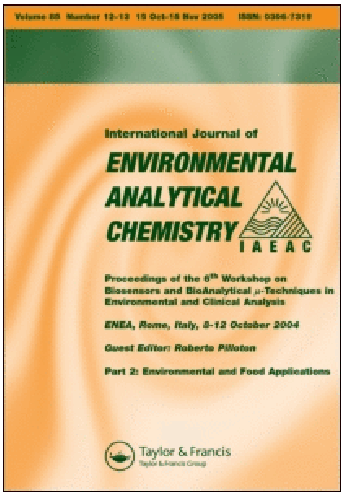

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Comparison and Evaluation of Extraction Media and Their Suitability in a Simple Model to Predict the Biological Relevance of Heavy Metal Concentrations in Contaminated Soils

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COMPARISON AND EVALUATION OF EXTRACTION MEDIA AND THEIR SUITABILITY IN A SIMPLE MODEL TO PREDICT THE BIOLOGICAL RELEVANCE OF HEAVY METAL CONCENTRATIONS IN CONTAMINATED SOILS

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The concentrations of heavy metals in agricultural soils are increasing gradually from various diffuse sources. The metals can transfer from contaminated soils into the growing plants or may affect the respiration rate of soil microorganisms.

In the first part of this paper, the following simple model to predict the biorelevant metal concentration in anthropogenically or artificially contaminated soils is reported:

$\log M_P = \alpha + \beta \log [M_{NaNO3}]$
$\log M_R = \alpha' + \beta' \log [M_{NaNO3}]$
$\log M_P$ = log Metal concentration in test plant (mg/kg dry weight)
$\log M_R$ = log percent Redution in the respiration or enzyme activity
$[M_{NaNO3}]$ = Metal concentration in NaNO3-soil extract (mg/kg soil)
α and β & $\alpha' + \beta'$ = Intercepts and slopes of the linear regression lines.

The model has been tested in laboratory experiments, in growth experiments either in a greenhouse or in the field. Also, an attempt is made to present a theoretical interpretation.

Critical concentrations are calculated with the help of the relationship between metal concentration in soil solution $[M_{NaNO3}]$ and metal concentration in plants. The critical concentration, which is able to induce either phyto- or zootoxic concentrations in the plant parts (productive or vegetative) or reduce their growth or to reduce significantly the growth of soil microorganisms, are found to be uniform in a wide range of soils (pH 3 to 8 and CEC 10-50 millimol/100g soil). the critical concentrations of $NaNO_3$ in soil extracts are comparable with the values obtained with plant growth experiments in nutrient solution and also with the concentrations obtained from soil respiration experiments reported in literature.

In the second part of this paper several salt extraction media such as $NaNO_3$, $CaCl_2$, NH_4NO_3, KNO_3 along with strong acid and chelating agent are evaluated, compared and discussed with respect to prerequisites for selection of an extracting medium. Chelating agent and strong acid extraction media extract more metal from soil than salt extraction media and are unsuitable for the prediction of either the phyto-and/or zootxic concentration in the plants or the reduction in respiration of soil microorganisms.

The evaluation clearly shows that in spite of the fact that the metal concentrations in $NaNO_3$-extracts are lower than in other salt extracts, $NaNO_3$ fulfils most of the prerequisites and is one of *the best available choices to predict* biorelevant metal concentration in soils and indicates that it is most suitable for the risk assement of metals in soils. Comparable data for other salt extraction media tested in this paper are not available in literature.

KEY WORDS: Heavy metals, ecotoxicity of metals, contaminated soils, soil microorganisms, extraction procedures.

INTRODUCTION

There are two types of interrelationships between soil and environmental pollution. Either soil can be a source of environmental pollution ("Source"-Function) or it can be a victim of environmental pollution ("Sink"-Function). The concentrations of heavy metals in agricultural soils are gradually increasing from various processes such as utilization or dumping of sewage sludge, use of fertilizers, creation of landfills, and atmospheric fall-out of contaminated dust particles. The general aspects of metal pollution, binding in soils, contents of metals in plants have been reviewed [1, 2, 3, 6, 10, 11, 12, 13]. The metals may transfer from contaminated soils into the growing plants and may retard the growth of plants and the growth of soil microorganisms. The bioavailability of metals in soil is determined by characteristics of both the soil and plants. Although some metals at low concentrations are essential for life since they are required for enzyme systems, larger concentrations may be toxic; general considerations of mineral uptake by plants and microorganisms have been been reviewed by Marschner [4] and Gupta [5], respectively.

In order to protect agricultural soils from heavy metals, it is essential to assess and predict the biological relevance of the metals e.g the rate of growth and/or activities of soil microorganisms, the growth of plants and leaching of heavy metals into the ground water. Gupta [21] and Gupta and Häni [17] proposed, on the basis of several laboratory, greenhouse growth, and micro-plot field experiments, the following generalized model to predict the critical metal concentration in soil, which could induce either the phyto- and/or zootoxic concentration of metal in two indicator plants i.e. rye grass (monocot) and lettuce (dicot) is proposed:

$$M_P = \alpha + \beta\,[M_l] \tag{1}$$

M_l = Metal activities in soil solution

α and β = Intercept and slope of the linear regression line

Metal activities in soil solutions $[M_l]$ can be approximated by metal concentratrion in neutral salt extract of soils $[M_{NaNO3}]$.

$$M_P = \alpha \text{ and } \beta\,[M_{NaNO3}] \tag{2}$$

M_P = Metal concentration in test plant (mg/kg dry weight)

$[M_{NaNO3}]$ = Metal concentration in NaNO3-soil extract (mg/kg soil)

α and β = Intercept and slope of the linear regression line

Experimental data generally fitted better by a log linear regression model than a simple linear regression model. The model can be written as follows [5, 6, 16]:

$$\log M_P = \alpha \text{ and } \beta \log [M_{NaNO3}] \quad (3)$$

This model is also successfully used for repiration rate studies with soil micro-organsims [5,16,21,26,29,31,32]

$$\log M_R = \alpha' + \beta' \log [M_{NaNO3}] \quad (4)$$

Where $\log M_P$ = % Redution in the respiration rate

$\alpha' + \beta'$ = Intercept and slope of the linear regression line

Other symbols are used as described above.

Stadelmann *et al* [16,] Gupta and Stadelmann [26] and Gupta *et al* [5] verified this model using respiration as an indicator and found it to be valid under laboratory, greenhouse and field experiments.

The specific effects of experimnental conditions, types and species of plant and selected soil properties are well accounted for by this model. The results, which substantiated the usefulness of this model have been published previously [5, 9, 19, 21, 26, 31, 33]. Thus it is possible to correlate the metal concentration in soils, as measured by a neutral salt extraction (e.g. $NaNO_3$), either with the respiration rate of soil microorganisms or with the Phyto- or zootoxic (ecotoxic) concentration in plants or plant growth. From the regression line, it is possible to calculate the metal concentration in soils, which could induce ecotoxic concentrations in plants or growth of organisms. These calculated concentrations are denoted as the critical concentration in soil solution, which are independent of soil properties except soil pH and total metal concentration. These critical concentrations are of very great help in the assessment of risk posed by metal accumulation.

