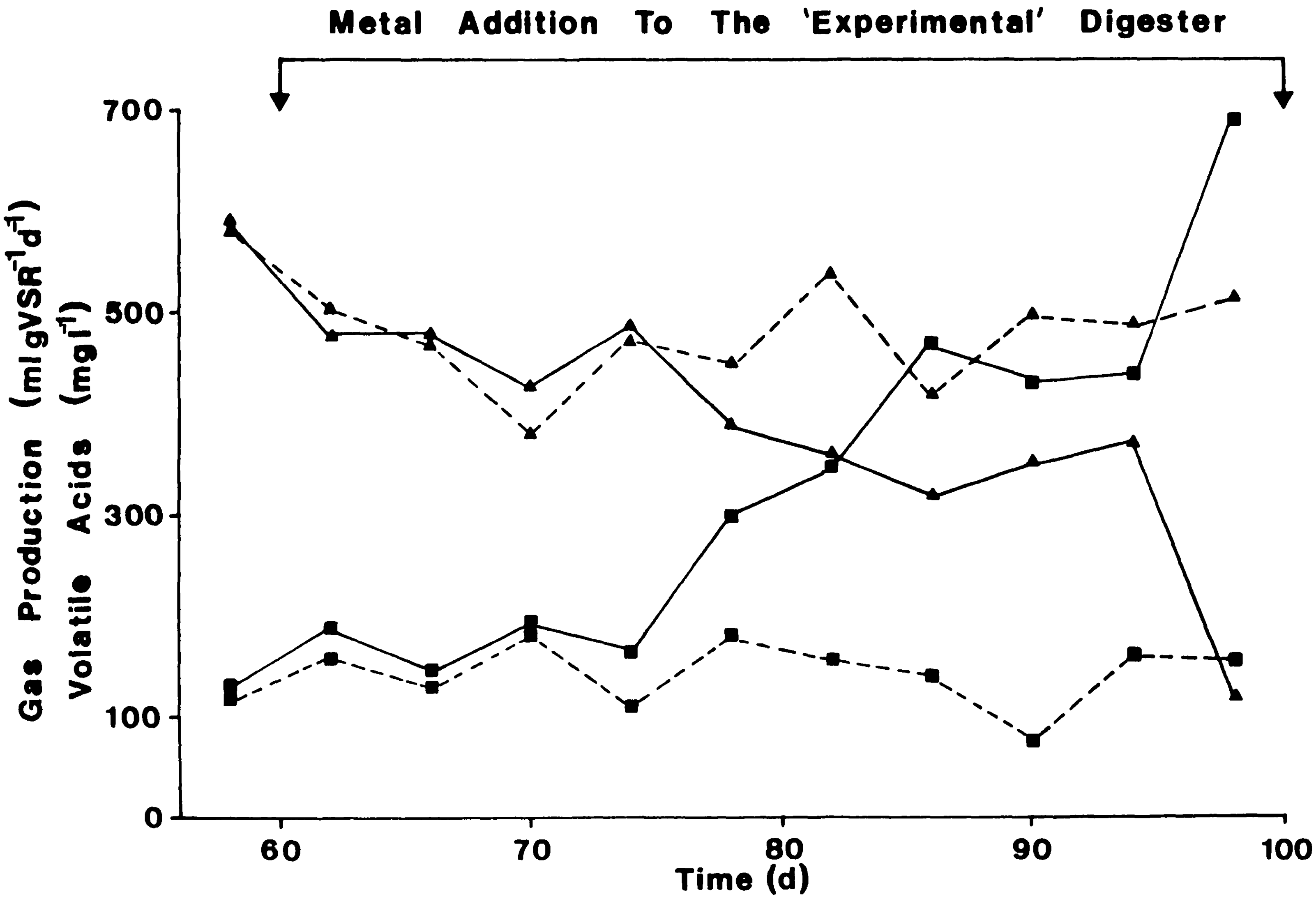
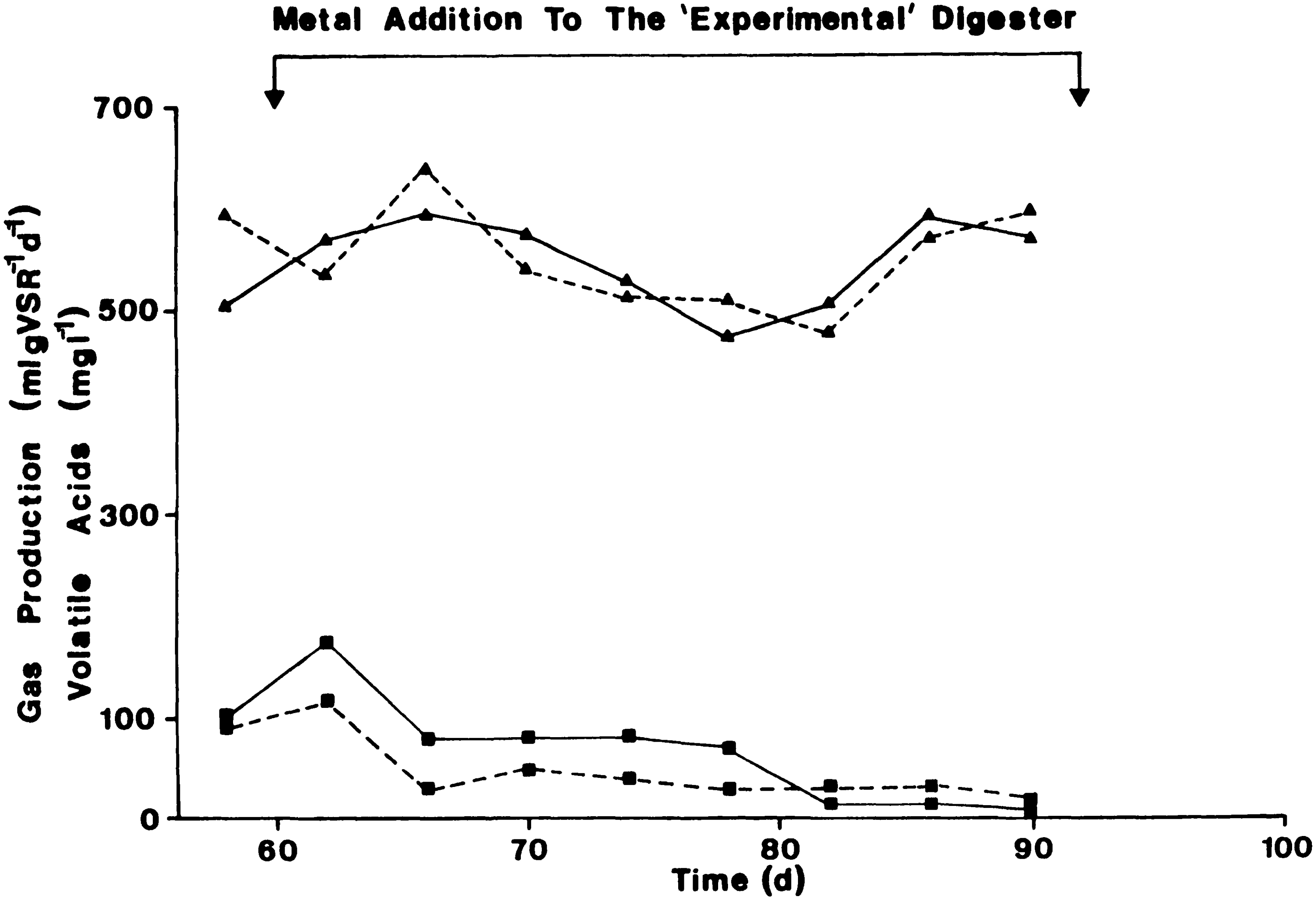


Figure 31 Gas production (▲) and volatile acids concentration (■) in 'control' (---) and 'experimental' (—) anaerobic digesters receiving primary sludge throughout the period of metal addition



centration, volatile solids reduction and alkalinity were similar in both the 'control' and 'experimental' digesters throughout the period of metal dosing. In addition, volatile acids concentrations remained consistently below 200 mgl^{-1} in both digesters, with the values obtained for the 'experimental' digester actually decreasing to slightly below those of the 'control' digester towards the end of the period of metal dosing.

In contrast, however, the volatile acids concentration in the 'experimental' digester receiving mixed primary sludge exhibited a marked increase from 164 mgl^{-1} on day 74 to 690 mgl^{-1} on day 98, which coincided with a reduction in gas production from 479 to 116 ml g VSR^{-1} d^{-1} over the same period (Figure 30). Concentrations of Cd, Cu, Ni, Pb and Zn present in the 'experimental' digester increased from approximately 30.0 to 71.0, 776 to 1120, 109 to 117, 920 to 1940 and 1970 to 3550 $mgkg^{-1}$ respectively from day 74 to day 98. Meanwhile, the 'control' digester displayed no such effects, consistently producing less than 250 mgl^{-1} of volatile acids but greater than 375 ml g VSR^{-1} d^{-1} of gas. Such discrepancies between the 'control' and 'experimental' digesters receiving mixed primary sludge suggest that the latter digester was exhibiting preliminary signs of inhibition as a result of heavy metal toxicity.

4.3 Elucidation of Metal-Solids Associations in Sewage Sludges

4.3.1 Investigation of sludge solids and heavy metal distributions using particle size filtration

Prior to determination of complexation parameters for complex formation between Cd, Cu, Pb and sludge solids of varying particle size it was necessary to evaluate the individual distributions of these metals and the total solids in each of the selected mixed primary raw, activated and digested sludge samples according to particle size. The resulting distributions among the < 0.22 µm, 0.22 - 10 µm, 10 - 100 µm, 100 - 1000 µm and > 1000 µm particle size fractions, expressed as percentages of the total solids and metal concentrations determined in bulk samples of each sludge type (Table 24), have been presented in Figure 32. Due to the large volumes of undiluted activated sludge required for filtration it was necessary to collect a second sample from Hogsmill Valley Sewage Treatment Works in order to study Pb complexation in this sludge type. Although the two activated sludge samples differed in terms of total solids concentration (Table 24), the particle size distributions were similar (Figure 32).

