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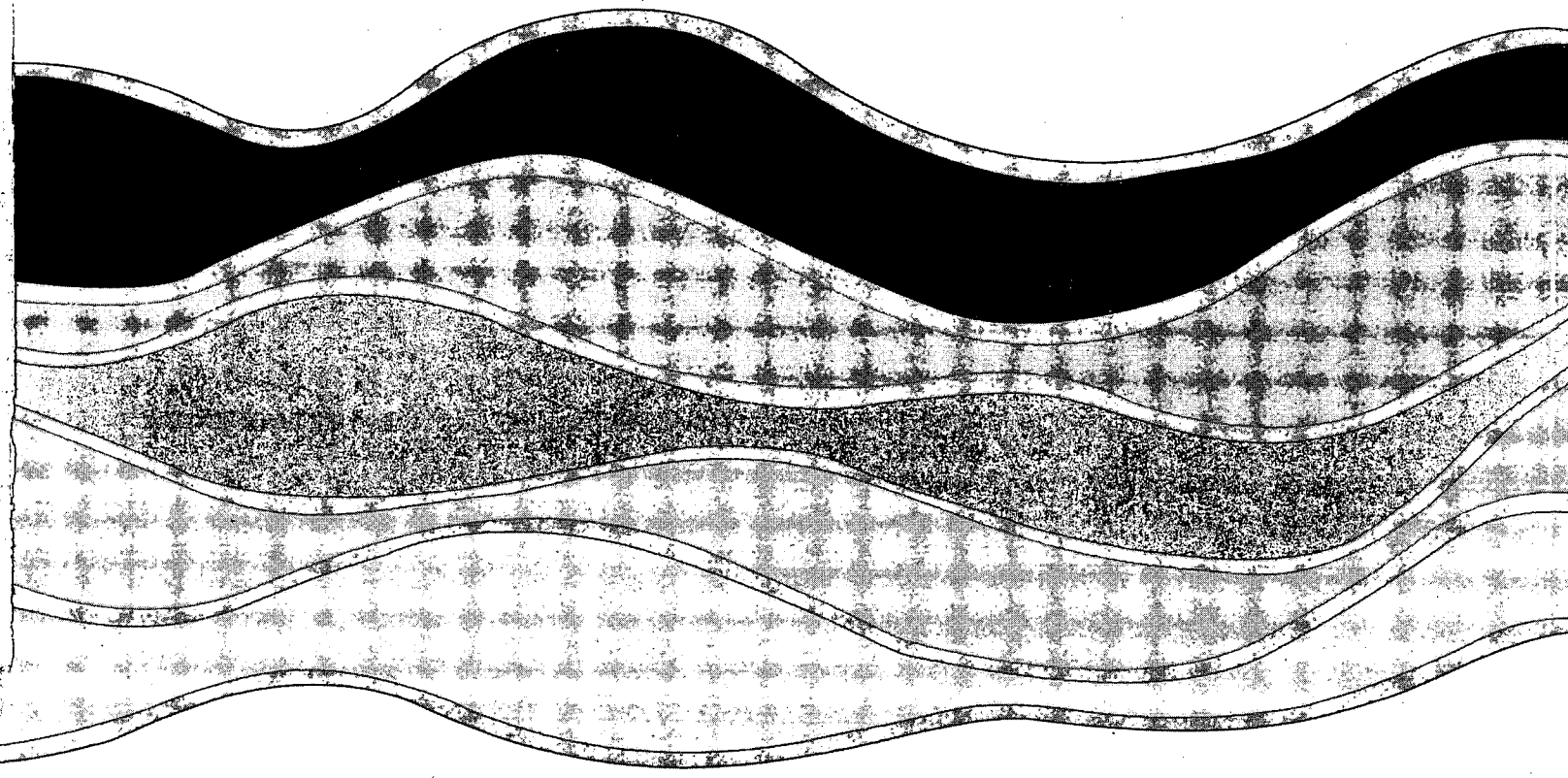

COMPARISON OF TOXICITY RESPONSES OF FOUR SOLID PHASE BIOASSAYS TO TWELVE SOLID PHASE CERTIFIED REFERENCE MATERIALS

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NWRI Contribution No. 97-55

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Management perspective

The lack of understanding of relationships between sediment contaminant concentrations and bioavailability requires the use of laboratory toxicity/genotoxicity bioassays and bioaccumulation tests to estimate the present and potential damage to biota.