During the last 5 years, there have been suggestions to use either other extracting salt media or complexing agents (DTPA, EDTA) for use with this predictive model. However, it should be mentioned that although in the literature very comprehensive experimental evidence is available, as far as $NaNO_3$ is concerned, for other salt extraction media either no critical concentrations are caculated using standard growth experiments and/or else they have not tested in anthropogenically contaminated soils under field conditions. Or the activities and rate of growth of soil microorganisms were not tested. Much information is missing.

The specific objectives of this paper are:

1. to provide a summary of the theoretical considerations and experimental facts in the development of a simple model to calculate critical metal concentrations, which could induce ecotoxic concentration in plants or significant reduction in the % respiration rate of soil microorganisms in anthropogenically contaminated soils,
2. to enumerate the reasons to replace the metal activity in a soil solution (M_l) with metal concentration in the neutral salt solution (e.g. M_{NaNO3}),
3. to summarize and evaluate the available literature in order to estimate the critical concentrations of metals in soils for the assessment of risks posed by them.
4. to compare and evaluate the usefulness of other extraction media for a variety of metals in order to predict the metal concentration in plants grown on different anthropogenically contaminated soils.

THEORETICAL CONSIDERATIONS FOR THE USEFULNESS OF THIS MODEL FOR CALCULATING CRITICAL CONCENTRATIONS OF METALS IN SOILS

Critical concentration of metals in soils

The critical concentration of metals in soil solution ($NaNO_3$ extract) is the concentration, which is able to induce either phyto-or zootoxic (ecotoxic) concentrations in the plant parts (productive or vegetative) or reduce their growth or to reduce significantly the growth or activities of soil microorganisms. The critical concentration is independent of growth media.

Soil as a reaction system

The soil is an open reaction system composed of three important phases i.e. Solid, Solution, and Gas. Total mineral analysis, in fact does not provide any information about the biochemical activity of the heavy metals in a soil.

The solution-Phase or soil-water, which consists of metals in ionic form (charged and/or neutral species) and as organic complexes, is the most important part of the soil system. The soil-solution pool is in equilibrium with the Solid-Phase. A part of the metal bound to Solid-Phase can be mobilized to the Solution phase either as a result of a decrease in soil pH or as a result of the decomposition of humus. However, so far, no standardized chemical or biological methods are available to assess the mobilizable part of the metal in the solid phase [1, 19, 23].

Choice of extraction media to meet the model conditions to estimate the critical metal concentrations in soils, which could induce phyto- or zootoxic metal concentrations in plants

i) Extracting media which simulate the solid phase of a soil Media (e.g. strong acids, buffered and unbuffered chealting agents, mixtures of acid and chelating agents), which extract either a large portion or almost all of the total metal from the solid phase are not suitable for this model because of reasons discussed in following paragraphs.

The regression lines caclulated from the growth or metal concentration in plants are different for different soils (Figure 2). Thus, the caculated critical concentration is different for different soils. The so called "total" metal content is an excellent criterion to define the extent of metal build up or degree of contamination in soil. It provides an estimate of the extent or degree of saturation of total cation exchange capacity of soils colloids through metal or metals. As the % of metal saturation increases, the selective binding strengths of metals with soil solids decreases and hence the metal concentration in the solution phase increases [15, 17, 21, 33].

To assess the change in the metal status of soil, the so called "total" metal content in soil is not suitable parameter. For example for two soils having the same total metal concentration but differing in pH, humus, clay and oxide contents, the metal concentration in the solution phase would be significantly different [9, 15, 19, 29] (Figure 2).

ii) Extraction media to obtain simulated solution phase of soils: The solution phase also designated as the soil solution is composed of water and dissolved soil salts and completely envelopes soil solids. A part of the total metals or nutrients is either in the form of charged or uncharged ions or charged and uncharged soluble organic complexes. It provides information about the net result of variety of reactions mediated through chemical, biological and a physical processes in soils. Howerver, it does not tell us the contribution of each individual reaction (Figure 1).

The Soil solution bathes the plant roots and forms the source from which roots and soil microorganisms obtain their water, as well as their inorganic and, for a part, their organic nutrition (Figure 1). The acidity of soil solution largely regulate the equilibria. In the following paragraph, it will be shown that the metal concentration in the solution phase of soil is the net result of the interactions between different solid components (clay, humus, oxides etc), and influencing factors such as soil pH, total metal content and soil oxygen content. Significant changes in any of the influencing factors are immediately reflected in the metal concentration in the soil solution.

In this way, analysis of the soil solution is a very sensitive means to assess chemical or biological change in a soil system; for all practical purposes it is independent of soil properties and it is similar to the metal activity in nutrient solution used for growth experiments.

iii) Calculation of metal distribution coefficient (K_d) with the help of a simple adsorption model (equilibrium model) and relationship of K_d with properties of solid phase

$$H\alpha\text{-}S_B + [M_l] \rightleftharpoons M\text{-}S_B + \alpha H^+ \tag{5}$$

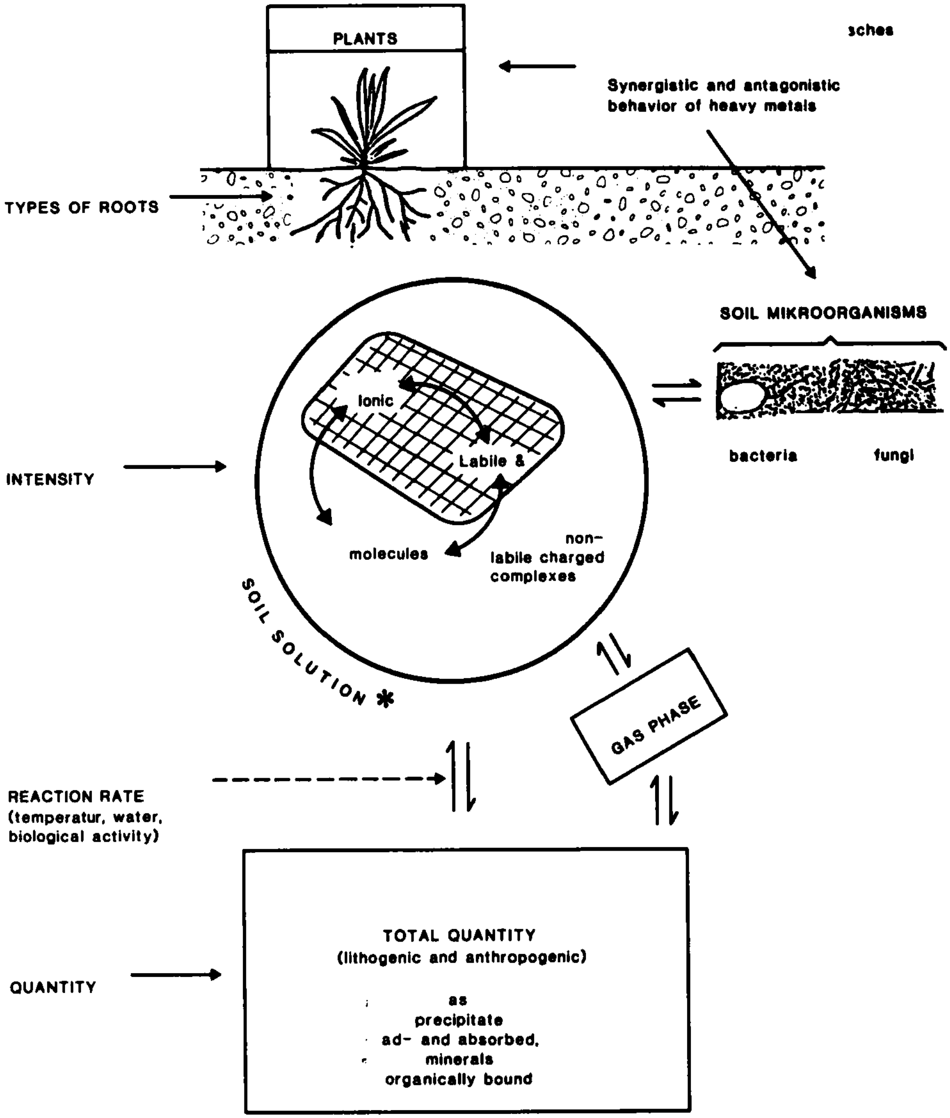
$H\alpha\text{-}S_B$ = Protonated metal binding sites on the solid surface [measured as Cation exchange capacity (CEC)]
$[M_l]$ = Metal (divalent) concentration in solution phase
$M\text{-}S_B$ = Bound metal on the soil surface
α = number of proton released per metal ion adsorption

