UNIVERSIDADE ESTADUAL PAULISTA
“Júlio de Mesquita Filho”
Instituto de Geociências e Ciências Exatas
Câmpus de Rio Claro

LAUREN NOZOMI MARQUES YABUKI

# UTILIZAÇÃO DE NOVOS AGENTES LIGANTES OBTIDOS A PARTIR DE BIOMASSAS PARA DETERMINAÇÃO DE ÍONS METÁLICOS POR MEIO DA TÉCNICA DE DIFUSÃO EM FILMES FINOS POR GRADIENTE DE CONCENTRAÇÃO

Rio Claro - SP
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Tese de Doutorado apresentado ao Programa de Pós-Graduação em GEOCIÊNCIAS E MEIO AMBIENTE – Instituto de Geociências e Ciências Exatas do Câmpus de Rio Claro, da Universidade Estadual Paulista "Júlio de Mesquita Filho", como parte dos requisitos para obtenção do título de Doutor em GEOCIÊNCIAS E MEIO AMBIENTE.

Orientador: Amauri Antonio Menegário

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*Dedico este trabalho à Cloe,*

*fruto do amor mais forte e verdadeiro,*

*minha continuidade.*

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Que os vossos esforços desafiem as impossibilidades,

lembrai-vos de que as grandes coisas do homem

foram conquistadas do que parecia impossível.

(Charles Chaplin)

## RESUMO

A técnica de difusão em filmes finos por gradiente de concentração (DGT) tem se consolidado como uma excelente ferramenta analítica capaz de fornecer concentrações lábeis por tempo, pré-concentração dos analitos e amostragem *in situ,* porém a aplicação desta técnica em águas de drenagem ácida de mina (DAM) é complicada devido à redução da adsorção do amostrador tradicional (resina ligante Chelex-100) para metais em baixo pH. Este estudo, avalia a utilização da técnica DGT para determinação das concentrações lábeis de Al, Cd, Cu, Mn, Ni, Pb U e Zn em águas de DAM, empregando novos agentes ligantes preparados a partir de biomassas (cascas de banana, borra de café e sementes de moringa) impregnadas em agarose. As medições DGT foram realizadas em soluções padrão em laboratório e *in situ*. Resultados laboratoriais mostram que os agentes ligantes podem ser empregados em águas com pH baixo (pH 3,5) para os íons Cd, Cu, Ni, Pb e Zn. Já os íons Al, Mn e U não apresentaram linearidade nas curvas de imersão para nenhuma biomassa pesquisada. Para a análise de amostras *in situ* de DAM, houve baixas recuperações dos íons Cd, Cu, Ni e principalmente para Zn com 5,7% da fração solúvel com o gel de agarose-moringa, podendo indicar a baixa eficiência dos agentes ligantes ou algum efeito matriz inerente durante a determinação por ICP-OES. Por outro lado, houve recuperações satisfatórias para a imersão em amostras do Rio Corumbataí e do Rio Piracicaba, ressaltando o emprego destes agentes ligantes pesquisados em matrizes aquáticas diferentes das matrizes de DAM. Este estudo mostra que o uso da técnica DGT pode ser estendido para águas com pH baixo (desde que sejam avaliados as limitações e condições de contorno avaliadas neste estudo) podendo ser uma ferramenta útil para o monitoramento em tempo integrado de concentrações lábeis em diferentes sistemas aquáticos.



**Palavras-chave:** Cascas de Banana. Borra de Café. Sementes de Moringa. Metais Traço. DGT.

## ABSTRACT

The diffusive gradients in thin films (DGT) technique has consolidated as an excellent analytical tool capable of providing labile concentrations by time, analytical preconcentration and *in situ* sampling, but the application of this technique in acid mine drainage waters (AMD) is limited by the reduction of adsorption of the traditional binding layer (Chelex-100 chelating resin) to metals at low pH. This study proposes the use of the DGT technique to determine the concentrations of Al, Cd, Cu, Mn, Ni, Pb U and Zn in AMD using new binding agents prepared from biomass (*Musa cavendishi* banana peel, spent coffee grounds and shelled *Moringa Oleifera* seeds) impregnated with agarose. DGT measurements were performed in laboratory standard solutions and *in situ*. Laboratory results showed that the binding agents can be used in waters with low pH (pH 3.5) for Cd, Cu, Ni, Pb and Zn. Al, Mn and U ions did not present linearity in the deployment curves for any biomass studied. For the analysis of *in situ* AMD samples, there were low recoveries of the Cd, Cu, Ni, and mainly Zn ions with 5.7% of the soluble fraction with the agarose-moringa gel, indicating the low efficiency of the binding agents at low values pH or some inherent matrix effect during determination by ICP-OES. On the other hand, there were satisfactory recoveries for deployment in samples from the Corumbataí River and the Piracicaba River, highlighting the use of these binding layers in aquatic matrices other than AMD matrices. This study shows that the use of the DGT technique can be extended to waters with low pH (provided that the limitations and contour conditions evaluated in this study are evaluated) and can be a useful tool for the integrated time monitoring of labile concentrations in different aquatic systems.



**Keywords:** Banana Peel. Coffee Grounds. Moringa seeds. Trace Metals. DGT.

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# SUMÁRIO

## 1. INTRODUÇÃO GERAL

A mineração juntamente com a geração de drenagem ácida é um dos problemas de maior impacto ambiental podendo causar comprometimento em longo prazo dos cursos de água e biodiversidade (AZAPAGIC, 2004), ocasionando problemas ambientais relacionados à águas com uma diversidade de metais dissolvidos e baixos valores de pH. Para a prevenção e minimização da drenagem ácida de mina (DAM) é necessário o gerenciamento das superfícies de rejeitos e/ou estéreis que contém minerais sulfetados evitando as condições oxidantes em presença de água (BORMA; SOARES, 2002).