Before the advent of short term solid phase bioassay techniques, there were a great variety of methods for preparing leachates (extracts) from solid or semi-solid samples. There was, understandably, no agreed-upon standard method to follow. Rationale for researchers choosing one particular technique or procedure over any other was usually lacking and thus researchers tended to adopt published methods piecemeal, often adding their own modifications. Thus, compounded with the great variability one finds in sediments and soils, it is very difficult to meaningfully compare data and results from other researchers.

Arising from these heterogeneous approaches for obtaining toxicological information is the awareness that there is a lack of understanding of all the processes which control the movement and bioavailability of resident pollutants. Traditional chemical-specific methods for assessing toxicity are not easily applied to sediments or soils and are not capable of determining the degree of contaminant bioavailability. If toxic contaminants can be identified , the selection of a remediation process is made easier.

In this study we were able to work with twelve highly-characterized freeze-dried reference sediments within which the concentration of many of the organic and inorganic toxic chemicals were known. These sediments have been and are still being used as Certified Reference Materials (CRM) for chemical analyses control. These materials provided us with an opportunity to be able to compare the toxicity /genotoxicity responses of four solid phase bioassays and to evaluate the possibility of relating the different bioassay responses to the known chemical composition of each of the CRMs. This study also showed us the difficulties and problems involved in assessing and identifying the toxicity potential of trace amounts of chemicals in sediments.

Sommaire à l'intention de la direction

En raison du manque de compréhension des relations entre les concentrations et la biodisponibilité des contaminants dans les sédiments, on doit utiliser des bio-essais de toxicité et de génotoxicité et des tests de bioaccumulation de laboratoire pour estimer les dommages actuels et potentiels imposés au biote.

Avant l'arrivée des techniques de bio-essais en phase solide à court terme, il y avait une grande variété de méthodes pour préparer les lixiviats (extraits) à partir d'échantillons solides ou semi-solides. Il n'y avait, on le comprend, aucune méthode généralement acceptée à suivre. Les chercheurs choisissaient une technique ou une procédure donnée sans raison particulière, et ils tendaient à adopter des méthodes publiées au gré des circonstances, en ajoutant souvent leurs propres modifications. Ainsi, en plus du fait de la grande variabilité dans les sédiments et les sols, il est très difficile de comparer correctement les données et les résultats des différents chercheurs.

Ces approches hétérogènes visant à obtenir des informations toxicologiques nous ont révélé le manque de compréhension de l'ensemble des processus qui gouvernent le déplacement et la biodisponibilité des polluants présents. Les méthodes classiques relatives à des produits chimiques spécifiques pour l'évaluation de la toxicité ne sont pas facilement appliquées aux sédiments ou aux sols et ne permettent pas de déterminer le degré de biodisponibilité des contaminants. Si les contaminants toxiques peuvent être identifiés, le choix d'une méthode de décontamination s'en trouve faciliter.

Dans la présente étude, nous avons pu travailler avec douze sédiments de référence lyophilisés hautement caractérisés dans lesquels la concentration de nombre des produits chimiques toxiques organiques et inorganiques était connue. Ces sédiments ont été utilisés et sont encore utilisés à titre de matériaux de référence certifiés pour le contrôle des analyses chimiques. Ces matériaux nous permettent de comparer les réponses de toxicité et de génotoxicité de quatre bio-essais en phase solide et d'évaluer la possibilité de relier les différentes réponses des bio-essais à la composition chimique connue de chacun des matériaux de référence certifiés. Cette étude nous a aussi montré les difficultés et les problèmes liés à l'évaluation et à l'établissement du potentiel de toxicité de quantités infimes de substances chimiques dans les sédiments.

Abstract

This study was initiated to illustrate the difficulties and problems involved in assessing and identifying the toxicity potential of trace amounts of chemicals in sediments. Usually in evaluating the toxicity of sediments, pore water and / or solvent extracts are used to estimate the level of soluble or extractable toxicants. However, it is often difficult or even impossible to detect the presence of the total bioavailable toxicants due to their low concentrations, low solubility and /or insolubility in the extracting solvents. Direct sediment toxicity testing (intimate contact between testing organisms and all solid and liquid parts of a sediment) can significantly circumvent these problems by directly detecting the total toxic response of soluble and insoluble organic and inorganic contaminants. In this study 12 Certified Reference Material (CRM) sediments, whose main constituents were known, were used with solid phase bioassays to try to evaluate the possibility of relating the different bioassay responses to the known chemical composition of each of the CRMs. Results and bioassays used and the difficulties and problems involved in assessing and identifying the toxicity potential of trace amounts of chemicals in sediments are described..