$$K = \frac{(M - S_B)(H^+)^{\alpha}}{(H_{\alpha} - S_B)\,[M_l]} = \frac{K_d\,(H^+)^{\alpha}}{(H_{\alpha} - S_B)} \tag{6}$$

Upon rearrangement:

$$\begin{aligned} \log K_d &= \log K\,(H_{\alpha} - S_B) + \alpha\,\mathrm{pH} \\ &= \log K\,(\mathrm{CEC}) + \alpha\,\mathrm{pH} \\ &= \log K + \log \mathrm{CEC} + \alpha\,\mathrm{pH} \quad (7) \end{aligned}$$

Thus with the results shown in Equation 3, the graph of log K_d vs pH gives:
Intercept = log K + log CEC
Slope = α
Adsorption experiment with Cd shows following relationship:



* Simulated by extracting soils with a neutral salt (eg. $NaNO_3$) using a ratio of 1:2,5.

Figure 1 Important parameters to characterize the bio-availability of soil heavy metals.

$\log K_d = -3.16 + 0.78\,\mathrm{pH}\ (r = 0.96)$ (8)
(21, 23)

iv) Interrelationship between metal distribution coefficient (K_d) and soil properties: The following equations, which are empirical in nature, help in understanding the contribution of soil components to the metal adsorption process in soils. This equation was tested with Cd metal.

$$\log\left[\frac{M_T}{[M_l]}\right] = \log \mathrm{Kd} = \mathrm{constant} + a \log \mathrm{CEC} + b \log \mathrm{pH} \tag{9}$$

Where Kd is the distribution coefficient between metal in solid (M_s) and metal in solution phase (M_l or M_{NaNO_3}) (23).

CEC = Cation exchange capacity of soil, which is significantly related to soil properties such clay, oxide, humus content
$\log \mathrm{CEC} = 3.38 + 0.48 \log (\mathrm{clay}\%) + 0.45 \log (\mathrm{Humus}\ \%)$
(21, 23)

The distribution coefficients K_d for Cadmium have been significantly correlated with soil properties as follows[23]:

$$\log \mathrm{Kd}_{cd} = -5.13 + 0.47 \log (\mathrm{CEC}) + 0.78\,\mathrm{pH}\ (r^2{=}0.89) \tag{10}$$
(21, 23)

v) Conclusions: The above presented discussion clearly shows that the metal concentration in the soil solution reflects soil properties. It means that the critical metal concentrations are comparable in different soils and it can be interpreted on its own.

With the help of Figure 2, an attempt has been made to illustrate the interrelationship between metal in soil solution, $[M_l]$ or $[M_{NaNO_3}]$, metal in solid phase, M_T, metal concentration in plants, M_p or growth rate and activities of soil microorganismens, M_R, and soil properties.

The following two important observations can be inferred from Figure 2.

A) Variable amounts of metal in the solid phase (M_T) are needed to induce a fixed ecotoxic concentration (phyto- or zootoxic) of metal in indicator plants (M_p). (M_T) is mainly depend upon soil pH and cation exchange capacities (CEC) as shown in equation 5 and 8 [Figure 2].
B) An uniform and comparable metal concentration in soil solution (M_l) should be able to induce a fixed amount of ecotoxic concentration of metals in plants irrespective of the soil properties such as pH, CEC, and total metal in solid phase (M_T) [Figure 2].

Reasons for the choice of neutral salt ($NaNO_3$) as a measure of soil solution to predict the biorelevant metal (metal-intensity) in different soils

The following characterstics of $NaNO_3$ led us to decide upon it as an extraction media to use in this model:

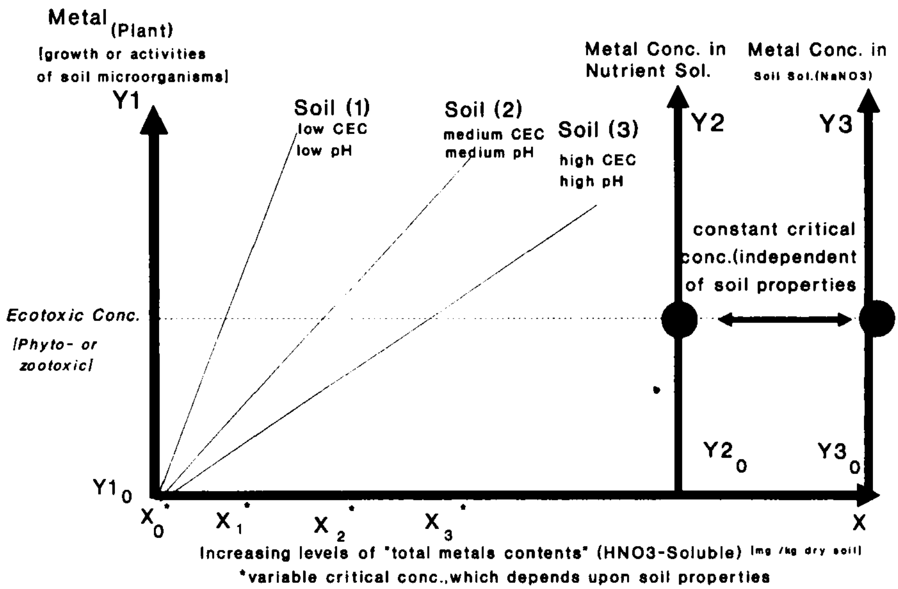


Figure 2 Influence of metal in HNO_3-extract, metal in nutrient solution, metal in soil solution ($NaNO_3$), and soil properties on the *critical concentration in soils*, which is capable of inducing phyto- or zootoxic conc. in plants and growth of organisms.

i) $NaNO_3$ is a neutral salt.