Para fins de um melhor conhecimento da labilidade/biodisponibilidade que ocorre em ambientes aquáticos, a técnica de Difusão em Filmes Finos por Gradiente de Concentração (DGT) está sendo utilizada devido à sua grande vantagem de realizar amostragens *in situ*, evitando que a natureza e a distribuição das espécies de metal presentes na água não sofram alterações, como ocorre em técnicas comuns de amostragem e armazenamento (ZHANG; DAVISON, 1995). Porém, esta técnica apresenta algumas limitações, dentre elas podemos citar, a limitação para a técnica DGT devido ao desempenho reduzido do agente ligante tradicional (resina quelante Chelex-100) em valores altos e baixos de pH, inviabilizando a aplicação desta técnica na drenagem ácida de mina que apresenta pH em torno de 3,0 (SØNDERGAARD, 2007).

O desenvolvimento de novos agentes ligantes para a técnica DGT tem o propósito de contornar as limitações que a técnica prevê, aliando à crescente conscientização dos problemas ambientais e a busca por tecnologias sustentáveis, este estudo prioriza o emprego de biomassas na técnica DGT. Com base neste enfoque biossustentável, foi avaliado o uso dos géis de biomassas, como a casca de banana nanica (*Musa cavendishi*), borra de café (*Coffea arabica L.)* e semente de moringa (*Moringa oleífera Lam.*) como agentes ligantes para a técnica DGT. A partir dos resultados satisfatórios deste trabalho, foi possível a determinação dos níveis de concentração de metais traço como, cádmio, chumbo, cobre, níquel e zinco, em águas de drenagem ácida de mina e para ampliar o leque de empregabilidade destas biomassas, a técnica também foi empregada em águas de rio.

As áreas de estudos consideradas para este trabalho foram:

- a Unidade de Tratamento de Minérios (UTM) Osamu Utsumi, pertencente às Indústrias Nucleares do Brasil (INB) localizada em Caldas – MG, em fase de descomissionamento desde 1995. Esta área foi escolhida por apresentar inúmeros problemas de alto impacto ambiental, sendo um deles, a geração de águas provenientes da drenagem ácida de mina dos minérios não aproveitados.
- Rio Corumbataí, por este rio ser responsável por todo o abastecimento público da cidade de Piracicaba e portanto, motivo de preocupação devido às cargas poluidoras que recebe em seu trajeto, principalmente esgotos domésticos e industriais oriundos das cidades de Analândia, Corumbataí e Rio Claro.
- Rio Piracicaba, em um ponto de amostragem oficial da CETESB, código PCAB 02192, localizado na cidade de Piracicaba que se encontra num trecho considerado crítico no enfoque de qualidade das águas do rio Piracicaba, segundo relatórios de águas superficiais da CETESB de 2011, 2014 e 2014.

A presente Tese de Doutorado foi organizada na forma de três artigos (Capítulos 2, 3 e 4) redigidos em inglês, a serem submetidos em revistas internacionais, tais como: Analytica Chimica Acta, Chemosphere e Talanta, portanto, o estilo das referências é diferente das normas ABNT ao qual esta tese está sendo apresentada. Anteriormente aos aritogs, há uma Introdução Geral, Revisão da literatura e Áreas de estudo e posteriormente finalizando com as Considerações Gerais e Referências.

## 2. HIPÓTESE E OBJETIVOS

O presente estudo teve como hipótese central: os agentes ligantes utilizados na técnica de Difusão em Filmes Finos por Gradiente de Concentração (DGT) preparados a partir de biomassas (borra de café, casca de banana e sementes de moringa) podem funcionar para a determinação de íons metálicos em baixos valores de pH.

Portanto, devido à investigação dessa hipótese, o objetivo principal deste trabalho foi a implementação de uma nova metodologia para a técnica de Difusão em Filmes Finos por Gradiente de Concentração (DGT) com a utilização de novos agentes ligantes a partir da casca de banana, borra de café e semente de moringa para determinação de íons metálicos presentes em águas oriundas de drenagem ácida na Unidade de Tratamento de Minérios (UTM) Osamu Utsumi operado pelas Indústrias Nucleares do Brasil (INB) localizada em Caldas – MG. Adicionalmente, com o intuito de ampliar a empregabilidade destes novos agentes ligantes, estes foram testados em água de rio (Rio Corumbataí e Rio Piracicaba). Neste contexto, os objetivos específicos estão relacionados abaixo:

- Analisar o desempenho dos géis de biomassa propostos através da técnica DGT.
- Comparação dos novos agentes ligantes frente ao agente ligante tradicional Chelex-100.
- Comparação da técnica DGT com programa de especiação Visual MINTEQ.

# 3. REVISÃO DA LITERATURA

## *3.1. Problemática dos metais no ambiente aquático*

A utilização dos metais é bem conhecida pela humanidade desde a pré-história e, provavelmente pode ser considerado como os elementos mais conhecidos pelo homem desde então. Os metais são quimicamente definidos como elementos que conduzem eletricidade e calor, têm um brilho metálico, são maleáveis e dúcteis e formam cátions ao perderem elétrons. A mais antiga definição acerca do termo “metal pesado” é a de Bjerrum (1936) que se baseia na densidade da forma elementar do metal e classifica esses metais como sendo aqueles com densidades elementares acima de 4 g/cm3. Atualmente de acordo com o artigo pulicado pela IUPAC, o critério de classificação para os metais pesados considera também suas propriedades toxicológicas (DUFFUS, 2002) e o termo elemento-traço ou metais-traço tem sido mais bem colocado, em diversas publicações que tratam de assuntos relacionados ao termo metais pesados.