Résumé

La présente étude a été entreprise pour illustrer les difficultés et les problèmes liés à l'évaluation et à l'établissement du potentiel de toxicité des quantités infimes de substances chimiques dans les sédiments. Habituellement, dans l'évaluation de la toxicité des sédiments, on utilise l'eau interstitielle ou des extraits de solvant pour estimer les taux de toxiques solubles ou extractibles. Cependant, il est souvent difficile ou même impossible de détecter la présence des toxiques totaux biodisponibles en raison de leurs faibles concentrations ou de leur faible solubilité ou de leur insolubilité dans les solvants d'extraction. Des tests directs de toxicité des sédiments (contact intime entre les organismes des tests et toutes les parties solides et liquides d'un sédiment) peuvent bien circonvenir ces problèmes en détectant directement la réponse toxique totale des contaminants organiques et inorganiques solubles et insolubles. Dans cette étude, 12 sédiments constituant des matériaux de référence certifiés, dont les principales composantes étaient connues, ont été utilisés avec des bio-essais en phase solide pour essayer d'évaluer la possibilité de relier les différentes réponses des bio-essais à la composition chimique connue de chacun des matériaux de référence certifiés. On décrit les résultats et les bio-essais utilisés, de même que les difficultés et les problèmes liés à l'évaluation et à l'établissement du potentiel de toxicité de quantités infimes de produits chimiques dans les sédiments.

Introduction

Sediment provides habitat for many aquatic organisms but it is also a major repository for many persistent chemicals that are introduced into surface waters. Sediments are also very heterogeneous, exhibiting high spatial and temporal variability. Most chemicals and waste materials , including organic chemicals and heavy metals from point and non-point sources may accumulate in sediment. Concentrations of chemicals are often several orders of magnitude higher in sediment than in the overlying waters, thus the long- term release of low concentrations of chemicals into water can result in elevated concentrations in water. Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain. While many chemicals, including petroleum hydrocarbons, other organic compounds and heavy metals, tend to sorb to sediment, bulk sediment concentration of these contaminants are not highly correlated to bioavailability (Ingersol, 1991). The lack of understanding of relationships between sediment contaminant concentrations and bioavailability requires the use of laboratory toxicity/genotoxicity bioassays and bioaccumulation tests to estimate the present and potential damage to biota.

Before the advent of short term solid phase bioassay techniques, there were a great variety of methods for preparing leachates (extracts) from solid or semi-solid samples. There was, understandably, no agreed-upon standard method to follow (Dombroski et al 1990). Rationale for researchers choosing one particular technique or procedure over any other was usually lacking and thus researchers tended to adopt published methods piecemeal, often adding their own modifications. Thus, compounded with the great variability one finds in sediments and soils, it is very difficult to meaningfully compare data and results from other researchers.

Arising from these heterogeneous approaches for obtaining toxicological information is the awareness that there is a lack of understanding of all the processes which control the movement and bioavailability of resident pollutants. Traditional chemical-specific methods for assessing toxicity are not easily applied to sediments or soils and are not capable of determining the degree of contaminant bioavailability. If toxic contaminants can be identified , the selection of a remediation process is made easier.
In this study we were able to work with twelve highly-characterized freeze-dried

reference sediments within which the concentration of many of the organic and inorganic toxic chemicals are known. These sediments have been and are still being used as Certified Reference Materials (CRM) for chemical analyses control (Quality Assurance Reference Materials and Services, 1995). These materials provided us with an opportunity to be able to compare the toxicity /genotoxicity responses of four solid phase bioassays and evaluate the possibility of relating the different bioassay responses to the known chemical composition of each of the CRMs. The bioassays used and the results obtained are discussed below.

METHODS

Bioassays

The solid phase CRM samples were tested by the following four direct bioassays (no extraction): Direct Sediment Toxicity Testing Procedure (DSTTP), which is commercially available as Toxi-Chromopad, EBPI, Brampton, Ont.(Kwan, 1993); SOS-Chromotest pad procedure (SCPP), a genotoxicity bioassay developed by Dutka et al. (1995); Microtox solid phase (SPT) test (Tung et. al. 1991); and the solid phase *Panagrellus redivivus* (SPR) bioassay (McInnis 1996). The CRM samples were also tested by the *Panagrellus redivivus* liquid phase test (Samoiloff, 1990). To perform this test, 5 grams of the freeze-dried sediment were vigorously mixed with 5 mL of Milli-Q water for three minutes, then centrifuged for 20 minutes at 10,000 rpm in a refrigerated centrifuge. The supernatant was used in the *Panagrellus* (nematode) bioassay.