This does not in any way affect the equilibrium between soil solution and soil solid neither by changing soil pH nor by forming complexes nor do its cations or anions take active part in exchange reactions. These characterstics are the most important and deciding factors in selecting any extraction medium for such a purpose (Figures 1 and 2). This is because of the fact that a fixed amount of extracting media would affect differently the soil pH (depending upon its buffering capacity) or would form varying amount of the complexes (metals are complexed or bound on exchange sites in different soils with different components and with different strengths). The critical metal concentrations, which induce ecotoxic metal concentrations in plants, especially in contaminated soils, comes mainly from the loosely bound metal pool, which is easily washed away by neutral salt.

ii) The $NaNO_3$ soluble metal concentrations, which could induce ecotoxic concentrations in indicator plants are found to be uniform specially in anthropogenically contaminated soils having different soil properties (clay, organic matter, oxides content and soils environment)[9,21,29].

iii) Critical metal concentrations (ecotoxic) extracted by $NaNO_3$ have been calibrated for different metals over a wide pH range (free calcium or with free calcium oxides) in artificially and anthropogenic contaminated soils (pH 3.5 to 8):

The metal concentrations in $NaNO_3$ extracting medium has been calibrated with ecological parameters such ecotoxic concentration in plants or growth of microorganisms, and in different types of soils (Table 1). For this purpose, greenhouse, laboratory, and mini-plot field experiments were carried out, and critical concentrations calculated for metals such Cd, Cu, Zn, Ni, Pb and finally they were tested in field conditions. In a mini plot field experiment in anthropogenically contaminated soils the following observations were made[8]. In a number of mini-plot locations, where the $NaNO_3$ soluble metal content lie above the doubled VSBo critical concentration (Table 1), it has been found that the plant concentrations are still below the ecotoxic concentrations (phyto- or zootoxic) are given in the following table.

The total number of mini plot locations are given in brackets.

Metal	Rye grass	Lettuce
Copper	1 (34)	3 (32)
Zinc	2 (35)	2 (33)
Cadmium	0 (43)	

In a recent unpublished study, samples of fodder corn, wheat and other edible plant parts along with soil samples were collected. The soil samples and different plants (edible parts) were analyzed for Zn and Cd. The results showed that the Cd and Zn concentrations in the edible parts are correlated with $NaNO_3$ solution (r_{Cd}= 0.447 and r_{Zn}= 0.764 n =19). The pH_{CaCl_2}

Table 1 Comparison of critical concentrations of $NaNO_3$ soluble metals with the critical concentrations ascertained with the help of other extraction media and substrates (nutrient solution).

Metals	*Concentration in Extractants (mg metal/l)*						*VSBo*	*Pot-Water (German guide–values)*
	$NaNO_3$				*Saturation Extract*	*Nutrient solution*		
	(1)	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*
Zinc	0.3	0.4	-	0.24	1.0	0.4	0.2	2.0
Copper	0.6	0.28	-	0.20	0.4	0.4	0.28	-
Cadmium	0.02	0.008	0.003	0.02	0.01	0.05	0.012	0.006
Nickel	0.08	-	-	0.16	-	0.03	0.08	0.04
Lead	-	-	-	-	-	0.8-2	0.4	0.4-

(1): Pot Experiments used to establish calibration curves and from these curves critical concentrations are estimated [5,8,9,15,21,29]

(2): Mini plot field experiments were conducted in anthropogenic contaminated soils. The concentrations are estimated from metal concentration in $NaNO_3$ extracts of 34 different soils vs metal concentration in indicator plants grown on them [33].

(3): These values are estimated irrespective of culture. The pH values ranged in between 7.4–7.9.

(4): Laboratory experiments carried out to calibrate the respiration rate of soil microorganisms and metal conc. in $NaNO_3$ extracts. The critical concentrations were estimated from these curves [16,31,32].

(5): Growth experiments with soil : Metal concentration in saturation extracts [33] of soils.

(6): Growth experiments in nutrient solution. The values are calculated from calibration curves between metal conentration in plants vs metal in nutrient solution [27].

(7): VSBo–1986 (Verordnung über Schadstoffgehalt im Boden [Swiss Ordinance on Pollutants in Soil]). The critical values in $NaNO_3$ were calculated from pot experiments. The values obtained were halved. In VSBo the values are given in g/t of dry soil. However, in order to compare the values with nutrient solution, the values were divided by a factor of 2.5 (Soil solid to solution is 2.5).

(8): German Guide values for Pot water [13].

of soil samples ranged between 7.4 to 7.9. Zn and Cd concentrations measured ranged in between 0.04-7.15 mg Zn/kg Soil and 0.3-52 mg Cd/kg soil, respectively. In spite of such a narrow pH range and low metal concentrations it is possible to predict the change in the metal concentration in the edible plant parts thus showing definitely usefulness of $NaNO_3$ in this model.

iv) Critical concentrations have been calibrated with growth rate and activities of soil microorganisms:

With the help of laboratory aerobic incubation growth studies in an acid soil, the critical concentrations of metals for the growth rate of microorganisms (respiration rate) have been estimated [5,9,16,32]. The results substantiate the critical concentrations obtained from the plant growth experiments (Table 1).

v) 0.1 Molar concentration of $NaNO_3$ is comparable to the commonly found molar concentration of soil solutions under field conditions, which ranges between 0.05 to 0.2M [36].

This provides another advantage in that the dilute $NaNO_3$ extracting medium does not contain excessive salt concentrations and does not extract very high amounts of soil borne salts as compared to concentrated extracting media such as 1 M solutions. This enables one to measure, with modern sensitive analytical instruments, the metal concentrations in the $NaNO_3$-extract without significant interference from salts. In the case of concentrated NH_4NO_3 extracting medium, the danger of explosion during the measurement can not be ruled out.

vi) Low molar concentration of $NaNO_3$ extraction medium is suitable for many metal detection techniques.

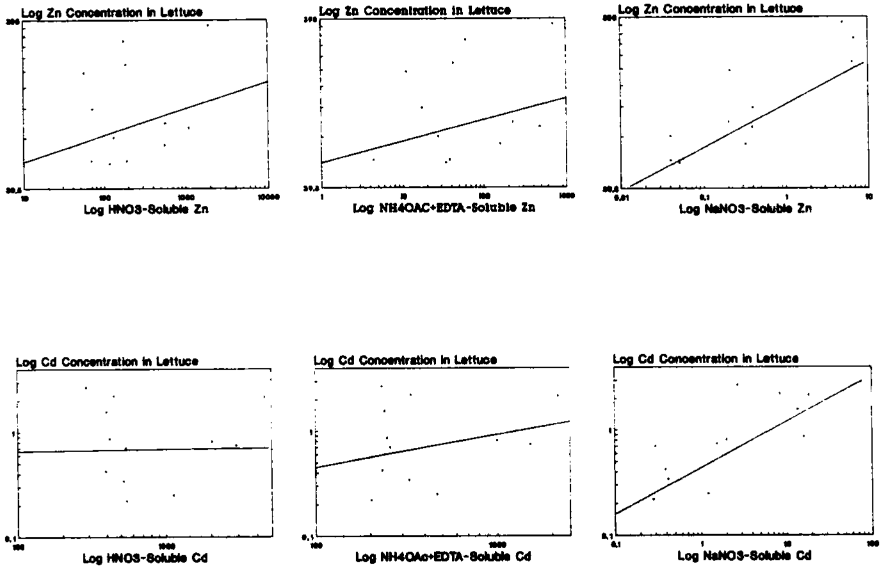


Figure 3 Relationship between metal concentration (log) in soils (extraction media) and metal concentration (log) in lettuce leaves.