Os metais existem, de forma natural, em baixas concentrações na crosta terrestre, sendo mencionados também como metais-traço ou elementos-traço (BAIRD, 1998). Contudo, a intensa atividade antropogênica destes elementos aumentou expressivamente suas concentrações principalmente, no meio ambiente aquático (CALLENDER, 2004). Portanto, a poluição por metais tornou-se um dos desafios a ser enfrentado pela humanidade nos dias atuais (IZQUIERDO et al., 2015).

A disponibilidade dos metais no meio aquático ocorre naturalmente através do intemperismo de rochas e solos, diretamente expostos à água, e também por fontes antrópicas como efluentes domésticos e industriais, mineração, pesticidas na agricultura, precipitação em áreas com poluição atmosférica, entre outros (COSTA et al., 2008). Os metais-traço apresentam-se sob diferentes formas em águas naturais. Eles podem estar complexados com ligantes inorgânicos simples, como água, haletos, carbonatos, sulfatos ou por ligantes orgânicos, tais como ácido húmico e fúlvico, açúcares, aminoácidos, carboidratos e polímeros. Posteriormente, são então incorporados ao sedimento pelo processo de sedimentação, resultando em níveis mais elevados de metais neste compartimento (BOTTÉ, FREIJE, MARCOVECCHIO, 2007). O conhecimento da forma dos metais-traço, em tais amostras, é importante

para entender sua toxicidade, biodisponibilidade e transporte, pois a toxicidade/biodisponibilidade dos metais-traço é influenciada por fatores físicos e químicos, entre eles:

- precipitação de hidróxidos, carbonatos e hidroxi-carbonatos insolúveis;
- formação de complexos inorgânicos solúveis com carbonatos, hidróxidos, fosfatos e sulfatos;
- competição entre cátions alcalinos-terrosos e elementos-traço pelos sítios de transporte nas membranas dos organismo (SALOMONS, FORSTNER, MADER,1995; TCHOUNWOU et al., 2012).

Os principais elementos químicos que são focos de maiores estudos e podem afetar o meio ambiente (podendo ser metais, semi-metais e mesmo não metais) são descritos abaixo na Tabela 1 com suas respectivas fontes e seus possíveis danos ao organismo humano.

Tabela 1. Principais metais-traço com suas respectivas fontes e possíveis danos ao organismo humano.

| Metais-traço | Danos ao organismo | Fonte |
|---|---|---|
| **Alumínio (Al)** | Anemia por deficiência de ferro; intoxicação crônica. | Produção de artefatos de alumínio, serralheria, soldagem de medicamentos (antiácidos) e tratamento convencional de água. |
| **Arsênio (As)** | Câncer (seios paranasais) | Metalurgia, manufatura de vidros e fundição. |
| **Cádmio (Cd)** | Câncer de pulmões e próstata; lesões nos rins. | Queima de carvão, indústrias de aço (soldas), pigmentos, fertizantes, pesticidas, tabaco, baterias e pilhas. |
| **Chumbo (Pb)** | Envenenamento, danos renais e cerebrais, anemia, doenças cardiovasculares, além de interferir no metabolismo ósseo e reprodução. | Fabricação e reciclagem de baterias de automóveis, indústria de plásticos e tintas, pinturas em cerâmicas, soldagem, fertilizantes e mineração. |
| **Cobalto (Co)** | Fibrose pulmonar que pode levar à morte | Preparo de ferramentas de corte e furadoras. |
| **Cobre (Cu)** | Intoxicação e lesões hepáticas | Indústrias de tubos, válvulas, ligas e algicidas. |
| **Cromo (Cr)** | Asma (bronquite) e câncer | Indústria de tintas, esmaltes, corantes, liga com aço e níquel e cromagem de metais. |
| **Manganês (Mn)** | Não é considerado muito tóxico | Indústria de vernizes, vidros, baterias e fertilizantes |
| **Mercúrio (Hg)** | Envenenamento, danos renais e ao sistema nervoso central e problemas em fetos. | Moldes industriais, indústrias de cloro-soda, mineração de ouro e lâmpadas fluorescentes. |
| **Níquel (Ni)** | Irritação gástrica, câncer de pulmão. | Indústria de baterias, moedas, pigmentos, refrigerantes, refinarias e mineração. |
| **Zinco (Zn)** | bioacumulativo | Indústria de borracha, protetores solares, vitaminas e esgotos |

Fonte: Adaptado de Ruppenthal, 2013.

### 3.2. Drenagem ácida de mina

O comprometimento dos sistemas aquáticos naturais, movido pelo desenvolvimento industrial, pode consumir recursos naturais e gerar resíduos que contém metais que podem afetar profundamente o meio ambiente. Estes metais introduzidos sem controle nos sistemas aquáticos (resíduos oriundos, principalmente de efluentes industriais, mineração e lavouras) podem ser tóxicos à vida aquática e tornar a água imprópria para consumo humano (MANAHAN, 2003).

DAM é um fenômeno que ocorre quando rochas usualmente (mas não exclusivamente) contendo minerais sulfetados são retiradas do interior terrestre e, quando dispostas na superfície terrestre, oxidam-se por reação com água e oxigênio atmosféricos. As soluções ácidas geradas por DAM, podem solubilizar alguns elementos químicos presentes nas rochas e solos (arsênio (As), cádmio (Cd), cromo (Cr), cobre (Cu), ferro (Fe), manganês (Mn), níquel (Ni), chumbo (Pb), zinco (Zn), dentre outros) contaminando águas superficiais e/ou águas subterrâneas. Esse fenômeno está associado a atividades de mineração, porém, a drenagem ácida pode ocorrer em qualquer operação que resulte em grande movimentação de terra e rochas que contenham minerais sulfetados (MELLO, et al., 2014).