Sediments

The CRM sediments were collected from a variety of sources in the Great Lakes basin (e.g. Hamilton Harbour, Lake Ontario, Niagara River plume, Toronto Harbour, Lake Erie, Lake St. Claire and a Sudbury area lake), which contained the contaminating chemicals of interest. These sediments were prepared as Sediment Reference Materials following procedures described by Lee and Chau, (1987), Lee et al. (1987), Lee et al. (1986) and Cheam and Chau, (1984). The following CRM sediments were part of this study: EC1, EC2, EC3, EC4, EC5, EC6, EC7, EC8, WQB1, WQB3, SUD1 and TH2.
The CRM EC2 was created by mixing three parts of sediment collected from EC3

area with one part sediment collected from EC1. EC2 and EC3 have elevated levels of dioxins and furans. EC8 was collected from the same site as EC3, but eight years later, and at the time of this study its composition was not fully known. Each of the CRMs contained a variety of other chemicals (not identified fully), none of which impacted on the use of these freeze-dried sediments as Certified Reference Materials.

RESULTS AND DISCUSSION

Table 1 presents the various reactions of the solid phase and liquid phase *Panagrellus redivivus* (nematode) tests to the 12 CRMs. From this information there are two major observations: (1) the reverse toxicity pattern shown by the Milli-Q extracts of EC3 and EC4 compared to the direct sediment toxicity testing of EC3 and EC4, and (2) the extreme toxicity shown by CRMs SUD1, EC8, EC6 and WQB1.

In the nematode test, percent survival is an indicator of acute toxicity. Historically, our control nematodes have shown greater than 96% survival. Thus any bioassay result showing less than 90% survival is considered to be the result of toxicants in the sample. Similarly, percent growth , the percent of nematodes reaching the J4 stage, has always been between 96-100% of the nematodes surviving in the control solution. One of the main reasons for the nematodes not reaching the J4 stage is the presence of low concentrations of toxicants which inhibit growth but do not kill. This is considered to be a chronic effect. The other endpoint is percent maturation. For a nematode to progress from the J4 stage to become an adult, a genetic change has to occur. Many known mutagens/genotoxicants can selectively inhibit the J4 to adult molt, and this inhibition of growth can be used as an indication of potential mutagens/genotoxicants in the sample (Samoiloff, 1990). Again, historically, we have found that 96-100% of control J4 animals become adults, therefore any results that show less than 90% maturation suggest the presence of genotoxic activity. However, there are a few instances where discretion in interpreting the data should be considered. An example can be seen in sediment samples EC8 and SUD1. In Table 1 it can be seen that there was 0% maturation. However no nematodes ever reached the J4 stage , thus the potential for genetic inhibition actually never occurred. Observations such as these led Samoiloff (1990) to develop a percent

fitness scale where each factor was given a calculated weight and a fitness scale was developed. If we accept that any percent fitness of less than 60% is a valid indication of a significant toxic effect , it can be seen that the solid phase tests on EC1, EC3, EC4, and EC5 suggest minimal toxicity for these samples while Milli Q extracts of EC3 and EC4 indicate a very low percent fitness and a strong toxicity effect.

Solid phase nematode results suggest that the CRMs can be arranged in three groups: very toxic, (SUD1, EC8, WQB1 and EC6); moderately toxic, (TH2, WQB3, EC7 and EC2); and minimal toxic effect,(EC1, EC3, EC4 and EC5). The Milli-Q extracts can be separated into two groups:very toxic/genotoxic,(EC3 and EC4); and little or no toxic effect,(the remaining CRMs).

While EC3 and EC8 were collected from the same area, but 8 years apart , EC8 in the nematode solid phase test is much more toxic than EC3, the older sample. It would appear that there may have been some spatial variability or that over the 8 years there has been a change in composition with a loss or decrease in a Milli-Q soluble component and an increase in a non water soluble toxicant component.

Table 2 presents a summary of the solid phase bioassays. The DSTTP results vary from 0.0625 to 0.5 grams of sediment required to produce an EC_{100} effect i.e. no reduction of the chromogen and thus no blue colour. While an EC_{50} effect can be established, usually 1:2 or 1:4 dilutions lower, the end point is based on the discrimination by eye between various shades of blue, thus the EC_{50} values would vary from person to person. From the DSTTP data it can be seen that WQB1, EC3 and EC4 are the most toxic CRMs, and EC6 and EC7 are the least toxic.