Molar salt concentrations are even important for measurement techniques such as photometer, ICP, AAS etc. They may either affect the sensitivity or choke the burner or interfere with color development etc. The salt content of the extracting media may interact with other soil borne salts and thus have unknown effects on soil solution-soil solid equilibrium.

vii) Quantity of extracted metal in the extraction media.

The quantity or total metal concentration in the extraction media is not so important as the relationship of extracted metal to the induced phytotoxic concentration in the indicator plants.

Very often it is said that the metal concentrations found in $NaNO_3$ extracts are low. However, this should not be used to argue against this extractant since, it predicts very successfully the ecotoxic concentrations. Modern sensitive analytical instruments are able to measure accurately the metal concentration range usually found in $NaNO_3$ extracts.

viii) Calibrated and field tested critical concentrations in $NaNO_3$ are comparable with the estimated critical concentrations found with other extraction media and other growth substrates as nutrient solution.

The results of Table 1 clearly show that the critical concentrations estimated for $NaNO_3$ extractable metals in soils are comparable with the results of saturation extracts.

The extracts were prepared by mixing 40 g soil with 100 mL extraction solution, the slurry was shaken for 2 hours at 120 rpm at room temperature, and after centrifugation 48 mL liquid was filtered through cellulose acetate (0.45 m)[38]. The filtrates were acidified to prevent bacteria or fungi from growing in the solutions. For ion chromatography the filtrate was used directly with no added acid. These solutions were refrigerated and were analyzed within a few days before conspicuous organism growth occurred. The physico-chemical properties[38] and locations of the soil samples are given in Table 2. The coded names of soils, which will be used in the discussion are given in Table 2. The list of selected extraction media and their composition are given in Table 3.

COMPARISON OF EXTRACTION EFFICIENCY OF $NANO_3$ WITH THAT OF OTHER SALT EXTRACTION MEDIA OR STRONG ACID MEDIA

In last five years several other salt solutions such $CaCl_2$[20, 30], NH_4NO_3[28], or some chelating agents such as NH_4OAc + EDTA, DTPA[14] have been proposed for use in this simple model, which correlates the extracted metal concentrations with the critical concentration for ecotoxic effects.

Experimental

For this experiment 13 soil samples were selected from a miniplot field experiment, in which lettuce (dicot) and rye grass (monocot) were grown on 35 different anthropogenic contaminated sites in Switzerland in order to assess the usefulness of $NaNO_3$ in predictive model[9].

Table 2 Physico-chemical characteristics of surface soil samples (0–20 cm) and abbreviations for Sites (on these sites Miniplot field growth experiment were carried out).

Sites	*Code*	*Physical and chemical characteristics of soils*								*Heavy metals (HNO₃-soluble)*			
		*pH**	*organic carbon*%*	*field bulk density*	*Clay %*	*Silt %*	*Al-oxide mg/kg*	*Fe-oxide mg/kg*	*CEC-total mVal/100g soil*	*Cu mg/kg*	*Zn mg/kg*	*Cd mg/kg*	*Pb mg/kg*
Reinach	Re	5.6±0.1	2.8±0.4	1.04	40.4	39.2	2006	3458	36.18	56.0	151	0.825	32.5
Progrero 1	P1	6.2±0.2	4.0±0.5	0.77	50.0	17.6	6643	2907	23.06	511	56	0.290	18.0
Progrero 2	P2	6.5±0.3	3.8±0.1	0.77	6.6	16.2	6047	2541	20.38	806	71	0.450	31.0
Dottikon	Do	6.2±0.1	1.7±0.4	1.26	18.8	50.4	1028	2939	13.63	513	187	0.420	30.0
Däniken	Dae	6.1±0.2	1.5±0.1	1.12	14.0	30.8	976	3056	12.75	13.5	176	0.400	25.0
Salez 0	SO	6.9±0.3	6.0±0.8	0.70	41.3	52.2	2036	8718	53.92	35.0	69	0.395	29.5
Salez 2	S2	7.2±0.3	4.2±1.1	0.94	21.7	61.6	1216	5800	33.36	33.0	132	0.540	27.5
Dornach B	DB	7.3±0.1	5.4±1.0	1.08	20.6	55.5	1288	3493	13.29	794	1092	2.910	149
Dornach F	DF	6.8±0.1	5.7±1.0	—	21.7	0.7	1438	2818	36.15	1950	1943	4.500	281
Dornach C	DC	7.6±0.2	3.8±0.8	1.15	20.3	59.8	1066	2044	28.13	53	116	0.520	33.0
Biberist	Bi	7.2±0.1	2.8±1.1	1.08	11.6	36.7	746	3266	17.52	82.5	552	1.120	200
Schafis	Sc	7.3±0.1	6.3±3.4	1.00	15.4	43.7	1225	3247	20.95	466	564	2.030	225
Nidau	Nu	7.5±0.2	4.4±2.3	1.08	10.2	49.7	821	2484	24.39	77.5	188	0.545	155

*Average values of three independent measurements carried out in 1987, 1988 and 1989
(Ref. 8)

Table 3 Composition of the Extraction Solutions.

Relative Extraction Strength						
Code	*Solute in Water*	*Cu*	*Zn*	*Cd*	*Pb*	*I*
HNO_3*	2 M HNO_3	950	2400	1300	100,000	
D	0.5 M NH_4OAc + 0.02 M EDTA	360	540	460	42,000	
F	0.1 M $NaNO_3$ + 0.05 M Calcium lactate	5.5	13	12.0	4.1	
K	0.1 M $CaCl_2$	1.6	8.6	31.0	5.1	0.548
E	0.1 M KNO_3	1.0	1.8	1.0	3.6	0.316
H	0.1 M $NaNO_3$	1.1	1.0	1.2	1.0	0.316
B3	0.1 M $NaNO_3$ + 0.5 M NH_4NO_3	2.2	3.9	4.2	6.0	0.775
B2	0.5 M NH_4NO_3	2.2	3.9	3.9	2.6	0.707
B1	0.1 M NH_4NO_3	1.0	1.8	1.6	1.6	0.316
C	0.1 M $NaNO_3$ + 0.1 M NH_4NO_3	1.5	2.0	2.1	6.3	0.447
A	0.05 M $CaCl_2$	1.1	3.9	13.0	4.1	0.387
L	0.01 M $CACl_2$	—	—	—	—	—

* Schriftenreihe der FAC Nr. (Ref. 8)
I = Ionic Strength

Soil samples were dried at ambient temperature, passed through a 2mm sieve and mixed. Approximately 5 kg of each soil was taken from each site.

Duplicates of each soil in each extraction media were analysed for the elements Cu, Zn, Cd and Pb by atomic absorption spectroscopy. The concentrations of Cu and Zn were determined in the flame, and Cd and Pb concentrations were determined in a heated graphite furnace. Each set of measurements was calibrated with standard solutions prepared in the extraction medium. Duplicate solutions in extraction media 0.1M $NaNO_3$+ 0.1 M NH_4NO_3, 0.05 M $CaCl_2$ and 0.1 M $NaNO_3$ were analyzed by ion chromatography for the elements Cu and Zn with the following apparatus and conditions:

- Dionex IC System 2010i
- Analytical Column: Stagroma AG KO2 Cation Exchange
- Concentrator Column Dionex HPIC-CG2
- Eluent 8.4 mM oxalic acid + 7.5 mM tartaric acid pH 4.3 (LiOH). Flow rate 0.8 mL min^{-1}.
- A postcolumn reactor mixed the column eluent with 4-(2-pyridylazo)-resorcinol (PAR) in NH_3 and NH_4OAC; detection was by absorbance at 520 nm.