Table 24 Total solids and metal concentrations determined in mixed primary raw, activated and digested sewage sludges

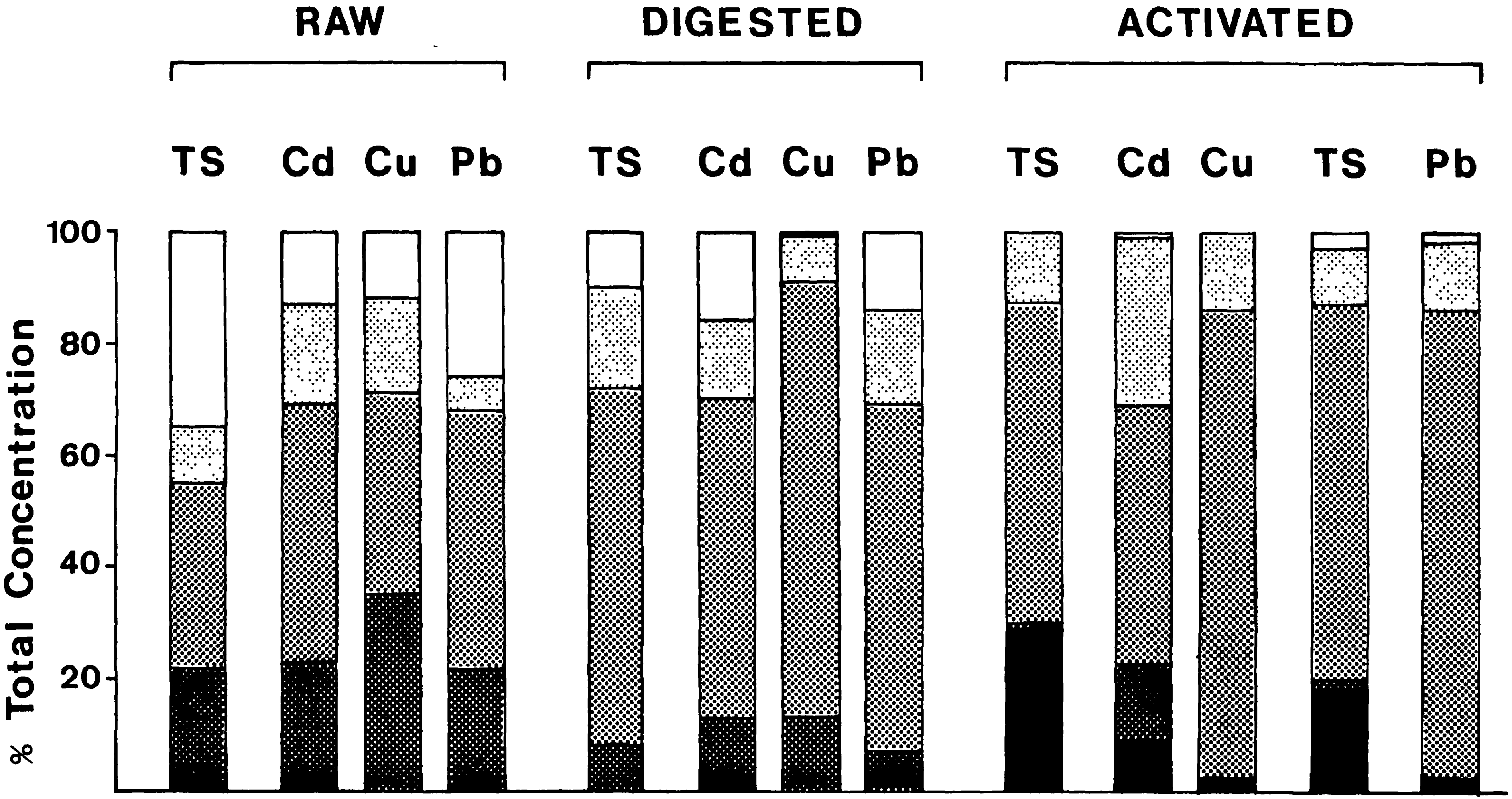
Sludge Type	Total solids (gl^{-1})	Metal concentration (mgl^{-1})		
		Cd	Cu	Pb
Raw	25.6	0.08	5.84	3.80
Activated I	2.12	0.01	0.78	-
Activated II	4.19	-	-	0.70
Digested	27.1	0.16	10.5	8.00

It is evident from Figure 32 that the solids content of the mixed primary raw sludge was fairly evenly distributed between the > 1000 µm, 100-1000 µm, 10-100 µm and 0.22 - 10 µm particle size fractions, with no one fraction predominating. Following sludge treatment, however, substantially greater proportions of the total solids were found to be less than 1000 µm in size, the 10-100 µm fraction predominating in both the activated and digested sludge samples. In all three sludge types, less than 30% of the total solids passed through the 10 µm filter, with significant quantities of dissolved solids being found in the activated sludge samples only.

The resulting metal fractionations (Figure 32) indicated that in all three sludge types Cu and Pb were present exclusively in the insoluble phase (ie. associated with particles of > 0.22 µm in diameter). In contrast, however, Cd was found to be present in the soluble phase, although this fraction represented only 9% of the total Cd content of the activated sludge and only 1% of that of both the raw and digested sludges.

The percentages of all three metals observed in the 0.22 - 10 µm

Figure 32 Distributions of the total solids (TS), Cd, Cu and Pb content of mixed primary raw, activated and digested sludges among the <0.22 µm (■), 0.22-10 µm (▩), 10-100µm (▨), 100-1000 µm (░) and >1000 µm (□) particle size fractions



fraction were lower in the activated and digested sludges than in the raw sludge. However, relatively higher percentages were generally observed to be associated with particles in excess of 10 µm, especially in the 10 - 100 µm range, in the activated and digested sludges than in the raw sludge. Such differences indicated that Cd, Cu and Pb preferentially associated with the relatively larger sludge particles during treatment. This was particularly apparent with respect to Cu and Pb in activated sludge.

It would thus appear that solids distribution is an important factor influencing the establishment of heavy metal fractionation equilibria in sewage sludges. In order to verify this, linear regression analyses between the solids concentration and the concentration of each of the three metals determined in each sludge sample and each filtrate produced by membrane filtration were performed. The resulting correlation coefficients (r-values), presented in Table 25, were consistently above + 0.970, indicating that a direct proportionality existed between the Cd, Cu and Pb concentrations and the corresponding total solids concentrations of bulk samples and filtrates of each sludge type.

Table 25 Correlation coefficients (r-values) determined for heavy metal concentrations of bulk sludge samples and filtrates versus respective total solids concentrations

Sludge Type	Metal		
	Cd	Cu	Pb
Raw	0.973	0.969	0.988
Activated	0.983	0.999	0.999
Digested	0.998	0.989	0.999

4.3.2 Determination of metal-sludge complexation parameters

Conditional stability constants (K') and complexation capacities

([L']) were determined in order to quantitatively assess the characteristics of complexes formed between Cd, Cu and Pb and sludge solids of varying particle size. Values of $[M_F]$ and $[M_B]$ obtained over a range of total metal concentrations by ISE determinations were used to calculate K' and [L'] values from a graphical solution of equation (6) (Section 3.2.4). Plotting $[M_F]/[M_B]$ against $[M_F]$ produced either a single straight line, with a slope of 1/[L'] and an intercept of 1/K' [L'], or a bi-phasic straight line relationship. Examples of both types of plot are presented in Figures 33 and 34 respectively. Resultant values of log K' and [L'] for complexes formed between Cd, Cu and Pb and solids of varying particle sizes in mixed primary raw, activated and digested sludges are presented in Tables 26-28. Values of [L'] obtained for the various samples have been expressed as the number of available binding sites per unit mass of total solids to enable assessment of the effect of the size of particles on this parameter.

Generally, two types of complex were formed by each metal, the second having relatively lower conditional stability constant values than the first (ie. log K'_2 values were lower than log K'_1 values). This indicates that at low metal ion concentrations the complexation was controlled mainly by stronger binding sites whereas at high metal ion concentrations the stronger sites became fully occupied and weaker sites started to effect complexation. However, the concentrations of binding sites available for participation in the second type of complex (ie. $[L'_2]$) were greater than those available for participation in the first type (ie. $[L'_1]$). Values of K'_1 and K'_2 obtained for Cu in the activated and digested sludges differed by approximately two orders of magnitude, whereas those obtained for Cu in the raw sludge and Cd and Pb in all three sludge types differed by less than one order of magnitude. This indicates that the two groups of ligands involved in Cu binding in the activated and digested sludges were more clearly distinguishable than those involved in all other complex formation.

It is clearly apparent from Tables 26-28 that the order of the stability of complexes formed in each sludge type was Cu>Pb>Cd.

Figure 33 Plot of free Pb/complexed Pb against free Pb over a range of concentrations in a 1000 μm filtrate sample of a raw sludge

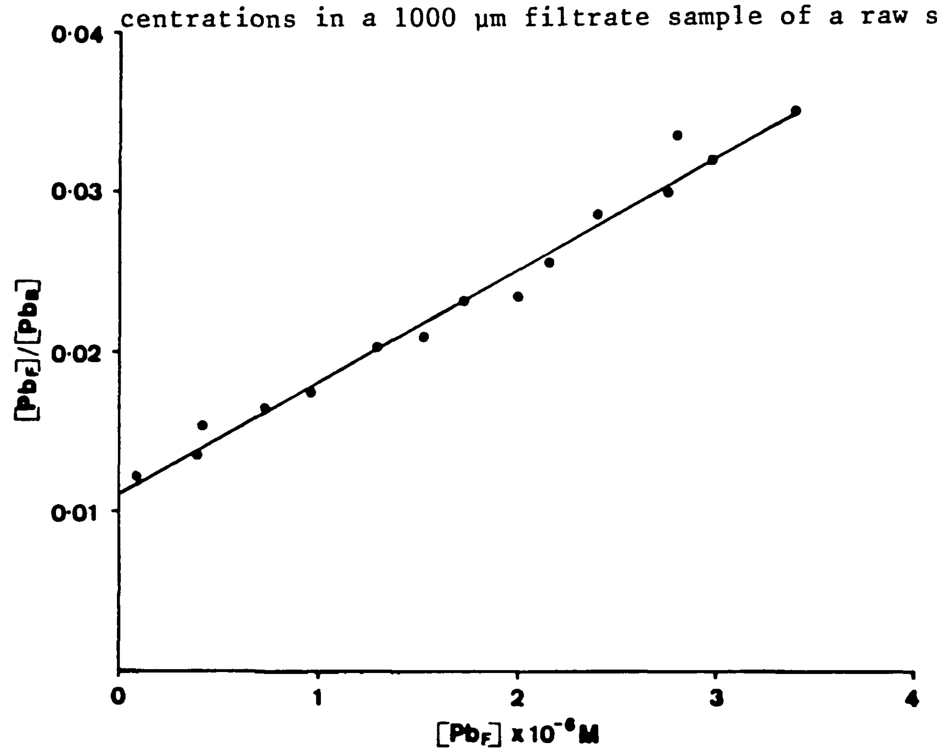


Figure 34 Plot of free Cd/complexed Cd against free Cd over a range of concentrations in a bulk activated sludge sample