The Microtox SPT results are based on the percent of the sediment sample which reduces light output by 50% in the indicator organism *Vibrio fischeri* . As there is no standard for this bioassay, users usually accept EC_{50} values produced by 1.0% or less of the solid phase samples as being a realistic assessment of sample toxicity. Therefore, the lower the percent of sample required to produce the EC_{50} effect, the more toxic the solid phase sample. Based on Microtox SPT results the most toxic samples are, in order of toxicity: SUD1, WQB1 and EC4.

The Colour Index Profile values shown in Table 2 under the heading Sed-SOS Chromotest were obtained in the following manner. After incubation of sample dilutions on the chromogen pads (Dutka et al. 1995) the solid particulates were washed of the pads with tap water using a wash bottle. The colour of each transfer spot was then visually observed. Based on the intensity of the blue colour developed in the positive control, a point rating scheme was used in which the most intensive blue colour (indicative of a strong genotoxic effect) was given a colour index value of 5, while no blue colour was given a colour index value of 0.

For each natural or 4-nitroquinoline-N-oxide spiked sample, eleven colour index values were recorded, corresponding to each of the 2-fold dilutions of sample material (Dutka et al. 1995) . The colour index values for each test sample were combined in a Colour Index Profile (CIP), which is an 11-digit number representing the colour index of the lowest dilution (or highest sample concentration) to the highest dilution. The first step in determining the genotoxicity was to subtract the digit values of the reference sample from the corresponding CIP digit values of the test sample, resulting in an 11-digit net-CIP number. In the second step, all digits that were ≥ 2 of the net-CIP were added up, giving a numerical genotoxicity value. The CIP genotoxicity value was used as a quantitative measure to compare the genotoxic response between samples. From Table 2. it can be seen that EC7, EC5, EC4 and WQB3 showed the greatest genotoxic effect with SUD1 having the least effect.

From Table 1 it appears that EC8 and SUD1 have maximum genotoxic effects, however it can be seen that none of the few surviving nematodes ever progressed beyond the J2 or J3 stages, thus it is impossible to know if these CRMs had any genotoxic potential. Interestingly the Sediment- SOS Chromotest indicates that SUD1 contains no detectable genotoxicants while EC8 is strongly genotoxic. Comparing the two genotoxic bioassay responses (Tables 1&2) It can be seen that both EC5 and TH2 CRMs appear to contain chemical mixtures which produce strong genotoxic effects. Similarly in both genotoxic indicating bioassays , EC2 and EC3 results indicate that these CRMs contain some of the lowest concentrations of genotoxic effects producing chemicals.

The trace metal concentrations in two of the sediment CRMs are shown in Table 3. Similarly, the concentrations of toxic organics in three of the CRMs are shown in

Table 4. It is very difficult if not impossible to relate a specific chemical to a specific bioassay response. The chemicals are too many and too varied in concentration.In the bioassay responses shown in Tables 1 and 2 we see specific responses due to heterogeneous mixtures of chemicals in various stages of bioavailability. In the recorded bioassay responses we are seeing the end effect of synergistic, additive and antagonistic effects as a result of coming in contact with a heterogeneous mixture of bioavailable known chemicals . Also added to this effect are the many unknown chemicals in each CRM.

There are many known and proposed mechanisms by which toxicants inhibit and eventually kill bacteria or other bioassay organisms. For example, toxicants may directly cause damage to the genetic material or as in the case of halogens,their presence may lead to protein denaturation. Toxicants such as phenol and quaternary ammonium compounds are known to disrupt cell membranes resulting in leakage of DNA, RNA, proteins and other organic materials. Acids and alkalis may displace cations such as Na^+ and Ca^+ from adsorption sites on bacterial cells. A more subtle action of toxic pollutants is their ability to block bacterial chemoreceptors which may lead to the inhibition of organic decomposition and self purification processes in sewage treatment plants and in waters receiving faecal material . However, it is believed that one of the most important effects of the toxic action of chemicals on bacteria is on enzyme activity. Also, in any toxicity study one must take into account the physico-chemical factors (presence of other cations, pH, oxidation-reduction potential, temperature, organic matter, clay particles, etc) that control the toxic action towards microorganisms (Babich and Stotzky, 1980; Bewley and Stotzky, 1983, and Levine and Black, 1996).