Under these conditions Cu and Zn eluted with clean, sharp, and well resolved peaks within the first 3.2 mL. The Pb signal was too weak and the Cd signal was too late (25 mL, broad, and subject to interference) to be useful. With a stronger eluent Cd could be eluted much faster and probably could be separated from the two interfering substances observed (retention volumes of 20 and 23 mL)

Comparative extraction efficiency

The chemical and physical properties of the 13 selected soils are presented in Table 2.

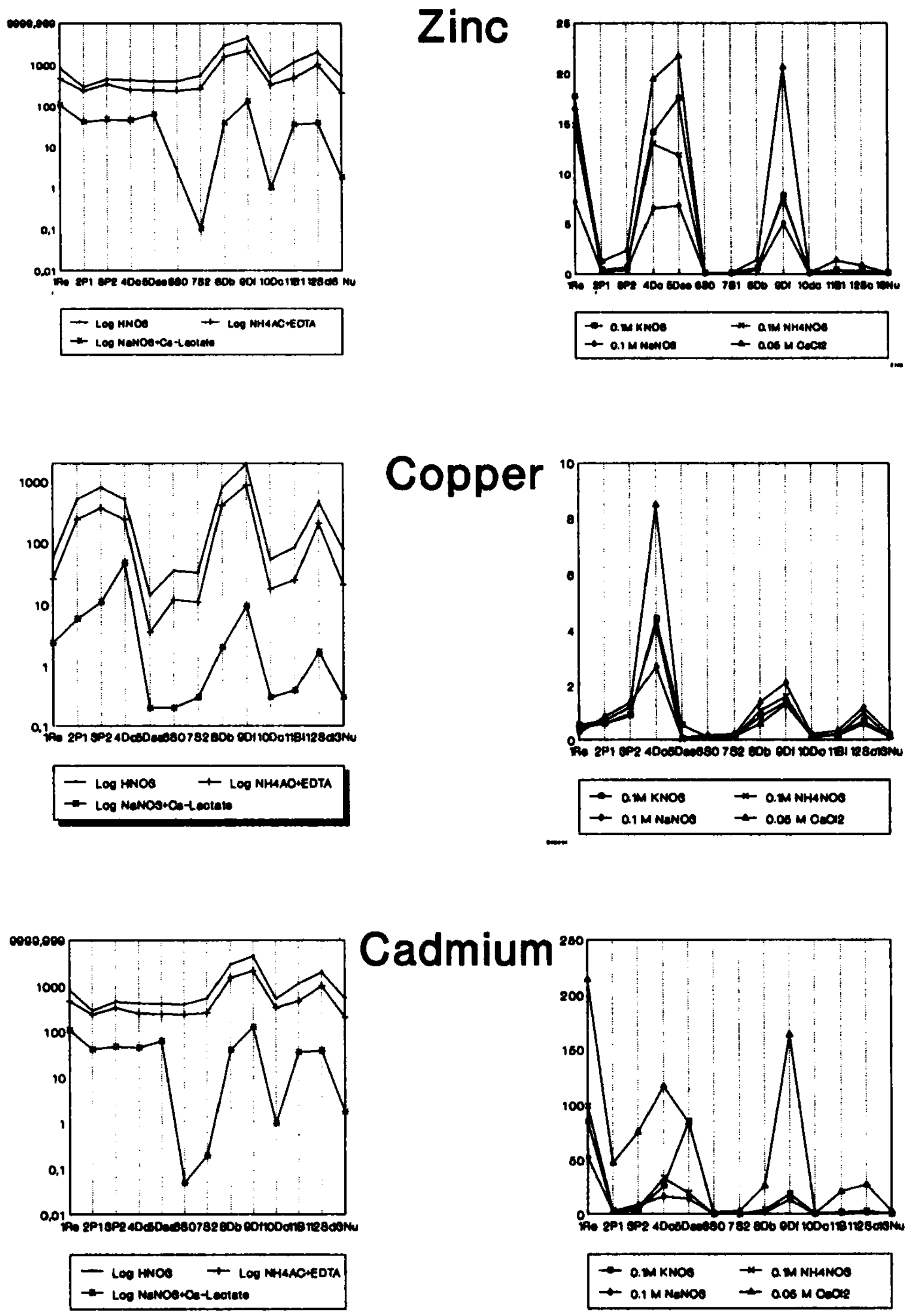


Figure 4 Comparision of Zn, Cu and Cd extraction efficiency of 7 extraction media in 13 soils.

The concentrations of each particular metal extracted from 13 soils by the various extraction media containing different salts are in approximately the same range for each soil (Figure 4) except for HNO_3 and NH_4OAc + EDTA. This implies the absence of specific interactions between the soils and the extractants and allows us to rank the extractants in order of strength. The relative extraction strengths are calculated as average extraction ratios (the ratio of concentrations for a pair of extractants for each soil, averaged over the soils) and are listed in Table 3.

The 2 M HNO_3 and 0.5 M NH_4OAc + 0.02 M EDTA are much stronger than the others and behave qualitatively differently with respect to the soils than the other extractants (Figure 4). Extraction with 0.1 M $NaNO_3$+ 0.05 M calcium lactate is somewhat anomalous since the patterns of extractions are similar to salt extractants for Cu and Zn although not for Cd and Pb. Apparently, the latter effect is due to lactate complexes of the Cu and Zn ions. The extractant calcium chloride ($CaCl_2$) which was used in three different molarities i.e. 0.1 M and 0.05 M and 0.01 removed higher concentrations of Cd and Zn from soils than expected from the equation perhaps because of formation of chloro-complexes. According to the studies of Verloo and Eeckhout [25] the complexing ability of chloride anions is very high (1meq/l Cl^- forms 5 mg $CdCl^+$ per liter). Copper and lead are usually not affected by chloride.

The NH_4NO_3 extracting solutions usually affect the soil equilibrium between soil solid and soil solution. Thus a part of the metal in solid phase, which is less of active and of less biological significance is mobilized into soil solution. This can occur due to following reasons:

- it decreases the initial bulk soil pH and thus metals from the solid phase of soil are mobilzed to the soil solution phase.
- the transformations of NH_4 ions to NH_3 which form strong complexes with the Cu, Ni and Cr are accelerated due to high soil pH and increased biological activity. The biological activity in the slurry during the 2 hours of shaking is normally high, and is significantly enhanced due to presence of free calcium ions.

Ionic and total metal concentrations in salt extracts

In three of the salt extractants the concentrations of Cu and Zn were measured by the ion chromatography (IC) in addition to AAS. The chromatographic technique estimates to a large extent free metal ions and ions bound weakly in complexes so that the ion-exchange resin in the concentrator column can compete with the ligands and retain the metal ions. Strong complexes would pass through the concentrator column and out of the apparatus, when the sample was loaded. In contrast, AAS measures total metal concentrations regardless of the ligands or strength of complexation. Concentrations of Cu determined by the two methods in three extraction media (Tables 4 to 7) were significantly correlated with the IC measurement being 44% of the AAS value. Thus, in each of these extractants about 44% of the Cu^{2+} was essentially free, while 56% was in strongly bound complexes. Concentrations of Zn^{2+} determined by IC in $CaCl_2$ and NH_4NO_3 correlate well with AAS measurements and showed 12 % of the Zn^{2+} to be free (Tables 4 to 7).