Os minerais pirita e marcassita (polimorfo da pirita) ($FeS_2$), são os principais causadores da drenagem ácida e ocorrem em diferentes tipos de rochas. Entretanto, a oxidação de outros sulfetos de ferro, como pirrotita (FeS), arsenopirita (AsFeS) e calcopirita ($CuFeS_2$), também pode gerar soluções ácidas. Salientando que nem todos os minerais sulfetados sofrem oxidação ácida, caso dos sulfetos: galena (PbS), a esfalerita (ZnS) e a calcocita (CuS) (MELLO, et al., 2014).

A oxidação da pirita é geralmente descrita pelas seguintes reações químicas estequiométricas globais que foram originalmente caracterizadas por Garrels e Thompson (GARRELS; THOMPSON, 1960):

- oxidação de bissulfeto de ferro ($FeS_2$), gerando Fe dissolvido, sulfato ($SO_4^{2-}$) e hidrogênio ($H^+$);

$$\mathrm{FeS_{2(s)}} + \frac{7}{2}\mathrm{O_{2(g)}} + \mathrm{H_2O_{(aq)}} \rightarrow \mathrm{Fe^{2+}_{(aq)}} + 2\mathrm{SO^{2-}_{4(aq)}} + 2\mathrm{H^{+}_{(aq)}} \quad (1)$$

- oxidação do $Fe^{2+}$ a $Fe^{3+}$;

$$\mathrm{Fe^{2+}_{(aq)}} + \frac{1}{4}\mathrm{O_{2(g)}} + \mathrm{H^{+}_{(aq)}} \rightarrow \mathrm{Fe^{3+}_{(aq)}} + \frac{1}{2}\mathrm{H_2O_{(aq)}} \quad (2)$$

Na ausência de bactérias, $Fe^{3+}$ não é produzido a um ritmo significativo (pH <4) ou é tão insolúvel que sua forma dissolvida é relativamente inexpressiva como um oxidante da pirita (pH> 4). Porém, a etapa de oxidação do $Fe^{2+}$ pode ser acelerada pela ação de bactérias, presentes naturalmente em ambientes aquáticos e que se desenvolvem melhor em condições de pH entre 2,8 e 3,2. Duas espécies de bactérias envolvidas nesse processo são *Acidithiobacilus thiooxidans*, que oxida enxofre, e *Acidithiobacilus ferroxidans*, capaz de oxidar ferro, havendo a possibilidade da existência de outros tipos de bactérias.

- Produção de precipitado de hidróxido férrico ($Fe(OH)_3$) por hidrólise, com liberação de prótons (íons $H^+$), tornando o meio ácido.

$$Fe^{3+}_{(aq)} + 3H_2O_{(aq)} \rightarrow Fe(OH)_3 + 3H^+_{(aq)} \quad (3)$$

Em valores baixos de pH, a reação (3) de hidrólise não ocorre, gerando uma disponibilidade ainda maior de $Fe^{3+}$ no meio que oxida a pirita, favorecendo novamente o ciclo da geração de ácidos, como mostrado na reação (4) (AKCIL; KOLDAS, 2006):

$$FeS_{2\,(s)} + 14Fe^{3+}_{(aq)} + 8\,H_2O_{(aq)} \rightarrow 15Fe^{2+}_{(aq)} + 2SO^{2-}_{4(aq)} + 16H^+_{(aq)} \quad (4)$$

A outra explicação para a geração de DAM sugere a oxidação da pirita na ausência de $O_2$, segundo duas vias que podem produzir $S_o$ ou $SO_4^{2-}$, dependendo do potencial eletroquímico. A reação inicial representada na Reação 5 ocorre na ausência de $O_2$, sendo que as reações 5 a 8 representam as semi-reações de oxidação da pirita pelo processo eletroquímico (AL-HASHIMI; EVANS; COX, 1996).

$$FeS_{2\,(s)} + 8\,H_2O_{(aq)} \rightarrow Fe^{2+}_{(aq)} + 2SO^{2-}_{4(aq)} + 16H^+_{(aq)} + 14\,e- \quad (5)$$

$$FeS_{2\,(s)} \rightarrow Fe^{2+}_{(aq)} + 2S_{0(s)} + 2\,e- \quad (6)$$

$$4Fe^{2+}_{(aq)} + O_{2(g)} + 4H^+_{(aq)} \rightarrow 4Fe^{3+}_{(aq)} + 2H_2O \quad (7)$$

$$FeS_{2\,(s)} + 8\,H_2O_{(aq)} \rightarrow Fe^{3+}_{(aq)} + 2SO^{2-}_{4(aq)} + 16H^+_{(aq)} + 15\,e- \quad (8)$$

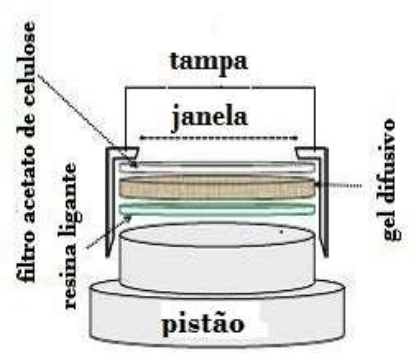
Apesar do $O_2$ não ser consumido diretamente na etapa da oxidação da pirita, ele é necessário para a regeneração do $Fe^{3+}$ para o ciclo de oxidação prosseguir. Dessa forma, a quantidade de $O_2$ na solução pode interferir na taxa de geração de DAM, mostrando que em águas pouco aeradas e oxigenadas e, consequentemente, pobres em $Fe^{3+}$, a oxidação da pirita pode ocorrer mais lentamente, acarretando numa menor acidez das soluções.