Thus, it is impossible to relate cause and effect with the CRMs. These conclusions are similar to those arising from toxicity assessments made on sediments , soils , sewage samples and many industrial effluents (Dutka et al. 1996, and Levine and Black, 1996). However each bioassay system has its own sensitivity range with some overlaps and this allows us to group the CRMs from most toxic/genotoxic to least toxic /genotoxic by bioassay and by the battery of test approach. Since the total chemical concentration of each CRM is not known, similar to working on a sediment sample , the grouping or ranking are only indications of the relative toxicity/genotoxicity of the group being compared.

The most simplistic approach for assessing the most toxic and genotoxic CRMs and

trying to relate them to the various chemical concentrations is to use a simple ranking approach, (e.g. the greatest or most toxic bioassay response and the highest chemical concentration = 1; and the lowest positive toxic response and third lowest chemical concentration = 3). Table 5 presents the results of this approach based on the three most toxic and genotoxic responses in each bioassay and using a selected group of chemicals believed to have greater environmental importance. In some instances, in the bioassay responses, an arbitrary decision was made in cases where more than one similar bioassay response was found (e.g. nematode maturation where there were four similar results, values of 2 and 3 were not given). Based on this arbitrary ranking scheme it can be seen that the four most toxic CRMs were SUD1, WQB1, EC4 and EC3. From this ranking comes an interesting observation that the two genotoxicity assessment bioassays seemed to respond to different mixtures of chemicals. Although Table 5 only presents selected chemicals, it can be seen that EC2 and WQB3 have the majority of chemicals which are in the higher concentrations and yet these two CRMs are two of the least toxic or genotoxic. These observation again confirm the observation that all organisms respond at different levels to toxicants (possibly related to the bioavailibility status of each within the mixture) and thus the need for the battery of tests approach.

Another interesting observation was the difference in bioassay responses between CRMs ,EC8 and EC3. These were collected from the same area with a time gap of 8 years. The Milli-Q extracts of EC3 were very toxic but EC8 Milli-Q extracts were only mildly toxic. From Table 5 it can be seen that the concentration of chemicals in EC3 and EC8 are almost completly different thus supporting the differences in toxicity/genotoxicity effects. Similarly the question is raised what water soluble toxic chemicals are only present in EC3 and EC4 in concentrations sufficient to produce an acute toxicity response in the nematode test.

This study shows the importance of using the "battery of tests" approach to show the presence of toxicants in solid and liquid phase samples. It also indicates the improbability of trying to relate "cause and effect"even when some or all the chemicals in a mixture are known. Long et al. (1995) report that "significant differences in toxicity can occur at similar toxicant concentrations over relatively small ranges in TOC and/or AVS concentrations and that it has been argued that sediment quality criteria are indefensible if they do not account for factors that control bioavailability." Thus on the rare occasions when all the chemicals are known or identified in mixtures or solid phase samples their bioavailability is not

known and thus false or inaccurate conclusions may be drawn by toxicity identification/reduction evaluations (TIE).

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Table 1 **Responses of Solid phase and Milli-Q water extracts of CRMS on the Panagrellus redivivus bioassay**

Sample	% Survival	% Growth	% Maturation	% Fitness
M9Y Control	100	100	100	100
EC 1	88	15	92	68
EC 2	63	14	83	52
EC 3	84	84	71	82
EC 4	92	63	46	77
EC 5	92	71	29	77
EC 6	12	18	0	12
EC 7	60	24	32	46
EC 8	12	0	0	7
SUD	6	0	0	3
TH2	40	27	0	31
WQB1	12	18	0	12
WQB3	22	78	42	41
EC 1 MQ*	92	96	66	89
EC 2 MQ	93	60	64	79
EC 3 MQ	18	33	0	20
EC 4 MQ	35	23	0	27
EC 5 MQ	94	100	83	94
EC 6 MQ	100	98	96	99
EC 7 MQ	92	100	100	95
EC 8 MQ	73	95	84	81
SUD MQ	100	98	71	95
TH2 MQ	100	98	82	97
WQB1 MQ	90	89	98	91
WQB3 MQ	96	98	94	96