Table 4 Relationship between Extractable Copper in 11 different Extracting Media (log) and Copper Concentration in leaves of Lettuce and Ryegrass (log) grown in Miniplot Field Experiment.

Messtechniques																*Plant*
Atomic Absorption													*Ion-Chromotograph*			
Extracting media	*2M HNO_3*	*0.5M NH_4OAc+ 0.02M EDTA*	*0.1M $NaNO_3$ +0.05M Ca-lactate*	*0.1M $CaCl_2$*	*0.1M KNO_3*	*0.1M $NaNO_3$ +0.5M NH_4NO_3*	*0.5M NH_4NO_3*	*0.1m NH_4NO_3*	*0.1M N_aNO_3 +0.1M NH_4NO_3*	*0.05m $CaCl_2$*	*0.1M $NaNO_3$*	*0.1M $CaCl_2$*	*0.1M $NaNO_3$ +0.1M NH_4NO_3*	*0.05M $CaCl_2$*	*0.1M $NaNO_3$*	*Lettuce*
		D	*F*	*K*	*E*	*B3*	*B2*	*B1*	*C*	*A*	*H*	*L*	*IC*	*IA*	*IH*	
HNO_3	/															
D	0.998	/														
F	0.848	0.852	/													
K	0.598	0.601	0.898	/												
E	0.685	0.690	0.882	0.966	/											
B3	0.872	0.878	0.889	0.756	0.804	/										
B2	0.891	0.895	0.886	0.723	0.770	0.997	/									
B1	0.935	0.938	0.910	0.720	0.773	0.982	0.990	/								
C	0.925	0.930	0.902	0.711	0.760	0.988	0.995	0.997	/							
A	0.829	0.835	0.980	0.888	0.882	0.949	0.942	0.944	0.942	/						
H	0.960	0.961	0.918	0.735	0.798	0.968	0.975	0.992	0.987	0.935	/					
L	0.900	0.904	0.982	0.862	0.887	0.949	0.945	0.962	0.956	0.742	0.962	/				
C-IC	0.826	0.840	0.928	0.823	0.836	0.949	0.937	0.924	0.940	0.959	0.925	0.845	/			
A-IC	0.837	0.839	0.974	0.928	0.936	0.869	0.858	0.880	0.869	0.947	0.902	0.784	0.892	/		
H-IC*	0.911	0.918	0.960	0.845	0.892	0.924	0.918	0.941	0.936	0.950	0.958	0.934	0.926	0.971	/	
Plants																
Lettuce	0.585	0.603	0.677	0.589	0.576	0.523	0.521	0.552	0.563	0.601	0.578	0.598	0.608	0.726	0.736	/
Ryegrass	0.894	0.891	0.882	0.736	0.779	0.797	0.799	0.844	0.839	0.829	0.885	0.867	0.820	0.910	0.941	0.811

*Results of 1987–1988, mean values (Ref. 8)

Table 5 Relationship between Extractable Zinc in 11 different Extractanting Media (log) and Zinc Concentration in leaves of Lettuce and Ryegrass (log) grown in Miniplot Field Experiment.

Messtechniques															*Plant*
Atomic Absorption													*Ion-Chromotograph*		
Extracting media	*2M HNO_3*	*0.5M NH_4OAc+ 0.02M EDTA*	*0.1M $NaNO_3$ +0.05M Ca-lactate*	*0.1M $CaCl_2$*	*0.1M KNO_3*	*0.1M $NaNO_3$ +0.5M NH_4NO_3*	*0.5M NH_4NO_3*	*0.1M NH_4NO_3*	*0.1M $NaNO_3$ +0.1M NH_4NO_3*	*0.05M $CaCl_2$*	*0.1M $NaNO_3$*	*0.01M $CaCl_2$*	*0.1M $NaNO_3$ +0.1M NH_4NO_3*	*0.05M $CaCl_2$*	*Lettuce*
		D	*F*	*K*	*E*	*B3*	*B2*	*B1*	*C*	*A*	*H*	*L*	*IC*	*IA*	
HNO_3	/														
D	0.957	/													
F	0.405	0.510	/												
K	0.215	0.321	0.973	/											
E	0.127	0.226	0.904	0.966	/										
B3	0.108	0.228	0.916	0.973	0.988	/									
B2	0.136	0.244	0.931	0.985	0.991	0.993	/								
B1	0.151	0.233	0.891	0.955	0.996	0.979	0.984	/							
C	0.173	0.274	0.936	0.984	0.996	0.992	0.997	0.991	/						
A	0.218	0.337	0.977	0.997	0.958	0.964	0.978	0.943	0.977	/					
H	0.203	0.301	0.943	0.985	0.994	0.988	0.993	0.989	0.999	0.978	/				
L	0.253	0.378	0.951	0.937	0.875	0.889	0.900	0.846	0.901	0.950	0.903	/			
C-IC	0.193	0.291	0.936	0.978	0.980	0.981	0.979	0.970	0.986	0.969	0.989	0.899	/		
A-IC	0.471	0.605	0.952	0.901	0.844	0.873	0.875	0.826	0.876	0.908	0.880	0.927	0.866	/	
Plants															
Lettuce	-0.123	0.021	0.780	0.867	0.883	0.879	0.901	0.854	0.874	0.877	0.861	0.831	0.834	0.746	/
Ryegrass	0.286	0.401	0.857	0.827	0.709	0.763	0.789	0.686	0.756	0.832	0.752	0.799	0.743	0.847	0.736

*Results of 1987 – 1988, mean values (Ref. 8)

Table 6 Relationship between Extractable Cadmium in 11 different Extractanting Media (log) and Cadmium Concentration in leaves of Lettuce and Ryegrass (log) grown in Minplot Field Experiment.

Messtechniques													*Plant*
Atomic Absorption													
Extracting media	*2M HNO_3*	*0.5M NH_4OAc+ 0.02M EDTA*	*0.1M $NaNO_3$ +0.05M Ca-lactate*	*0.1M $CaCl_2$*	*0.1M KNO_3*	*0.1M $NaNO_3$ +0.5M NH_4NO_3*	*0.05m NH_4NO_3*	*0.1M NH_4NO_3*	*0.1M $NaNO_3$ +0.1M NH_4NO_3*	*0.5M $CaCl_2$*	*0.1M $NaNO_3$*	*0.1M $CaCl_2$*	*Lettuce*
		D	*F*	*K*	*E*	*B3*	*B2*	*B1*	*C*	*A*	*H*	*L*	
HNO_3	/												
D	0.948	/											
F	0.174	0.322	/										
K	0.027	0.201	0.942	/									
E	-0.212	-0.092	0.740	0.907	/								
B3	-0.098	0.073	0.842	0.955	0.941	/							
B2	-0.100	0.084	0.844	0.947	0.917	0.994	/						
B1	-0.116	0.059	0.818	0.943	0.943	0.997	0.993	/					
C	-0.200	-0.020	0.823	0.939	0.940	0.992	0.991	0.990	/				
A	-0.041	0.108	0.894	0.948	0.877	0.928	0.896	0.905	0.907	/			
H	-0.214	-0.036	0.812	0.941	0.942	0.980	0.971	0.973	0.979	0.928	/		
L	-0.314	-0.291	0.553	0.745	0.916	0.890	0.877	0.900	0.906	0.962	0.987	/	
Plants													
Lettuce	-0.336	-0.076	0.482	0.634	0.603	0.623	0.607	0.615	0.645	0.676	0.683	0.673	/
Ryegrass	0.128	0.359	0.756	0.764	0.584	0.713	0.702	0.691	0.707	0.787	0.669	0.732	0.761

*Ref. 8

Table 7 Relationship between Extractable Lead in 11 different Extractanting Media (log) and Lead Concentration in leaves of Lettuce and Ryegrass (log) grown in Miniplot Field Experiment.