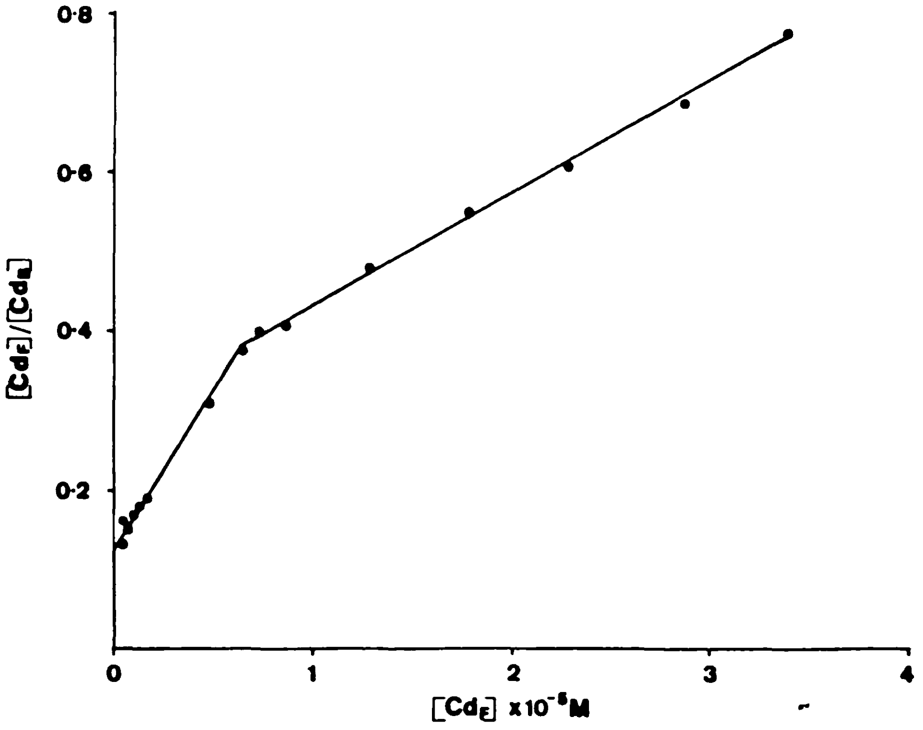


Table 26 Conditional stability constants and complexation capacities for Cu-sludge complex formation

(Ionic strength = 0.1 M; pH = 6.0, 7.2 and 7.0 for raw, activated and digested sludges respectively)

Sludge sample	Log K'_1	Log K'_2	$[L'_1]$ (mmol g^{-1} TS)	$[L'_2]$ (mmol g^{-1} TS)
Raw				
Bulk	6.99	6.29	0.484	0.808
1000 μm filtrate	6.94	6.35	0.788	1.27
100 μm filtrate	6.90	6.29	0.803	1.35
10 μm filtrate	6.24	5.77	1.83	2.84
0.22 μm filtrate	6.12	5.53	9.50*	17.4*
Activated				
Bulk	9.86	8.33	0.327	0.557
1000 μm filtrate	9.44	8.30	0.345	0.519
100 μm filtrate	8.98	8.30	0.284	0.390
10 μm filtrate	8.15	6.76	0.055	0.157
0.22 μm filtrate	8.09	6.16	0.039	0.257
Digested				
Bulk	9.82	7.59	0.140	0.515
1000 μm filtrate	9.80	7.64	0.161	0.620
100 μm filtrate	9.79	7.84	0.208	0.668
10 μm filtrate	8.01	6.83	1.14	2.76
0.22 μm filtrate	7.83	7.10	5.90*	11.3*

* [L'] calculated using the limit of detection of the solids concentration i.e. 0.1 gl^{-1}

Table 27 Conditional stability constants and complexation capacities for Cd-sludge complex formation

(Ionic strength = 0.1 M; pH = 6.0, 7.2 and 7.0 for raw, activated and digested sludges respectively)

Sludge sample	Log K'_1	Log K'_2	$[L'_1]$	$[L'_2]$
			(mmol g^{-1} TS)	
Raw				
Bulk	5.22	4.85	0.297	0.463
1000 µm filtrate	5.01	4.61	0.410	0.586
100 µm filtrate	4.85	-	0.337	-
10 µm filtrate	4.53	-	0.306	-
0.22 µm filtrate	NC	NC	NC	NC
Activated				
Bulk	5.53	4.70	0.115	0.327
1000 µm filtrate	5.52	4.78	0.114	0.327
100 µm filtrate	5.54	4.70	0.135	0.374
10 µm filtrate	NC	NC	NC	NC
0.22 µm filtrate	NC	NC	NC	NC
Digested				
Bulk	4.90	4.55	0.577	0.891
1000 µm filtrate	4.84	4.46	0.574	0.840
100 µm filtrate	4.88	4.55	0.713	1.01
10 µm filtrate	3.89	-	2.93	-
0.22 µm filtrate	NC	NC	NC	NC

NC - No complex formation detected since $[M_F] = [M_T]$

Table 28 Conditional stability constants and complexation capacities for Pb-sludge complex formation

(Ionic strength = 0.1 M; pH = 6.0, 7.2 and 7.0 for raw, activated and digested sludges respectively)

Sludge sample	Log K'_1	Log K'_2	$[L'_1]$	$[L'_2]$
			(mmol g^{-1} TS)	
Raw				
Bulk	5.90	-	2.34	-
1000 µm filtrate	5.81	-	3.42	-
100 µm filtrate	5.80	-	3.91	-
10 µm filtrate	ND	ND	ND	ND
0.22 µm filtrate	ND	ND	ND	ND
Activated				
Bulk	8.67	8.11	0.138	0.294
1000 µm filtrate	8.68	8.11	0.141	0.301
100 µm filtrate	8.67	7.94	0.122	0.325
10 µm filtrate	ND	ND	ND	ND
0.22 µm filtrate	ND	ND	ND	ND
Digested				
Bulk	6.52	5.84	5.63	6.97
1000 µm filtrate	6.43	5.79	6.29	7.64
100 µm filtrate	6.43	5.79	7.87	9.42
10 µm filtrate	6.32	5.52	55.9	70.4
0.22 µm filtrate	ND	ND	ND	ND

ND - Log K' and [L'] values not determinable using the plot of Ruzic (1982)

Cadmium exhibited similar stability constants in each sludge type, while greater variation was apparent between sludge type with respect to Cu and Pb. Both Cu and Pb formed more stable complexes in the treated sludges than in the raw sludge, with the highest stability constants being observed in activated sludge samples. Trends exhibited by [L'] values were more variable than those exhibited by K' values.

Values obtained for each complexation parameter in each particle size fraction displayed several trends. Very stable complexes were formed between Cu and both soluble and insoluble ligands in all three sludges, the stability of these complexes increasing with increasing particle size (Table 26). Values of [L'] obtained for Cu in the activated sludge similarly increased with increasing particle size, indicating both an increase in the stability of these complexes and in the number of binding sites available for complexation per unit mass of solids with increasing particle size. Conversely, however, [L'] values obtained for Cu in the raw and digested sludges were smallest in the bulk samples and largest in the 0.22 µm filtrates, indicating an increase in the stability of these complexes but a reduction in the concentration of available binding sites with increasing particle size.

In comparing the data presented in Table 26 with that presented in Table 27, it would appear that the size of particles has less effect on the characteristics of Cd-sludge complexes than on those of Cu-sludge complexes, particularly with respect to the activated sludge. The lack of complex formation between Cd and particles of less than 10 µm in this sludge type suggests that Cd preferentially complexes with activated sludge solids exceeding 10 µm in diameter. Complexation parameters were similar for the 100 µm and 1000 µm filtrates in addition to the bulk sample, possibly indicating that the same ligands participate in Cd binding in all three fractions. Characteristics of complexes formed between Cd and digested sludge solids in the 100 µm and 1000 µm filtrates and bulk sample also exhibited little variation. However, in contrast to observations made with respect to activated sludge, particles of between 0.22 µm and

10μm appeared to participate in Cd complexation in the digested sludge. Such complexes were weak relative to those formed with particles exceeding 10 μm in size, although a greater concentration of binding sites appeared to be available for participation in the weaker complexes. Similar to Cu, Cd exhibited an increase in complexation stability with increasing particle size in the raw sludge. Values of $[L'_1]$ and $[L'_2]$ obtained for Cd complexation in each sludge did not exhibit such clear trends as those obtained for Cu complexation; activated sludge values being almost constant in the bulk, 1000 μm and 100 μm fractions.

Similar to observations made with respect to Cd, complexation characteristics of Pb in activated sludge were more dependent on the total solids concentration than on the size of particles. However, in raw and digested sludges, Pb complexes exhibited an increased stability with increasing particle size, although such increases were less pronounced than those observed for Cu in these sludges. These increases in complex stability were accompanied by reductions in complexation capacities with increasing particle size.