### *3.3. Técnica DGT*

Diversos estudos têm comprovado a necessidade do conhecimento sobre a especiação de metais traço em águas para a compreensão da toxicidade, bioacumulação e particionamento destes metais nas interfaces água/fase coloidal/fase particulada (FLORENCE; MORRISON; STAUBER, 1992). Técnicas que combinem alta sensibilidade com recursos de especiação são significativamente necessários para análise de metais traço em águas (GONZALVEZ *et al.*, 2010).

Um método analítico capaz de realizar a especiação de metais é a técnica de difusão em filmes finos por gradientes de concentração (DGT) (ZHANG; DAVISON, 1995) que permite avaliar quantitativamente a concentração lábil dos metais por meio da imersão de um dispositivo no sistema aquático. O dispositivo consiste em um filtro, uma camada difusiva e um agente ligante (Figura 1). As camadas são fixadas em um suporte plástico com uma janela que permite o contato da camada difusiva com a solução no qual foi exposto. Convencionalmente, o gel de poliacrilamida-agarose é utilizado como camada difusiva e como filtro, a membrana filtrante de ésteres mistos de celulose. O agente ligante convencional é a resina Chelex-100 suportada no mesmo gel (poliacrilamida-agarose).

Figura 1. Esquema do dispositivo DGT

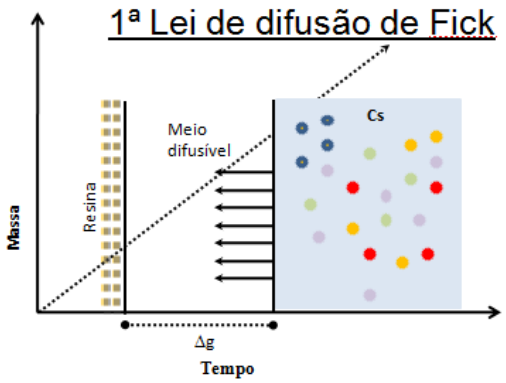



Fonte: Adaptado de Zhang e DAVISON (1995).

A técnica DGT utiliza um adsorvente, geralmente imobilizada em um hidrogel de poliacrilamida-agarose (gel ligante), para adsorver íons metálicos a partir de uma solução. O gel ligante é separado da solução por um gel difusivo com espessura (Δg) conhecida. O transporte de espécies a partir da solução para o gel ligante

ocorre por difusão molecular através do gel difusivo (1° Lei de Fick, Figura 2). O gel difusivo controla a taxa global de transporte de massa para o gel ligante, independentemente da hidrodinâmica da solução. A ligação do analito ao adsorvente garante a formação de um gradiente de concentração entre a solução e o gel ligante.

Figura 2. Gradiente de concentração de íons pela técnica DGT.




Fonte: Adaptado de Zhang e DAVISON (1995).

Uma grande vantagem da técnica DGT é a sua implantação *in situ* visto que na amostragem e armazenamento convencionais podem ocorrer alterações na natureza e distribuição das espécies metálicas. Além disso, o dispositivo DGT fornece uma medida das concentrações do metal integrada no tempo; tem a capacidade de determinações multielementares; capacidade de pré-concentração dos metais, melhorando os problemas de contaminação e baixa detecção dos metais traço. Desde o primeiro artigo da técnica em 1994, a técnica tem sido objeto de estudo para uma diversidade de metais e semimetais em diversos sistemas: aquáticos fluviais (DENNEY; SHERWOOD; LEYDEN, 1999; DIVIŠ, PAVEL *et al.*, 2007); marinhos (DE SOUZA, JOÃO M. *et al.*, 2014; STARK *et al.*, 2006); solos (SONG *et al.*, 2015; ZHANG, 2003); sedimentos (DIVIŠ, P. *et al.*, 2005); condições controladas em laboratório (GARMO, OYVIND AABERG *et al.*, 2003; ZHANG; DAVISON, 1995, 1999); biodisponibilidade (MENEGÁRIO; TONELLO; DURRANT, 2010; TONELLO et al., 2007) e especiação (WEIJIA et al., 2006; PESCIM et al., 2012; SONDERGAARD; ELBERLING; ASMUND, 2008; ZHANG, 2004)

A resina convencional Chelex-100 foi avaliada como agente ligante da técnica DGT para 55 elementos, sendo que destes elementos, apenas 24 foram determinados quantitativamente de forma eficiente (GARMO et al., 2003). O

desempenho desta resina é reduzido para valores altos e baixos de pH, provavelmente devido à competição entre metais e íons hidrogênio no agente ligante. Para a maioria dos metais, a performance desta resina funciona melhor em valores de pH acima de 4,5. Entretanto, alguns autores relatados a seguir, obtiveram resultados aceitáveis empregando a resina tradicional Chelex-100. Gimpel et al. (2001) conseguiram resultados com exatidão para as concentrações de Co, Mn e Zn entre os valores de pH 3,5 e 10; Cu entre pH 2,0 e 10 e Cd entre pH 5,0 e 10. Nos estudos de Sondergaard (2007) em área de drenagem ácida de uma mina de carvão em Svalbard (Noruega), os resultados para Al foram satisfatórios para valores de pH entre 2,5 e 4,5 porém para Mn, foi necessário aplicar um fator de correção para a dependência do pH abaixo de 4,0.