Messtechniques												*Plant*
Atomic Absorption												
Extracting media	*2M HNO_3*	*0.5M NH_4OAc+ 0.02M EDTA*	*0.1M $NaNO_3$ +0.05M Ca-lactate*	*0.1M $CaCl_2$*	*0.1M KNO_3*	*0.1M $NaNO_3$ +0.5M NH_4NO_3*	*0.5M NH_4NO_3*	*0.1M NH_4NO_3*	*0.1M $NaNO_3$ +0.1M NH_4NO_3*	*0.05M $CaCl_2$*	*0.1M $NaNO_3$*	*Lettuce*
		D	*F*	*K*	*E*	*B3*	*B2*	*B1*	*C*	*A*	*H*	
HNO_3	/											
D	0.966	/										
F	-0.179	-0.044	/									
K	-0.673	-0.595	0.654	/								
E	-0.421	-0.374	0.421	0.878	/							
B3	-0.462	-0.409	0.772	0.768	0.542	/						
B2	-0.400	-0.361	0.875	0.821	0.604	0.909	/					
B1	-0.192	-0.106	0.916	0.707	0.541	0.849	0.914	/				
C	-0.326	-0.208	0.730	0.592	0.249	0.694	0.693	0.727	/			
A	-0.542	-0.452	0.873	0.837	0.539	0.889	0.936	0.902	0.830	/		
H	0.045	0.181	0.951	0.542	0.393	0.745	0.795	0.905	0.691	0.757	/	
Plants												
Lettuce	-0.462	-0.395	0.103	0.028	-0.335	0.001	0.015	-0.193	0.258	0.130	-0.076	
Ryegrass	*-0.077*	*0.113*	*0.361*	*-0.044*	*-0.315*	*-0.075*	*-0.029*	*0.031*	*0.328*	*0.177*	*0.249*	*0.645*

Relationship between metal concentration in extraction media and metal concentration in indicator plants

The relations among the concentrations of metal ions removed from the soils by the various extracting media and by the two plant types lettuce (dicot) and rye grass (monocot) are summarized in four tables (log vs log Pearson correlation coefficients) (Tables 4 to 7).

In the following discussion Pb will not be included because the plants grown in none of these 13 soils were found to exhibit zootoxic concentrations (10 mg/kg). Further, the ranges of Pb concentration were 0.4 to 2.2 and 0.5 to 2.3 mg/kg for lettuce and rye grass, respectively. Since the mean uncertainity in measurements is 1 mg/kg of plant materials, it is not possible to obtain reliable correlations in the data.

Since the correlations between lettuce and rye grass are not very high (0.811, 0.736, 0.761 for Cu, Zn and Cd, respectively) any extraction media which yields higher correlation coefficients for both plant types offers a potentially useful means of predicting the concentration of the metal.

Among the correlation coefficients for the extraction media similar results summarized in Table 8 may be seen clearly; the two strongest extractants correlate well with each other and poorly with the others for each metal as is shown in Table 8. The salt extractants correlate very well for Cu, Cd and moderately well for Zn.

Extraction with strong acid and the chelating agent i.e. 2 M HNO_3 and 0.5 M NH_4OAc + EDTA) agree well with each other and correlate slightly better with copper concentrations in both lettuce and rye grass than the plants do with each other. In addition, the weak neutral salt extractant (0.1M $NaNO_3$) correlates significantly with the copper concentration in both plant species. The other salt extractants agree poorly with the plants. The range of copper concentration was 8-25 mg/kg dry matter for lettuce and 8-39 mg/kg for rye grass[8].

The correlation between lettuce and rye grass is only 0.54. All of the salt extractants correlated rather well with lettuce and lot poorly with rye grass. The range of zinc concentration was 56-369 mg/kg for lettuce and 31-360 mg/kg for rye grass.

Although the extractants containing strong acid and chelating - agents agree well with each other and the weak extractants agree fairly well among themselves, the correlations among the plants and the $NaNO_3$ soluble Cd is better correlated with the cadmium concentration of lettuce and rye grass.

Table 8 The relationship between extractable Copper, Zinc and Cadmium (log) in 5 selected important extraction media and Cu, Zn and Cd concentration in lettuce and rye grass (log).

Extraction Media	*Molarity (M)*		*Plants*					
			Lettuce			*Rye grass*		
			Copper	*Zinc*	*Cadmium*	*Copper*	*Zinc*	*Cadmium*
HNO_3	2.0	M	0.585	-0.123	-0.336	0.894	0.286	0.128
NH_4OAc+ EDTA	0.52	M	0.603	0.021	-0.076	0.891	0.411	0.359
$NaNO_3$	**0.1**	**M**	**0.578**	**0.861**	**0.683**	**0.885**	**0.752**	**0.669**
$CaCl_2$	0.05	M	0.601	0.877	0.676	0.829	0.832	0.787
NH_4NO_3	0.1	M	0.552	0.854	0.615	0.844	0.686	0.691
KNO_3	0.1	M	0.576	0.883	0.603	0.779	0.709	0.584
$CaCl_2$	0.01	M	0.598	0.831	0.673	0.867	0.799	0.732

AVAILABLE INFORMATION TO EVALUATE THE SUITABILITY OF EXTRACTION MEDIA TO PREDICT CRITICAL [PHYTOTOXIC] CONCENTRATION OF METALS IN PLANTS OR THE GROWTH OF SOIL MICROORGANISMS

The important characterstics of three important salt extractant are presented in Table 9. These results summarize the available information to find a compromise extractant to be used in the simple model to prdict either the expected concentration in the plants or reduction in growth and activities of soil microorganisms grown on a given anthropogenic contaminated soil. The final goal of such extraction medium is to predict the toxicity (either to plant or soil microorganisms or water and air) and thus to help in assessment of risk posed by the presence of metals in anthropogenically contaminated soils without carrying out the time consuming growth experiments.

The following conclusions can be drawn from Tables 8 and 9:

- Strong acid (2M HNO_3) and strong acidified Chelating agents (NH_cOAC+EDTA) are unsuitable for the prediction of either critical (phytotoxic) concentration in the plants or the growth of micro-organisms.
- The correlations coefficients between $NaNO_3$ soil extractable Cu, Zn and Cd are for both indicator plants are significantly correlated with the Cu, Zn and Cd concentrations, in spite of the fact that it extracts comparatively lesser quantity of metals from soil compared to other extractants. However, in case of other salt extractants the correlation coefficients for all metals and both plants are not always significant.

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