5. DISCUSSION

There is an increasing awareness that the environmental impact of toxic heavy metals depends to a significant extent on their physicochemical forms. In natural waters, obtaining data amenable to biological interpretation is probably the most common reason for studying metal speciation (Neubecker & Allen, 1983), while in more complex matrices, such as sewage sludge, approaches to speciation have been concerned more with an understanding of the partition of heavy metals into various forms or fractions which control their mobility and environmental dispersion. Although insoluble forms of heavy metals generally predominate in sewage sludges (Sterritt & Lester, 1984b), they have been studied to a much lesser extent than the soluble phase forms. This is mainly because they are of less toxicological significance and they are not as amenable to study (Sterritt & Lester, 1984b). However, the range of types of particulate matter with which heavy metals may become associated in sewage sludges is probably as complex as the soluble phase ligands. Thus, much information relating to particulate forms of heavy metals present in this matrix remains to be discovered. While no comprehensive or reliable speciation schemes for determining discrete heavy metal species or groups of species in the solid phase of sewage sludges have yet been developed, two types of approach, involving either a physical separation by means of membrane filtration or a chemical separation by means of selective extractants, afford considerable insight into solid phase distributions.

The results obtained from the initial sequential extraction of Cd, Cu, Ni, Pb and Zn in seven different types of sewage sludge showed that each metal had a characteristic fractionation profile. For Cu, Ni and Zn, such distributions were essentially independent of sludge type, indicating that major mechanisms of metal retention, such as ion-exchange, adsorption, complexation and precipitation, were common to all types of matrix. In contrast, mechanisms of retention of Cd and Pb were found to be variable depending on the physicochemical properties of the sludge. There was some indication that these latter metals tended towards more stable forms in the digested sludges than in the raw and activated samples; this phenomenon has previously been observed by Legret et al. (1983).

The fractionation rankings of Cu, Pb and Zn observed in the original sludge samples were almost identical to those obtained for raw, activated and digested sludges by Oake et al. (1984) and for digested sludge by Stover et al. (1976). With respect to Cd and Ni, however, much less agreement was apparent, although the fractions in which these metals were found to predominate were similar to those reported by Oake et al. (1984) for activated and digested sludge types. A summary of the predominant theoretical metal species obtained during the current investigation and those reported by other workers using sequential extraction techniques has been presented in Table 29.

The predominance of all five metals studied in either $Na_4P_2O_7$-, EDTA-or HNO_3-extractable forms in all three sludge types indicated the importance of precipitation and organic binding as mechanisms responsible for the association of heavy metals with insoluble sludge solids. Organically associated forms appeared to be of particular significance in the aerobic sludges, Zn being the only metal predominantly present in the $Na_4P_2O_7$ fraction in the anaerobically digested sludge samples. Copper was predominantly identified in all three sludge types in the HNO_3 fraction, which was originally designed to extract sulphide forms by Stover et al. (1976). Although Cu has been demonstrated to have a high affinity for sulphide ligands (Florence, 1982), the predominance of such forms in activated sludges in particular would appear to be anomalous, as sulphides are not generally formed under the aerobic conditions which prevail in the activated sludge system (Painter, 1983). This would seem to suggest that HNO_3 was not completely selective for Cu sulphides but may have been extracting other forms. Some doubt has been expressed as to the efficacy of $Na_4P_2O_7$ as an extractant for Cu-organic species (McBride & Bouldin, 1984), therefore the possibility exists that the HNO_3 fraction may contain some organic forms of Cu. The organically bound fraction has been identified as the most significant for Cu by other workers using different sequential chemical extraction techniques

Table 29 Predominant theoretical metal species identified in sewage sludges by means of sequential chemical extraction

Reference	Stover et al. (1976)	Emmerich et al. (1982b)	Legret et al. (1983)	Oake et al. (1984)			This* study		
Sludge Type	Anaerobically digested			Raw	Activated	Anaerobically digested	Raw	Activated	Anaerobically digested
Metal									
Cd	CO_3	CO_3	CO_3	CO_3	CO_3	CO_3/S	Org.	CO_3	S
Cu	S	Org.	Org.	S	S	S	S	S	S
Ni	CO_3	CO_3	Org.	Exch.	CO_3	CO_3	CO_3	CO_3	CO_3
Pb	CO_3	-	Res.	Org.	Org.	CO_3	Org.	Org./CO_3	CO_3
Zn	Org.	CO_3	Oxide	Org.	Org.	Org.	Org.	Org.	Org.

CO_3: Carbonate, Exch.: Exchangeable, Org.: Organically bound, Oxide: bound to Fe and Mn oxides
Res.: Residual, S: Sulphide

* Predominant metal species based on original definition of fractions by Stover et al. (1976)

(Emmerich et al., 1982b; Legret et al., 1983) and concurs with observations of its increased affinity for organic material in both activated (Rudd et al., 1984; Forster et al., 1985) and digested sludges (Gould & Genetelli, 1984; Sterritt & Lester, 1984c) in comparison to other metals. Further elucidation of the nature of the Cu species extracted by HNO_3 would thus seem necessary for a more accurate interpretation of the mechanisms by which this metal is retained within the sludge matrix.

One interesting aspect of Ni fractionation was that the most readily extractable KNO_3 fraction was of greater significance than for any other metal studied, comprising an average of 21% of the total Ni content. The KNO_3 reagent was originally incorporated into the sequential extraction scheme by Stover et al. (1976) in order to extract exchangeable metal. According to Adams & Sanders (1984), exchangeable forms of Ni are predominantly associated with organic surfaces in the sludge matrix. It is likely, however, that KNO_3 would also extract readily soluble Ni species. Since Ni has been observed to have a particular affinity for readily soluble organic matter in activated sludges (Rudd et al., 1984), the KNO_3 extract could possibly contain readily soluble Ni-organic complexes. Since metal mobility and bioavailability are considered to decrease approximately in the order of the extraction sequence, the predominance of Ni over the other metals studied in its occurrence in the KNO_3 fraction implies that it will be the most readily available for plant uptake.

In comparing the behaviour of the liquid and dried sludges under sequential extraction, it was apparent that although qualitatively similar, the fractions yielded by the two forms differed in their magnitude. Drying appeared to result in a higher proportion of Cu and Pb as KF-extractable forms, although this may have been caused by desorption to the soluble phase during storage of the liquid samples. Oake et al. (1984) observed a transfer of approximately 20% of Pb and Zn from the $Na_4P_2O_7$ extract to the EDTA and HNO_3 extracts following storage of liquid sludges for one month. The reduction in the $Na_4P_2O_7$-extractable Cd, Pb and Zn in the liquid sludges as opposed to the dried forms in this study suggests the occurrence of a similar phenomenon, possibly due to loss of specific binding sites on the organic material. For Cd, Pb and Zn in particular, drying of the sludges effectively reduced the magnitude of the most readily extractable KNO_3 fraction. In contrast, however, the dried samples tended to release these metals into solution at higher pH values than the liquid samples when subjected to progressive acidification. The contrasting responses of the liquid and dried preparations seems significant as many studies on metal extractability and availability are conducted using dried sludge samples. Since the water content of sludge as disposed to land usually exceeds 65% even after dewatering treatment (Oake _et al._, 1984), it may be more realistic to use the original liquid sludge for experimentation. However, the reduced homogeneity and gradual alteration of the liquid sludge characteristics on storage (Oake _et al._, 1984) renders the use of dried sludge samples preferable.

The patterns of solubilisation which resulted from subjecting sludge samples to progressive acidification were found to be characteristic of the individual metals studied but essentially independent of sludge type, even for Cd and Pb. The solubilities observed largely confirmed the relative ease of extraction shown by each metal when subjected to sequential extraction. Copper showed very little solubility consistent with its emergence in the refractory HNO_3 fraction following sequential extraction. This was similar to observations made by Adams & Sanders (1984) for an unamended sludge sample, where no threshold pH value for Cu would be identified. Initiation of Ni solubilisation at near neutral pH values was in accordance with its emergence in the KNO_3 (soluble/exchangeable) extract, while Zn, which was also solubilised at high pH values, was found to be largely extracted by $Na_4P_2O_7$, specific for organically bound forms of this metal. Although Pb was found to be associated primarily with organic and carbonate ligands, its observed threshold at pH 2.0 in the liquid samples suggested that organic interactions may have been controlling the solubility of this metal, since carbonates may be expected to dissolve at pH values of approximately 5.0 (Tessier _et al._, 1979). The different threshold values exhibited by Pb and Zn, although both apparently retained by interactions with organic material, reflect the relative strength of binding of these metals to organic ligands in sewage sludge (Patterson & Hao, 1979; Sposito et al., 1981; Sterritt & Lester, 1984b). With respect to Cd, solubilisation began at slightly higher pH values in the dried raw sludges than in the dried activated and digested sludges. Such differences would appear to confirm the relatively greater resistance shown by Cd present in the treated sludges when subjected to sequential extraction.

A comparison of the threshold pH values obtained during this investigation with those observed in samples of raw sludge (Adams & Sanders, 1983; 1984; Adams, 1985) and sludge-amended soils (Sauerbeck & Rietz, 1983; Kiekens & Cottenie, 1984) using similar techniques is given in Table 30. Fairly good agreement is apparent, although the existence of a threshold pH value for Ni solubilisation appears to be questionable. A further comparison of the average percentage total metal extracted at approximately pH 2 (arbitrarily defined as 'available' metal (Sterritt & Lester, 1985)) from a range of sewage sludges, ie., 78% Zn, 73% Ni, 62% Cd, 42% Pb and 24% Cu, with the data obtained here shows a similar series of relative availability of the five metals (Scott & Horlings, 1975; Hayes et al., 1979; Jenkins et al., 1981; Wozniak & Huang, 1982; Logan & Feltz, 1985). Copper and Pb emerged as the metals most resistant to the effects of decreasing pH, as previously observed in single acetic acid (Berrow & Webber, 1972) and HCl (Stover et al., 1976; Logan & Feltz, 1985) extractions. Gerritse & Van Driel (1984) similarly found that Cu and Pb exhibited less reactivity to both decreasing pH and increasing ionic strength in comparison to Cd and Zn. Such findings indicate a similarity in the mechanisms by which Cu and Pb are retained in sludge, while different mechanisms are likely to be responsible for the retention of the more-easily extractable Cd, Ni and Zn.

It has been suggested that Cu and Pb are largely associated with organic materials (Kiekens & Cottenie, 1984). Observations made following sequential extraction of the acidified residues would seem to support this in respect of Pb, large increases in the solubilisation of this metal at pH 0.5 resulting primarily from the direct dissolution of the predominant $Na_4P_2O_7$ (organically bound) fraction.