A fim de aumentar e complementar a utilização da técnica DGT, alguns materiais ligantes alternativos têm sido propostos. Alguns autores têm sido pioneiros na investigação do uso de biomassas microbianas como agente ligante para a técnica DGT. Menegário, Tonello e Durrant (2010) utilizaram a levedura *Saccharomyces cerevisiae* imobilizada em gel de agarose como agente ligante para a técnica DGT para determinação de Cd(II) em águas do mar e rio. Posteriormente, Pescim et al. (2012) utilizaram novamente a *Saccharomyces cerevisiae* imobilizada em gel de agarose como agente ligante para a técnica DGT para determinação de chumbo (Pb) em águas do mar e rio, obtendo resultados concordantes com a técnica convencional.

Atualmente, há um número crescente de estudos que utilizam a técnica DGT com seu agente ligante convencional Chelex-100, para a avaliação da fração lábil em áreas impactadas de mineração ativas e/ou inativas para os íons Cd, Co, Cu, Ni, Mn, Pb, U e Zn (INAP, 2002; BALISTRIERI et al., 2007; SONDERGAARD, 2007; YAPICI et al., 2008; CASIOT, 2009; CHAKRABORTY et al., 2009; CONESA, SCHULIN, NOWACK, 2010; de OLIVEIRA et al., 2013; OMANOVIĆ et al., 2015). Drozdzak et al. (2016) avaliaram um novo agente ligante preparado a partir de uma resina de troca aniônica impregnada em polyphenol (PIWBA) para determinação de U nas proximidades de locais de extração de urânio na França, obtendo resultados superiores ou similares comparados à resina tradicional Chelex-100. Pedrobom et al. (2017) avaliaram a especiação de U utilizando múltiplos dispositivos DGT (membranas P81 e DE81 e a resina tradicional Chelex-100) em águas tratadas de drenagem ácida da mina inativa de urânio Osamu Utsumi (Caldas/MG/Brasil), com a

resina Chelex-100 retendo todas as espécies de U, a membrana DE81 retendo as espécies negativas de U e a membrana P81 apresentando baixa eficiência na retenção de qualquer espécie de U.

A comparação dos resultados da técnica DGT com softwares de modelagem de especiação pode ser uma maneira de complementar os resultados apresentados pela técnica, para fins de validação de dados. O Windermere Humic Aqueous Model (WHAM VI), o Visual MINTEQ (vMINTEQ), o CHemical Equilibria in AQuatic Systems (CHEAQS) são as plataformas de modelagem de especiação química com maior número de estudos comparativos com a técnica DGT, apesar de haverem outros softwares de modelagem como o Joint Expert Speciation System (JESS), MINEQL+, ou PHREEQC.

As concentrações dissolvidas de metais inorgânicos previstos pelas modelagens CHEAQS e WHAM VI foram comparadas com as concentrações lábeis da técnica DGT com resultados concordantes para Al, Cu, Fe, Mn, Pb e Zn em estudos de Gimpel et al. (2003), Jansen, Mulder e Verstraten (2003) e Pescim et al. (2012). Os estudos de Meylan et al. (2004) compararam os resultados da DGT para Cu e Zn com medições voltamétricas e previsões realizadas com os programas de especiação WHAM, vMINTEQ e SHM. As previsões de Cu livre e inorgânico foram superestimadas visto que três modelos não estão considerando uma forte ligação de Cu. As concentrações de Zn calculadas por estes modelos estavam de acordo com os resultados da DGT e da voltametria. Unsworth et al. (2006) obtiveram resultados pela técnica DGT para Cd, Cu, Ni e Pb e estes resultados foram comparados utilizando modelos WHAM e vMINTEQ, obtendo concordância somente para Cd com o modelo WHAM. Estudos utilizando águas de drenagem ácida de mina apresentaram similaridades com as modelagens descritas acima para Al, Cd, Co, Cu, Mn, Ni, Pb, U e Zn (SONDERGAARD, 2007; SONDERGAARD; ELBERLING; ASMUND, 2008; YAPICI et al., 2008; BALISTRIERI; BLANK, 2008; de OLIVEIRA et al., 2013; PEDROBOM et al., 2017).

### *3.4. Espectroscopia de emissão de emissão ótica por plasma indutivamente acoplado (ICP-OES)*

Técnicas analíticas suficientemente sensíveis são requeridas para determinação de metais em baixas concentrações. A espectroscopia de emissão de

emissão ótica por plasma indutivamente acoplado (ICP-OES) pode ser utilizada para atender a essa necessidade, por ser uma técnica analítica com uma série de vantagens, tais como: análise multielementar simultânea, alta sensibilidade e precisão, rapidez e ampla faixa de linearidade em diversos tipos de amostras (KOLA; PERÄMÄKI, 2004; SAPKOTA *et al.*, 2005).

O uso do plasma (nuvem de gás parcialmente ionizado e com elevada temperatura) como fonte de atomização na espectroscopia de emissão foi desenvolvido principalmente nos últimos 25 anos. Na espectroscopia de emissão de plasma, o gás, normalmente o argônio, se ioniza em um campo elétrico forte por uma corrente direta ou por radiofreqüência. Ambos os tipos de descarga produzem um plasma, o plasma de corrente direta (Direct Current Plasma-DCP) ou o Plasma de Acoplamento Indutivo (Inductively Coupled Plasma-ICP).

O princípio fundamental da técnica consiste na propriedade dos átomos emitirem radiação eletromagnética quando submetidos ao plasma de argônio acoplado indutivamente (gerando uma temperatura entre 7000 – 10000K). A esta temperatura, todos os elementos se tornam termicamente excitados e emitem luz em comprimentos de onda característicos. Esta luz é recolhida pelo espectrômetro e passa através de uma rede de difração que serve para dividir a luz em um espectro de comprimentos de onda. Dentro do espectrômetro, esta luz difratada é então amplificada produzindo um sinal de intensidade que pode ser convertido para uma concentração elementar por comparação com padrões de calibração (GINÉ-ROSIAS, 1998).