MQ* = Milli-Q water extract 1:1

TABLE 2

SUMMARY, SOLID PHASE BIOASSAY RESULTS

Sample	DSTTP g sample =EC100	Microtox STP % sample=EC50	Nematode % survive	Sed -SOS Chromo C I P *	Nematode % mature
EC1	0.25	0.15	88	14	92
EC2	0.25	0.27	63	9	83
EC3	0.0625	0.39	84	6	71
EC4	0.125	0.08	92	24	46
EC5	0.25	0.28	92	25	29
EC6	0.5	0.5	12	21	0
EC7	0.5	1.5	60	28	32
EC8	0.25	0.53	12	21	0
SUD 1	0.25	0.043	6	0	0
TH 2	0.25	0.3	40	22	0
WQB 1	0.0625	0.067	12	9	0
WQB 3	0.25	0.16	22	24	42

CIP* =colour index profile

TABLE 3 . TRACE METAL CONCENTRATIONS, ug/g and PERCENT, SELECTED CRMs

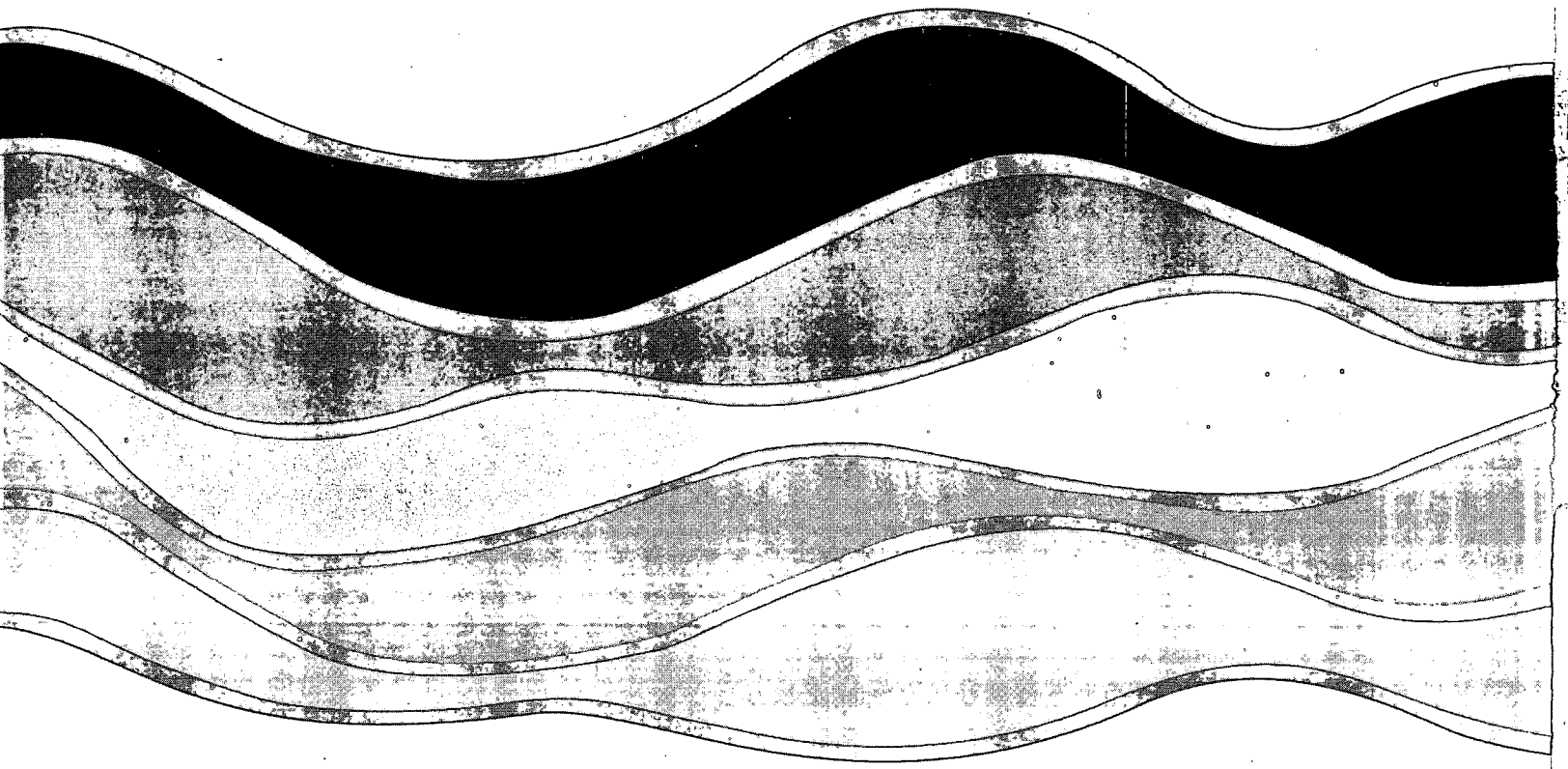
ELEMENTS	WQB 1	SUD 1
Al %	7.31	5.35
Ca %	1.13	1.19
Fe %	4.8	3.32
Mg %	1.42	1.1
Mn %	0.215	0.057
P %	0.168	0.071
K %	3.08	2.11
Na %	0.93	1.95
Sb ug/g	<15	<15
As ug/g	23	29.1
Ba ug/g	613	482
Be ug/g	2.44	1.42
Bi ug/g	<50	<50
Cd ug/g	2.1	2.3
Cr ug/g	101	92.4
Co ug/g	23.1	44.8
Cu ug/g	78.3	565
Pb ug/g	85.7	58
Mn ug/g	2197	564
Hg ug/g	1.09	0.113
Mo ug/g	2.7	2.3
Ni ug/g	62.1	946
Se ug/g	1.02	2.65
Ag ug/g	1.14	1.3
Sr ug/g	145	202
Sn ug/g	16.9	10.5
V ug/g	127	72.1
Zn ug/g	294	772