Table 30 Comparative threshold pH values obtained for solubilisation of metals from sewage sludges and sludge-amended soils by progressive acidification

Reference	Adams & Sanders (1983)	Adams & Sanders (1984)	Adams (1985)	This study		Sauerbeck & Rietz (1983)	Kiekens & Cottenie (1984)
Sample	Metal-enriched filter-pressed raw sludge	Thawed, metal-enriched filter-pressed raw sludge	Thawed, filter-pressed raw sludge	Raw, activated & digested sludge		Air-dried sludge-amended soil	Air-dried sludge-amended soil
				Liquid	Dried		
Metal							
Cd	-	-	-	2.0	4.0	4.0	4.0
Cu	4.0	4.5	<4.5	No solubilisation	2.0-4.0	-	2.0-4.0
Ni	6.0	6.3	6.4	No threshold	No threshold	-	No threshold
Pb	-	-	-	2.0	4.0	2.0	2.0
Zn	6.0	5.8	6.1	4.0	6.0	5.0	4.0

Since a high percentage of the sludge organic matter is likely to remain insoluble throughout acidification (Adams & Sanders, 1984), hydrogen ion substitution may play an important role in the solubilisation of this metal at low pH values. Although the predominance of Cu in the HNO_3 fraction in the acidified residues would seem to refute the association of this metal with sludge organic material, some evidence that Cu was bound to organic matter was provided by its observed association with precipitated humic material in the ambient pH filtrates. Two types of Cu binding to humic materials occur, either hydrogen exchange or, through the formation of intermolecular bridges mediated by the metal ions, coagulation and flocculation of the humic material (Pagenkopf, 1978). Any Cu thus precipitated during the experimental periods at low pH would be lost to the residual pellet and would not appear as soluble metal. The slight increase in Cu solubilisation at pH 0.5 may also have been due to hydrogen ion substitution. Sequential extraction of the pH 0.5 residues revealed that interactions at this pH value were dominated by ion-exchange. The contrast observed in the concentrations of Cu and Pb solubilised at pH 0.5 could possibly indicate that Cu is more strongly bound to sludge organics than is Pb.

The release of Cd, Ni and Zn to solution at comparatively higher pH values than either Cu or Pb indicated that the former three metals were less strongly retained within the sludge solid phase than the latter two. Sequential extraction of the acidified residues subsequently revealed that dissolution of the $Na_4P_2O_7$ fraction commenced between pH 6.0 and 4.0 with respect to Zn and between pH 4.0 and 2.0 with respect to Cd and Ni, suggesting that all three metals were less strongly bound to the sludge organics than Pb and, possibly, Cu. Stability constant values determined previously for complexes formed between each of these metals and organic constituents of sludge (Patterson & Hao, 1979; Sposito _et_ _al_., 1981; Sterritt & Lester, 1984c) confirm such rankings. It was apparent, however, that the increased soluble concentrations of Cd, Ni and Zn observed upon decreasing the pH of the sludge samples did not result from the direct dissolution of the $Na_4P_2O_7$, EDTA and HNO_3 fractions which dominated at ambient pH values since metal species present in the acidified residues indicated a progressive shift towards the more easily extractable forms. The predominance of Cd and Ni in the KNO_3 fraction and of Zn in both the KNO_3 and KF fractions in the pH 0.5 residues suggested that adsorption-desorption processes, particularly those involving cation-exchange, were controlling the solubility of these three metals at low pH values. According to Riffaldi et al. (1983) and Adams & Sanders (1984), exchange sites and other weak adsorption sites located on organic material play an important role in determining the solubility of Cd, Ni and Zn in sewage sludges at low pH values.

The sequential extraction of the acidified residues gave some indication of the potential behaviour of metals under conditions of reduced pH. Thus although Cu appeared to be immobile even at pH 0.5, it had been transferred to less stable forms in the residual sludge matrix. Cadmium would seem likely to be relatively stable down to pH values of 4.0, but will then change its distribution considerably between pH 4.0 and 2.0, while Pb may be directly solubilised below pH 2.0 with little redistribution occurring. In terms of sludge disposal to land, however, the most interesting transition is likely to be that from ambient to pH 4.0; the results obtained here imply that this may be of significance for Ni and Zn, two of the metals critical in determining limits for sludge application. The solubility of Zn at low H^+ concentrations emphasises its importance as a limiting metal, as its threshold pH approaches those recommended for arable land (pH 6.5) and grassland (pH 6.0) by the Department of the Environment & National Water Council (1981). This is of particular concern since factors not considered in guidelines, such as soil properties and rhizosphere effects (including carbon dioxide respiration by roots and associated microorganisms in addition to the production of acidic organic exudates), may further reduce the pH in the vicinity of plant roots, resulting in increased uptake of Zn by plants (Adams & Sanders, 1984). Although a definite threshold pH could not be identified for Ni, percentage concentrations of this metal solubilised between ambient pH values and pH 4.0 were higher than those of any other metal studied. This indicates that the minimum pH values recommended for soils to which sludge is applied are likely to be least effective in minimising the availability of Ni to plants.

Guidelines for sludge application to agricultural land are restrictive for raw sludges (Department of the Environment & National Water Council, 1981) and stabilisation of sludge prior to such disposal is likely to become mandatory throughout the EEC (Commission of the European Communities, 1982). The most widely applied sludge stabilisation process in the UK is mesophilic anaerobic digestion (Department of the Environment & National Water Council, 1981). A knowledge of the changes in heavy metal form incurred by this process is therefore essential in estimating their bioavailability and mobility in soils to which the digested sludge is ultimately disposed. Application of sequential extraction to 'grab' samples of raw and digested sludges indicated few differences in metal distribution between these two matrices. Elucidation of the transitions in metal form which occur following anaerobic digestion was therefore undertaken under more controlled conditions within laboratory-scale anaerobic digesters, this system additionally permitting the influence of total metal concentration on metal form to be investigated.

Once they had equilibrated, the four digesters were found to be operating satisfactorily within normally accepted limits (Pohland & Ghosh, 1971; Metcalf & Eddy, 1979; Stein & Malone, 1980) in terms of pH, volatile solids reduction, volatile acids concentration, alkalinity, gas production and gas composition. It was apparent, however, that the two digesters operating on a feed of primary sludge were performing more efficiently than the two operating on a feed of mixed primary sludge. This was probably related to the refractability of the organic fraction of the waste-activated sludge, which has been reported to be only 30 to 45% digestible in conventional anaerobic treatment (Malina, 1961; Garrison _et al._, 1978). It is possible, however, that the presence of an unidentified inhibitory agent may have contributed to the relatively lower efficiency of the mixed primary digesters. In particular, at a pH above 7.4 to 7.6, an increasing proportion of ammonium ions is converted to toxic dissolved ammonia gas which can inhibit biological activity (McCarty & McKinney, 1961; Garrison _et al._, 1978; Mosey, 1983).

The results obtained from sequential extraction of Cd, Cu, Ni, Pb and Zn in primary and mixed primary raw sludges indicated that both types of sludge displayed many similarities in metal form. The predominance of Cd, Pb and Zn in forms extracted by $Na_4P_2O_7$ was consistent with results obtained for the raw sludges used in the previous experiment. However, the predominance of Cu in the EDTA extract and Ni in both KNO_3-extractable and residual forms was not observed previously. Oake et al. (1984) similarly found KNO_3-extractable forms of Ni to predominate in raw sludges (Table 29). Literature relating to residual metal determinations in raw sludges is currently unavailable for comparison, but the importance of this fraction with respect to Ni in soils has been reported (Emmerich et al., 1982b; Chang et al., 1984; Hickey & Kittrick, 1984).

Significant changes in the distribution of each metal occurred following anaerobic digestion. Both sludge types exhibited similar changes in metal distribution although certain differences were evident in the magnitude of these changes. In contrast to Cd and Pb, concentrations of Cu, Ni and Zn in the KNO_3-extractable (soluble/exchangeable) fraction were markedly reduced as a consequence of digestion. This is consistent with the increase in water solubility of the former two metals and reduction of the latter three observed by Bloomfield & Pruden (1975) following anaerobic incubation in digested sludge for three months. The greater significance of soluble and/or exchangeable forms of Cu, Ni and Zn in aerobic as opposed to anaerobic sludge has also been reported by Jenkins & Cooper (1964), Legret et al. (1983) and Oake et al. (1984). According to Mosey (1976), this reduction in heavy metal solubility may be attributed to the formation of heavy metal sulphide precipitates.

In general, however, the overall effect of anaerobic digestion was to cause a shift away from the more-readily extractable forms which predominated in the raw sludges towards the less-readily extractable precipitated forms. This was particularly evident with respect to Cd, Cu and Pb. According to Mosey (1976), Cd, Cu, Ni, Pb and Zn form extremely insoluble sulphide salts under the reducing conditions of anaerobic digestion, via the reduction of sulphur compounds including sulphate and the amino acids cysteine and methionine.

Solubility products of metal sulphides relevant to this study range from 1 x 10^{-24} for NiS and ZnS to 1 x 10^{-48} for Cu_2S (Mosey et al., 1971). Although each of the metals studied exhibited an increase in forms extracted by HNO_3 following digestion, this fraction was pre-eminent for Cu alone, which is thought to be precipitated predominantly in the Cu_2S form (Mosey, 1976; Hayes & Theis, 1978).

The predominance of Cd and Pb as carbonate precipitates does not concur with their expected predominance as CdS and PbS respectively (Hayes & Theis, 1978). However, the relatively high pH value observed in both 'experimental' digesters may explain this distribution, at least with respect to Cd, since this latter metal has been found to be precipitated as the sparingly soluble carbonate salt under reducing conditions where the pH value exceeds 7.2 (Mosey, 1971).

In contrast to the other metals studied, Zn remained predominantly in the organically bound form in both the raw and digested sludges. Hayes & Theis (1978) reported Zn to be present in anaerobically digested sludge in a predominantly intracellular form, suggesting that Zn is actively taken up by the microbial population within the digesters.