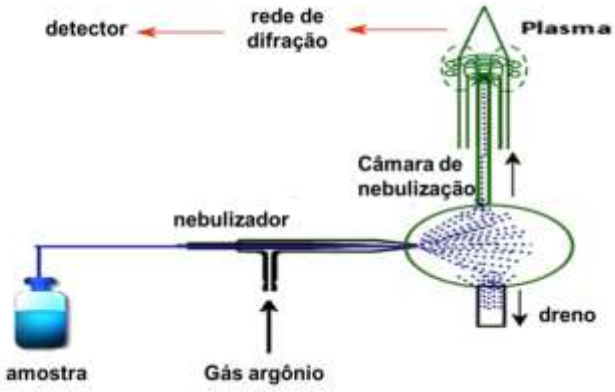
Abaixo segue uma ilustração do princípio de funcionamento do ICP-OES (Figura 3) e seus principais componentes:

• Sistema de Introdução de Amostra: geralmente formado por um nebulizador-câmara de nebulização, tocha de quartzo (geração do plasma) e fonte de radiofrequência.

• Sistema Óptico: serve para permitir a eficiente separação dos diferentes comprimentos de onda. A separação das linhas emitidas é feita utilizando um policromador que contém uma ou duas redes de difração.

• Sistema de Detecção: os detectores mais usados em ICP-OES são fotomultiplicadores e detectores de estado sólido (Charge Coupled Device – CCD) que possuem a função de converter os fótons incidentes em sinal elétrico(GINÉ-ROSIAS, 1998).

Figura 3. Diagrama de funcionamento do ICP OES.




Fonte: Adaptado de Rouessac (1994)

### 3.5. Biossorção

A biossorção é um termo que geralmente descreve a remoção de íons metálicos através da ligação passiva de biomateriais ou biomassas a partir de uma solução aquosa, devido aos grupos funcionais presentes na parede celular da biomassa que oferecem certas forças de atrações para os íons metálicos proporcionando uma alta eficiência de remoção (ASHKENAZY; GOTTLIEB; YANNAI, 1997; FAROOQ et al., 2010). Embora altamente promissor, o mecanismo da biossorção ainda não está bem entendido, pois não é baseada num único mecanismo, podendo ser troca iônica, quelação, adsorção, aprisionamento de íons em capilares e espaços da rede de polissacarídeos estruturais das biomassas (VOLESKY, 1990; GADD, 2009).

Um fator que tem incentivado a investigação de novas biomassas biossorventes é por seu baixo custo e por isso tem sido foco de diversos estudos nos últimos anos. Essas biomassas podem ser usadas tanto na forma in natura quanto modificada, ou seja, tratada com agentes químicos tais como ácidos carboxílicos, ácido fosfórico e bases, com o objetivo de melhorar a eficiência desses materiais na remoção dos metais (WAN NGAH e HANAFIAH, 2008).

Os diversos tipos de materiais biológicos que têm sido investigados como biossorventes incluem biomassa microbiana (bactérias, archaea, cianobactérias,

fungos filamentosos e leveduras), algas e microalgas, resíduos industriais e agrícolas (resíduos de frutas e vegetais), resíduos naturais (resíduos vegetais, serragem, cascas de árvores, sementes, ervas daninhas, musgo de turfa) e outros (PARK et al., 2010). Alguns biossorventes e seus analitos removidos que foram investigados são apresentados na Tabela 2.

Tabela 2 – Principais biossorventes pesquisados na literatura

| **Biossorvente** | **Analito** | **Referência** |
|---|---|---|
| Borra de café | As (V), Cu e P | (HAO; WANG; VALIYAVEETTIL, 2017) |
| | Ag | (JEON, 2017) |
| | Cu | (DÁVILA-GUZMÁN *et al.*, 2013) |
| | Cd | (DUTTA *et al.,* 2016) |
| Casca de amendoim | Cu | (JOHNSON *et al.*, 2002) |
| Casca da banana | Cu e Pb | (CASTRO *et al.*, 2011) |
| | Urânio (U) | (BONIOLO; YAMAURA; MONTEIRO, 2010) |
| | Cr | (MEMON *et al.*, 2009) |
| | Cu | (LIU, CONG *et al.*, 2012) |
| | Cd e Pb | (ANWAR *et al.*, 2010) |
| | Mn | (ALI, 2017) |
| | Cr (VI) | (ALI; SAEED; MABOOD, 2016) |
| | Cd, Ni e Pb | (INCE et al., 2016) |
| Casca de maçã | Cu e Ni | (MARAÑÓN; SASTRE, 1992) |
| Casca de melancia | Cu | (LIU, CONG *et al.*, 2012) |
| Bagaço da cana-de-açúcar | Cu, Cd e Pb | (GURGEL; GIL, 2009) |
| Bagaço da laranja | Cu, Ni, Pb e Zn | (MONTANHER, 2009) |
| Levedura *Saccharomyces cereviseae* | Sb | (MENEGÁRIO, AMAURI A. *et al.*, 2006) |
| | Cd, Cr(III), Cr(VI), Cu, Pb, Zn | (MAPOLELO; TORTO; PRIOR, 2005) |
| | Sn | (CALDORIN; MENEGARIO, 2007) |
| | Cd | (MENEGÁRIO; TONELLO; DURRANT, 2010) |
| | As | (SMICHOWSKI *et al.*, 2000) |
| | Pb | (PESCIM et al., 2012) |
| | MetilHg | (TAFURT-CARDONA et al., 2015) |
| Sementes de moringa | Ag | (ARAÚJO *et al.*, 2010) |
| | Cd | (SHARMA, PARUL *et al.*, 2006) |
| | Cd e Pb | (SHARMA, PARUL, 2008) |
| | Cu | (RODRIGUEZ et al., 2016) |
| | Cd, Cu, Fe, Mg, Mn, Pb e Zn | (MAINA; OBUSENG; NAREETSILE, 2016) |

Fonte: Dados da pesquisa.