TABLE 4. SELECTED CRMs ORGANIC PARAMETERS

PARAMETERS	EC-3	EC-5	EC-7
PAHs (ug/g)			
Naphthalene	0.035	0.026	0.04
Acenaphthylene	0.025	0.041	0.013
Acenaphthene	0.022	0.029	0.003
Fluorene	0.042	0.084	0.016
Phenanthrene	0.293	0.612	0.18
Anthracene	0.059	0.113	0.022
Fluoranthene	0.558	0.823	0.196
Pyrene	0.436	0.987	0.306
Benzo(a)anthracene	0.312	0.503	0.11
Chrysene/Triphenylene	0.458	0.619	0.182
Benzo(b)fluoranthene	0.505	0.481	0.09
Benzo(k)fluoranthene	0.271	0.419	0.084
Benzo(e)pyrene	0.451	0.441	
Benzo(a)pyrene	0.386	0.449	0.103
Perylene	0.195	0.187	
Indeno(1,2,3-c,d)pyrene	0.359	0.386	0.062
Dibenz(a,h)anthracene	0.109	0.195	0.034
Benzo(g,h,i)perylene	0.348	0.333	0.095
TOTAL PCBs (ug/g)	0.661	0.597	0.021
CHLOROBENZENES (ng/g)			
1,2-dichlorobenzene	20.7	7.4	7.8
1,3-dichlorobenzene	105.4	7.1	5.7
1,4-dichlorobenzene	108.2	29.1	22.4
1,2,3-trichlorobenzene	8.9	3.8	4.4
1,2,4-trichlorobenzene	141.2	8.3	5.7
1,3,5-trichlorobenzene	113.6	6.8	13.6
1,2,3,4-tetrachlorobenzene	44.3	2.5	1.6
1,2,3,5-tetrachlorobenzene	13.6	0.64	
1,2,4,5-tetrachlorobenzene	155.6	3.3	19.2
Pentachlorobenzene	65.4	2.2	9.1
Hexachlorobenzene	279.1	2.4	53.3
Hexachloroethylene			1.1
Octachlorostyrene	41.1	0.89	17.5
Hexachlorobutadiene	61.3	0.88	7.1

TABLE 5. RANKING OF CRMs BASED ON TOP 3 MOST TOXIC/GENOTOXIC BIOASSAY RESPONSES AND RELATIVE CONCENTRATIONS OF TOXIC PARAMETERS (1 HIGH > 3 LOW)

BIOASSAYS	EC1	EC2	EC3	EC4	EC5	EC6	EC7	EC8	SUD1	TH2	WQB1	WQB3
Nematode % survival						2		2	1		2	
Nematode Milli-Q % survival			1	2								
DSTTP			1	2							1	
Microtox				3					1		2	
Sediment Chromotest				3	2		1					3
Nematode % maturation						1			1	1	1	
PAHs	1	2		3						3		2
PCBs	1	2	3	3	3			3		3		2
Chlorobenzenes		2	1					3				2
Dioxins/Furans		1	1					2				1
Hg		1		3	3			1		3	2	1
Cu		3		2	3			3	1	2	3	3
Mn		2		3	3			2	3	3	1	2
Ni		3		3	3			2	1	3	2	3
Pb		1		1	2			3		2	3	1
Zn		2		2	3			1		3		2



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