The predominant forms in which Cd, Cu, Pb and Zn were found to occur in both the primary and mixed primary digested sludges are comparable to those reported for the digested sludges investigated by Stover et al. (1976) and Oake et al. (1984) (Table 29). With respect to Ni, however, there are large discrepancies. During this investigation, the response of Ni to anaerobic digestion differed from that of the other metals studied in that no one fraction predominated in either digested sludge. Although markedly reduced as a consequence of digestion, a significant proportion of the total Ni (averaging 18.7% between the two sludges) remained in the KNO_3 fraction. This figure is comparable to the 16.5% of the sum total Ni found in this fraction in digested sludges extracted by Oake et al. (1984) and is within the range (2.5% - 27.0%) reported by Stover et al. (1976).

Distribution patterns observed for Cu, Pb and Zn during anaerobic digestion of both sludge types were little affected by increasing total metal concentrations, the HNO_3, EDTA and $Na_4P_2O_7$ fractions respectively remaining pre-eminent throughout. According to Hayes & Theis (1978), Zn remains predominantly intracellular up to a total Zn concentration of around 400 mgl^{-1} (ie. approximately four times the maximum concentration reached during this study), after which this predominance diminishes.

In contrast, distribution patterns observed for Cd and Ni were markedly affected by increasing total metal concentrations, increased percentages of Cd extracted by KNO_3 from mixed primary digested sludge and Ni extracted by the same reagent from both digested sludges being the most significant effects. As mentioned previously, it is likely that KNO_3 extracts both soluble and exchangeable metal species. According to Masselli et al. (1961) and Mosey (1971), the solubility of heavy metals in anaerobic digesters is regulated by the solubility products of their sulphide salts. However, the significant positive correlations observed between the increasing concentrations of Cd and Ni in both HNO_3 and KNO_3 forms in the mixed primary sludge during the period of metal addition would seem to support the view of Callander & Barford (1983) that the kenetics of precipitation are unlikely to contribute significantly to increasing the concentration of soluble metals in an anaerobic digester. It would appear, however, that the increase in KNO_3-extractable forms of Cd in the mixed primary sludge and of Ni in both sludge types was associated with a reduction, in terms of percentage concentration, in corresponding $Na_4P_2O_7$-extractable forms. Since the actual concentrations of Cd and Ni present in the $Na_4P_2O_7$ fraction in these sludges had decreased at the end of the experimental period, it would seem that the reduction of this fraction in terms of percentage of total metal was the result of a release of Cd and Ni to the KNO_3 fraction rather than attainment of the maximum binding capacity between each of these metals and organic complexing sites. Concentrations of Pb and Zn present in the $Na_4P_2O_7$ fraction were greatly enhanced in both the primary and mixed primary digested sludges as total metal concentrations increased. Therefore, it is possible that competition by Pb and Zn for complexation sites may have reduced the concentration of organically complexed Cd and Ni, resulting in a release of these metals onto exchange sites or into solution. The disparate effects observed with respect to Cd fractionation in the two sludge types as total Cd loading increased suggested that the Cd binding characteristics of these sludges differed.

While increasing total metal concentrations did not adversely affect the performance of the digester treating primary sludge, the digester treating mixed primary sludge exhibited preliminary signs of inhibition as a result of heavy metal toxicity. This was unexpected since individual metal concentrations attained in the mixed primary 'experimental' digester at the end of the period of metal dosing were below reported individual toxic inhibitory values (Hayes & Theis, 1978; Anthony & Breimhurst, 1981). However, little information is available on possible antagonistic, stimulatory , synergistic or acclimation effects for combinations of metals (Kugelman & Chin, 1971). K-values, indicating the additive effects of Cd, Cu, Ni, Pb and Zn (Mosey, 1976), were calculated (by deletion of the Cd term as pH values were above pH 7.2) at the end of metal dosing to be 160 and 149 meqkg^{-1} for the 'experimental' digesters receiving mixed primary and primary sludge respectively. The similarity of these values would tend to imply that no significant difference in metal toxicity should be present. In addition, both are significantly less than the K-value proposed by Mosey (1976) to be indicative of potential digester failure ie. 400 meqkg^{-1}. It would thus appear that the inhibited digestion of the mixed primary sludge cannot be explained by total metal concentrations alone.

The most pronounced difference between the two sludges in terms of metal fractionation was the concentration of Cd present in the KNO_3 fraction. At the end of metal dosing, 0.12 mgl^{-1} of Cd was extracted by KNO_3 from the mixed primary sludge, which represents approximately five times the concentration extracted by this reagent from the primary sludge. Although not determined experimentally, it is possible that soluble Cd in the mixed primary sludge may have reached the threshold inhibitory concentration of 0.02 mgl^{-1} (Mosey, 1971; Anthony & Breimhurst, 1981). Such inhibition demonstrates that operation of the digestion process can itself be a valuable safeguard against heavy metal contaminated sewage sludges being spread on land inadvertently.

The data obtained from sequential chemical extraction indicated that stabilisation of metal forms in sewage sludges occurs as a result of anaerobic digestion. Hence it follows that heavy metals present in sewage sludge disposed of to land would be of less immediate concern following anaerobic digestion. However, the observed influence of both total metal concentration and sludge characteristics on metal speciation, particularly with respect to Cd and Ni, emphasises the necessity for consideration of all three factors in the efficient treatment and safe disposal of sewage sludges.

Sequential extraction of Cd, Cu, Ni, Pb and Zn present in the various sewage sludges investigated during this study revealed that these metals were predominantly associated with the insoluble phase, in agreement with the findings of Gould & Genetelli (1978a) and Legret et al. (1983). Such associations were verified for Cd, Cu and Pb using membrane filtration, in excess of 90% of the total Cd content and 99% of the total Cu and Pb content of the sludges studied being retained by a 0.22 µm filter.

Using elutriation and filtration, Gould & Genetelli (1975) investigated the insoluble phase distribution of heavy metals in a digested sludge and found in excess of 90% of each metal studied to be associated with particles exceeding 100 µm in diameter, which comprised a similar percentage of the total sludge solids. During the current investigation, examination of the particle size distributions of Cd, Cu and Pb present in the insoluble phase of raw, activated and digested sludges collected from the same sewage treatment works indicated that the major proportion of each metal was attributed to the size range 0.22 to 100 µm, coincident with the total solids distribution. While metals in the activated and digested sludges exhibited a preferential association with particles in the size fraction 10 to 100 µm, associations with particles of 0.22 to 10 µm were of relatively greater importance in the raw sludge. These metal distributions similarly reflected corresponding total solids distributions, differences between the solids distribution observed in the raw sludge and those observed in the activated and digested sludges indicating that increased homogeneity was conferred by these treatment processes. The correlations observed between the Cd, Cu and Pb concentrations and corresponding total solids concentrations of bulk samples and filtrates for each sludge type further indicated a close association between these metals and particulate matter.

Although filtration through a series of filter pore sizes provides a comprehensive speciation of particulate forms of heavy metals, the actual differentiation between physical types is difficult (Sterritt & Lester, 1984b). However, utilisation of this separation technique in combination with ion-selective electrode potentiometry provided a quantitative assessment of the characteristics of the complexes formed between heavy metals and sludge solids of varying particle size. Of the three metals studied, Cu was the only one observed to form complexes with soluble ligands. Conditional stability constants (log K'_1 and log K'_2) obtained for complexation between Cu and dissolved activated sludge particles compare well with those previously determined for Cu complexation with activated sludge extracellular polymers by Rudd et al. (1983) (Table 31), suggesting that extracellular polymers predominated in the soluble phase complexation of this metal. Similarly, stability constants determined for Cu complexation with dissolved raw sludge particles are comparable to those obtained for fulvic acid extracted from digested sludge by Sterritt & Lester (1984c), although the values reported by these authors are lower than those determined for Cu complexes in the soluble phase of the digested sludge investigated. The lower pH at which Sterritt & Lester (1984c) conducted their experiments (Table 31) may be responsible for this discrepancy, however, since the pKa of the ligand and the pH of the system affect the potential availability of sites on the ligand (Tan et al., 1971; Gould & Genetelli, 1978b), especially those of a heterogeneous nature such as are derived from sewage. While visible precipitation prevented derivation of complexation parameters for soluble Pb complexes, Cd appeared to predominate in solution in the free cationic form. Literature relating to the nature of soluble Cd species in sewage sludges is scarce, but the free

Table 31 Conditional stability constants for complexes formed between some organic ligands and metals

Ligand	Cd		Cu		Pb		pH	Reference
	Log K'_1	Log K'_2	Log K'_1	Log K'_2	Log K'_1	Log K'_2		
Digested sludge solids	5.19	-	4.67	-	-	-	7.0	Gould & Genetelli (1978a)
Activated sludge extracellular polymer	6.52	4.77	8.32	6.71	-	-	7.0	Rudd *et al.* (1983)
Digested sludge fulvic acid	4.70	3.80	6.51	5.10	6.30	5.40	6.5	Sterritt & Lester (1984c)
Activated sludge solids	-	-	7.10	-	6.30	5.50	7.0	Sterritt & Lester (1985)

cationic form has often been found to predominate in the solution phase of sewage sludge-amended soils (Mahler et al., 1980; Butterworth & Alloway, 1981; Mattigod, 1981). The different forms in which Cd and Cu appear to predominate in the sludge soluble phase suggests that Cd is likely to be the most available for uptake by plants following sludge disposal.

The association of metals with sludge solids is often studied after the removal of the indigenous soluble ligands from the sludge sample (Gould & Genetelli, 1978a; Sterritt & Lester, 1985). Stability constant values obtained for Cd complexation in 'intact' bulk samples of raw, activated and digested sludge were similar to the value obtained for Cd complexation with digested sludge solids by Gould & Genetelli (1978a). This would seem to substantiate the finding that soluble sludge ligands do not participate in Cd complexation to any great extent. However, log K' values determined for Cu and Pb in 'intact' bulk samples of activated sludge were approximately three orders of magnitude higher than those found by Sterritt & Lester (1985) for complexes formed between these metals and activated sludge solids, suggesting that soluble ligands may make a significant contribution to the overall capacity of activated sludge to complex both Cu and Pb. Since the equilibrium between complexation by soluble and particulate sludge ligands may be an important regulatory factor in the ultimate behaviour of heavy metals in the receiving environment, separate investigation of these two phases would not appear to be totally realistic.

The capacities of the raw and digested sludges to complex all three metals studied, in terms of unit mass of solids, generally decreased with increasing particle size, suggesting a surface-area effect. Conversely, the complexation capacity of the activated sludge for these metals, particularly for Cu, tended to increase with increasing particle size, indicating that metal binding in this matrix may not be entirely a surface-area related phenomenon. The low $[L'_1]$ values obtained for Cu complexation in the 10 and 0.22 µm filtrates suggests that some degree of site specificity occurs in the complexation of this metal with activated sludge particles of less than 10 µm.

Results generally indicated the relatively greater importance of particles in excess of 10 µm in Cd, Cu and Pb binding in activated sludge; such particles are likely to be in the form of bacterial flocs (Hawkes, 1983). Kempton et al. (1983) demonstrated that activated sludge biomass exceeds 20 µm in diameter.

The stabilities of Cu and Pb complexes in both raw and activated sludge were greater than those of Cd, generally reflecting the relative removal of these three metals during sewage treatment (Lester, 1983). Anaerobic digestion did not appear to affect this ranking. Sterritt & Lester (1984c) similarly found that complexes formed between Cd and the soluble fulvic acid fraction of an anaerobically digested sludge were less stable than those formed between Cu and Pb and the same ligand (Table 31). Cadmium exhibited similar values of log K' and [L'] in all three sludge types, indicating the association of this metal with similar binding sites in each matrix. However, log K' values obtained for Cu and Pb were found to vary between the sludge types, in the order: activated > digested > raw. This ranking further emphasises the conclusion drawn from sequential extraction regarding the necessity for digesting raw sludge prior to disposal in order to control the mobility and environmental dispersion of constituent heavy metals.

The physical and chemical speciation techniques investigated generally indicated that Cu and Pb prevail in sewage sludges in more stable forms than Cd, Ni or Zn. Hence, the former two metals are likely to exhibit less reactivity than the latter three with regard to plant uptake following land disposal. This is of particular concern with respect to Cd, due to the ability of this metal to enter and accumulate in the human food chain (Davis & Coker, 1980; Logan & Feltz, 1985).

Sequential chemical extraction, progressive acidification and particle size fractionation used in conjunction with ion-selective electrode potentiometry appear to have much potential as research methods of characterising heavy metals in sludges and of evaluating both their short and long term bioavailability and mobility. However, limitations imposed by interferences, selectivity and sensitivity currently prohibit the use of these techniques on a routine basis. A major restriction of sequential extraction, for example, is the apparent non-selective nature of some of the component reagents. Clearly, until the nature of the metal forms extracted are empirically defined, possibly through utilisation of model species or phases (Stover _et al._, 1976; Rapin & Förstner, 1983; Meguellati _et al._, 1983), they can only be operationally defined in terms of the extractants used. With improved definition of extracted metal forms and standardisation of the extraction scheme, however, this technique could prove to be suitable for routine species identification.

Despite the recognised limitations of the techniques used in this study to characterise heavy metals in sewage sludge matrices, the importance of this concept in terms of predicting the behaviour of heavy metals following sludge disposal makes such approaches essential. Further development of these techniques will lead to an assessment of sludge disposal guidelines based on metal form rather than total metal.

6. CONCLUSIONS

6.1 The profiles obtained for a range of domestic/industrial sewage sludges subjected to sequential chemical extraction and progressive acidification were characteristic of the individual metals but essentially independent of sludge type.

6.2 Dried and liquid forms of sludges differed in their response to sequential extraction and progressive acidification, but the former provided better reproducibility and ease of handling.

6.3 According to sequential chemical extraction, the major operationally-defined forms in which each metal occurred in the sludges were HNO_3-extractable for Cu, EDTA-extractable for Cd and Ni and $Na_4P_2O_7$-extractable for Pb and Zn. Nickel predominated over the other four metals in its occurrence in the KNO_3 fraction, implying that it would be the most readily available for plant uptake.

6.4 Threshold pH values below which metals were significantly solubilised in liquid sludges were pH 2.0 for Cd and Pb and pH 4.0 for Zn. Copper displayed negligible solubility and Ni, although solubilised, exhibited no clear pH threshold. Dried forms of the same sludges had thresholds of pH 4.0 - 2.0 for Cu, pH 4.0 for Cd and Pb, pH 6.0 for Zn and similarly no quantifiable threshold value for Ni.

6.5 Investigation of the changes in metal form in the sludge residues following acidification indicated that Cu, Cd and Pb speciation was relatively constant at pH values above 4.0 whereas that of Zn and Ni was likely to become less stable at pH values below ambient.

6.6 Laboratory-scale anaerobic digesters operating on a feed of primary sludge were found to be more efficient than those operating on a feed of mixed primary sludge under similar volatile solid loadings.

6.7 According to sequential chemical extraction, the major forms of metals in primary and mixed primary raw sludges were $Na_4P_2O_7$ - extractable for Cd, Pb and Zn, EDTA - extractable for Cu and both KNO_3 - extractable and residual forms for Ni.

6.8 Both sludge types exhibited similar transitions in metal distributions following anaerobic digestion. In general, there was a shift away from the more-easily extractable forms which predominated in the raw sludges towards the less-readily extractable precipitated forms. This was particularly evident with respect to Cd and Pb, which predominated in the digested sludges as EDTA-extractable forms, and for Cu, for which HNO_3-extractable forms were pre-eminent. Zinc, however, remained essentially in $Na_4P_2O_7$-extractable forms while Ni was distributed evenly among the fractions.

6.9 Distribution patterns observed for Cu, Pb and Zn during anaerobic digestion of both sludge types were little affected by increasing total metal concentrations, in contrast to those observed for Cd and Ni. Increased percentages of Ni present in the KNO_3-extractable fraction in both digested sludges and of Cd in mixed primary digested sludge were the most significant effects of increased total metal concentrations. The disparate effects observed with respect to Cd fractionation in the two sludge types as total Cd loading increased suggested that the sludges possessed different binding characteristics with regard to Cd.

6.10 Increased metal concentrations, although below individual and cumulative inhibitory concentrations, resulted in inhibition of the anaerobic digestion of mixed primary sludge. Considerations of inhibitory concentrations should therefore include an assessment of metal form.

6.11 Particle size distributions of Cd, Cu and Pb in raw, activated and digested sewage sludges were demonstrated to be correlated to corresponding total solids distributions. Although slight variations were apparent in the distributions of these metals according to sludge type, the major proportion of each metal in each sludge investigated was attributed to the size range 0.22 to 100 μm, coincident with the total solids distributions. The influence of both the size and concentration of sludge particles on heavy metal distribution was therefore demonstrated.

6.12 Particle size was also observed to influence the conditional stability constant and complexation capacity values determined for complexation of Cd, Cu and Pb by raw, activated and digested sludge solids using titration in combination with ion-selective electrode potentiometry. In raw and digested sludges, all three metals generally exhibited an increase in complex stability but a reduction in complexation capacity, in terms of unit mass of solids, with increasing particle size. With respect to activated sludge, stability constant and complexation capacity data indicated a preferential association of each metal with particles exceeding 10 μm in diameter.

6.13 Of the three metals investigated using ion-selective electrode potentiometry, only Cu was found to form complexes with soluble sludge ligands.

6.14 The stabilities of complexes formed in raw, activated and digested sludges were in the order: Cu>Pb>Cd. Cadmium exhibited similar complexation parameter values in each sludge type, while Cu and Pb formed complexes of relatively greater stabilities in activated and digested sludges as opposed to raw sludge.

6.15 The physical and chemical techniques used to fractionate and characterise heavy metals in various types of sewage sludge generally demonstrated the prevalance of Cu and Pb in more stable associations within this matrix than either Cd, Ni or Zn. Hence, the former two metals would be expected to exhibit less reactivity than the latter three with regard to their availability to plants following land disposal.

6.16 In addition, such procedures emphasised the necessity for anaerobically digesting raw sludge prior to disposal in order to stabilise metal forms and hence moderate their environmental impact.

6.17 Although the techniques investigated appeared to have much potential as research tools for characterising heavy metals in sewage sludges, analytical limitations currently prohibit their use on a routine basis. However, further development of these techniques, in respect of improved definition and standardisation, will lead to an assessment of disposal guidelines based on metal form rather than total metal.

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8. APPENDICES

Appendix I

The formula developed by Mosey (1976) to calculate the total metal load of a digesting sludge (K) was:

$$K = \frac{\frac{Zn}{32.7} + \frac{Ni}{29.4} + \frac{Pb}{103.6} + \frac{Cd}{56.2} + \frac{0.67\ Cu}{31.8}}{\text{solids concentration}} \qquad \text{meq kg}^{-1}$$

where Zn, Ni, Pb, Cd and Cu are the concentrations of these metals expressed as mgl^{-1}; 32.7, 29.4, 103.6, 56.2 and 31.8 are the equivalent weights of the respective metals when in the divalent state; 0.67 is a factor to allow for the partial reduction of Cu to the cuprous state and 'solids concentration' is the dry solids content of the digested sludge expressed as kgl^{-1}.

This equation assumes the pH value of the digesting sludge to be in the range 6.9 to 7.3. However, if the pH value is above 7.2 the term for Cd is deleted and if above 7.7, that for Zn is also deleted (Mosey, 1976).

Appendix II

Table 32 Percentage total metal content of the supernatants decanted from liquid sludge samples prior to sequential chemical extraction

Sludge Sample	Metal Content (% total)				
	Cd	Cu	Ni	Pb	Zn
R1	3.12	1.81	16.7	1.13	1.74
R2	3.14	2.49	12.0	1.85	1.32
A3	3.41	2.34	3.33	1.49	2.41
A4	0.92	2.69	6.88	1.05	1.23
D5	*1.80	1.44	3.96	0.70	1.14
D6	2.36	2.58	5.61	1.26	1.36
D7	3.00	5.76	5.02	3.55	2.04

* Means of five replicates, data for other sludges being means of three replicates

Appendix III

Table 33 Percentage total metal content extracted from dried samples of raw (R), activated (A) and digested (D) sludges subjected to progressive acidification and subsequent sequential chemical extraction

		Metal Extracted (%)											
		R2			A4			D5			D6		
Metal	Extractant pH	4.0	2.0	0.5	4.0	2.0	0.5	4.0	2.0	0.5	4.0	2.0	0.5
	KNO_3	1.6	31	40	22	36	39	1.6	18	16	2.8	34	39
	KF	4.6	3.0	3.5	<1	<1	1.2	<1	<1	<1	2.8	2.3	3.0
Cd	$Na_4P_2O_7$	50	1.3	0	63	41	40	18	23	2.5	39	13	2.7
	EDTA	15	0	0	15	2.5	1.5	52	6.0	<1	33	3.8	0
	HNO_3	58	50	46	27	4.0	1.5	47	23	<1	49	35	19
	RES	<1	0	0	8.3	5.2	5.2	1.4	2.0	2.4	1.5	0	0
	KNO_3	3.0	9.7	20	9.0	30	63	2.0	5.6	66	2.2	6.2	20
	KF	2.5	2.2	3.0	3.4	3.1	5.0	1.8	1.9	6.4	2.0	1.6	3.0
Cu	$Na_4P_2O_7$	17	14	10	15	12	8.8	6.9	8.4	26	16	26	29
	EDTA	16	11	6.2	20	17	2.0	11	12	13	17	13	9.8
	HNO_3	70	42	6.0	76	42	<1	100	100	7.1	76	60	4.8
	RES	13	12	7.7	3.0	2.7	<1	4.7	5.0	2.9	8.2	7.1	4.3
	KNO_3	9.3	36	35	34	61	61	16	18	20	6.6	21	35
	KF	2.3	<1	<1	2.7	<1	<1	2.9	0	0	2.5	1.0	<1
Ni	$Na_4P_2O_7$	20	6.8	8.7	12	5.3	4.4	21	7.6	2.3	27	24	16
	DTA	4.6	1.0	<1	22	3.3	4.4	13	2.1	<1	11	3.5	<1
	HNO_3	15	8.7	3.9	6.0	2.7	1.4	8.0	3.4	0	20	11	3.4
	RES	4.4	2.5	3.7	5.5	3.9	3.0	5.9	3.9	<1	4.5	4.1	1.4
	KNO_3	<1	10	3.0	21	31	13	<1	10	18	<1	5.5	9.0
	KF	<1	<1	<1	<1	<1	4.0	<1	<1	<1	<1	<1	<1
Pb	$Na_4P_2O_7$	77	67	8.8	85	31	9.8	86	87	21	49	53	6.3
	EDTA	46	26	3.1	17	3.4	1.5	37	15	1.8	43	21	1.9
	HNO_3	13	10	<1	<1	<1	<1	13	11	<1	15	12	3.1
	RES	1.7	1.6	1.0	<1	<1	0	1.8	1.5	1.1	1.2	<1	<1
	KNO_3	5.6	14	13	28	42	36	15	32	33	1.6	20	27
	KF	16	26	31	14	21	51	18	12	26	29	32	35
Zn	$Na_4P_2O_7$	35	4.1	1.3	50	6.5	2.7	68	13	1.9	34	9.7	3.0
	EDTA	9.0	1.4	<1	3.7	1.7	1.1	11	1.2	<1	16	2.4	<1
	HNO_3	12	7.2	<1	12	3.3	1.9	35	20	<1	19	9.1	1.3
	RES	2.2	2.1	2.3	2.6	1.2	1.5	1.9	1.9	1.4	2.9	4.5	3.2

Appendix IV

Table 34 Initial and final metal concentrations in operationally-defined fractions in the 'experimental' digester receiving mixed primary sludge

	Fraction Concentration ($mgkg^{-1}$)											
	KNO_3		KF		$Na_4P_2O_7$		EDTA		HNO_3		Residual	
Metal	I	F	I	F	I	F	I	F	I	F	I	F
Cd	0.04	4.55	0.02	0.60	1.63	9.41	2.96	34.4	0.65	19.1	0.13	0.21
Ni	9.20	65.8	1.25	3.65	8.45	6.27	9.50	52.3	7.65	35.4	8.90	5.10
Pb	1.93	2.78	6.19	2.30	125	619	227	1290	33.1	28.0	5.58	6.83
Zn	9.13	38.7	26.8	28.0	641	2970	104	446	65.3	485	15.2	14.4

I = Initial Concentration

F = Final Concentation

Appendix IV

Table 35 Initial and final metal concentrations in operationally-defined fractions in the 'experimental' digester receiving primary sludge

	Fraction Concentration (mgkg^{-1})											
	KNO_3		KF		$Na_4P_2O_7$		EDTA		HNO_3		Residual	
Metal	I	F	I	F	I	F	I	F	I	F	I	F
Cd	0.30	0.95	0.04	0.40	1.84	17.0	3.33	30.5	0.74	16.4	0.09	0.13
Ni	10.9	57.4	2.16	6.56	8.43	13.6	10.3	50.6	11.0	17.3	9.85	9.47
Pb	1.50	2.55	5.20	16.4	87.0	591	134	1160	13.8	27.0	4.61	8.72
Zn	7.75	21.0	45.6	49.6	955	2790	151	376	79.7	531	11.0	12.0

I = Initial Concentration

F = Final Concentration

Appendix V

Table 36 Metal distribution in the 'control' digester receiving mixed primary sludge over the period of metal addition (mean ± SD, n = 6)

Metal	Fraction						Determined Total ($mgkg^{-1}$)
	KNO_3	KF	$Na_4P_2O_7$	EDTA	HNO_3	Residual	
	% Determined Total						
Cd	0.78 ± 0.14	ND	29.6 ± 2.75	51.3 ± 2.90	12.2 ± 0.85	1.78 ± 0.50	5.35 ± 0.25
Cu	4.41 ± 0.32	2.46 ± 0.21	5.25 ± 0.40	21.2 ± 0.56	64.4 ± 1.86	1.60 ± 0.13	611 ± 29.2
Ni	19.2 ± 0.98	2.40 ± 0.20	16.4 ± 1.01	17.8 ± 0.78	16.5 ± 1.50	16.3 ± 0.42	50.3 ± 2.89
Pb	0.50 ± 0.09	1.51 ± 0.10	30.1 ± 0.76	55.2 ± 1.13	7.85 ± 0.16	1.32 ± 0.08	436 ± 12.1
Zn	1.11 ± 0.16	2.91 ± 0.14	77.4 ± 4.37	13.0 ± 0.78	7.88 ± 0.29	1.74 ± 0.15	806 ± 30.2

ND - not determinable

Appendix V

Table 37 Metal distribution in the 'control' digester receiving primary sludge over the period of metal addition (mean ± SD, n = 5)

	Fraction						Determined Total (mgkg^{-1})
Metal	KNO_3	KF	$Na_4P_2O_7$	EDTA	HNO_3	Residual	
	% Determined Total						
Cd	4.50 ± 0.33	0.61 ± 0.06	29.1 ± 1.51	47.4 ± 1.32	11.1 ± 0.57	1.04 ± 0.14	6.97 ± 0.20
Cu	7.32 ± 0.68	9.07 ± 0.47	9.11 ± 0.39	28.7 ± 1.13	44.1 ± 3.44	1.32 ± 0.48	631 ± 27.8
Ni	20.1 ± 0.59	4.01 ± 0.20	15.4 ± 0.51	19.1 ± 0.47	20.6 ± 0.62	17.8 ± 1.25	52.2 ± 1.60
Pb	0.55 ± 0.04	1.99 ± 0.07	34.9 ± 0.78	53.1 ± 1.71	5.48 ± 0.20	1.82 ± 0.06	254 ± 6.69
Zn	0.65 ± 0.08	3.74 ± 0.43	77.8 ± 2.94	11.2 ± 0.85	7.11 ± 0.42	0.91 ± 0.14	1227 ± 31.1

Appendix VI

Table 38 Routine parameter values obtained for the 'experimental' and 'control' digesters receiving mixed primary sludge throughout the period of metal addition

	pH		Total Solids (gl^{-1})		Volatile Solids Reduction (%)		Alkalinity (mgl^{-1})		Gas Composition % CH_4	
Day	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control
58	7.52	7.52	24.9	25.2	47.4	46.5	4627	4491	65.2	78.4
60*										
62	7.44	7.47	24.9	25.2	48.8	48.0	4378	4275	46.8	56.0
66	7.49	7.46	26.3	26.6	45.1	44.6	4372	4254	56.4	62.0
70	7.53	7.53	26.3	26.1	45.7	46.3	4236	4005	57.2	52.8
74	7.54	7.55	24.8	24.9	47.4	48.0	4318	4171	57.6	62.0
78	7.51	7.52	25.6	24.3	45.6	48.0	4136	4033	56.6	61.5
82	7.48	7.50	25.9	25.6	44.6	44.2	4315	4186	55.4	62.0
86	7.52	7.59	25.8	23.5	46.7	49.7	4123	4019	54.2	58.0
90	7.43	7.56	24.1	24.4	48.0	47.5	3861	3665	46.8	52.4
94	7.44	7.47	25.4	24.3	46.8	49.6	4141	4051	47.5	51.3
98	7.42	7.46	25.7	24.0	47.2	50.6	4128	4033	44.2	52.2

* Commencement of metal addition to the 'experimental' digester

Appendix VI

Table 39 Routine parameter values obtained for the 'experimental' and 'control' digesters receiving primary sludge throughout the period of metal addition

	pH		Total Solids (gl^{-1})		Volatile Solids Reduction (%)		Alkalinity (mgl^{-1})		Gas Composition % CH_4	
Day	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control
58	7.41	7.31	24.4	24.2	54.5	54.6	3124	3038	54.0	52.8
60*										
62	7.49	7.40	25.2	21.4	52.2	58.5	2880	2922	62.0	61.4
66	7.46	7.45	26.3	23.9	50.4	55.3	3044	2986	65.6	66.4
70	7.43	7.39	23.4	23.0	54.0	56.4	3026	2954	64.4	65.4
74	7.38	7.31	24.7	23.8	52.8	55.0	3084	2994	62.4	63.2
78	7.41	7.38	23.5	23.0	55.9	57.5	2994	2901	51.4	55.2
82	7.36	7.32	24.0	23.0	55.5	56.0	2929	2859	48.4	53.2
86	7.31	7.27	24.8	22.8	53.7	56.6	2777	2701	45.8	45.8
90	7.32	7.29	24.2	22.6	54.5	57.3	2674	2653	45.6	46.0

* Commencement of metal addition to the 'experimental' digester