O reaproveitamento da borra de café (além dos trabalhos citados na tabela acima) tem sido conduzido em diferentes partes do mundo, como por exemplo, no Japão que tem sido pesquisada a remoção de chumbo das águas de abastecimento,

confirmando a possibilidade do uso deste material em estações de tratamento de água (TOKIMOTO et al., 2005) A utilização da borra de café foi avaliada por Kyzas (2012) para a remoção de Cu (II) e Cr (VI) de soluções aquosas, conseguindo uma capacidade máxima de adsorção de 70 mg $g^{-1}$ para Cu(II) e 45 mg $g^{-1}$ para Cr(VI). Em um estudo recente de 2016 em Taiwan, a borra de café foi considerada um adsorvente efetivo na remoção de Ni em águas residuárias, obtendo uma capacidade máxima de adsorção q(m) de 4,29 mg $g^{-1}$ (YEN; HUANG, 2016).

A banana é uma das frutas mais consumidas no mundo e o descarte gigantesco dessas cascas pode causar problemas ambientais devido ao grande volume de geração e os trabalhos na literatura mencionando o reaproveitamento destes resíduos estão sendo mais pesquisados atualmente (BUGIERECK et al., 2014; ZHANG et al., 2005). Castro et al. (2011) empregou a casca da banana associado a extração em fase sólida para a remoção de cobre e chumbo provenientes de amostras de rios. Achak et al. (2009) investigaram o uso das cascas de banana para remover compostos fenólicos provenientes de efluentes de moinhos de azeite, indicando as cascas de banana como um biosorvente de baixo custo efetivo no tratamento neste tipo de água residuária.

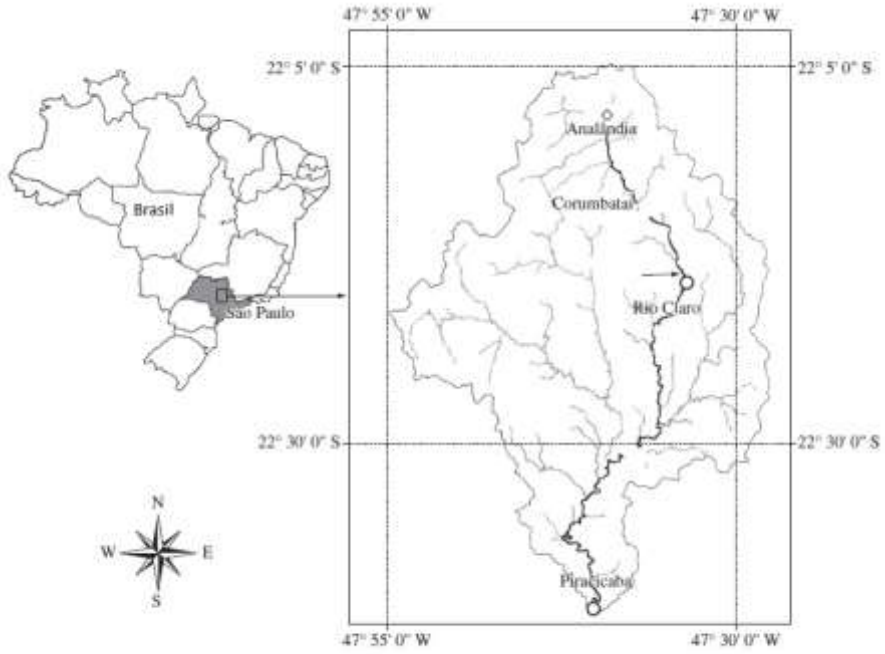
*Moringa oleifera* é uma planta nativa da Índia, que cresce principalmente em áreas semi-áridas tropicais e subtropicais. Esta planta prefere solos secos e arenosos e tolera solos pobres. No momento, o seu cultivo está espalhado por toda a África, América Central, América do Sul, Sri Lanka, Índia, Malásia e Filipinas (“Moringa”, [S.d.]). Várias partes da planta são identificadas para inúmeras propriedades farmacológicas (DANGI; JOLLY; NARAYANA, 2002; MARUGANDAN et al., 2001). Também foram empregadas sementes de M. oleifera para tratamento de água devido às suas propriedades de floculação (GASSENSCHMIDT et al., 1995; MEGAT, 2001). A semente de Moringa oleífera é uma das biomassas mais atraentes para a remoção de As, Cd, Cr, Ni e Pb como evidenciado em trabalhos anteriores (KUMARI et al., 2005; SHARMA, P et al., 2006, 2007; SHARMA, PARUL, 2008).

# 4. ÁREA DE ESTUDO

## 4.1. Rio Corumbataí

A bacia do rio Corumbataí (integrante da bacia do Rio Piracicaba) abrange uma área de 1710 km$^2$, localizada no estado de São Paulo, Brasil (Figura 4) tem em sua formação quatro rios principais: o Corumbataí (rio principal da bacia), o rio Passa Cinco, o rio Cabeça e o Ribeirão Claro. O Rio Corumbataí, com extensão aproximada de 120 km, nasce no município de Analândia, passando pelas cidades de Corumbataí e Rio Claro, desembocando no rio Piracicaba (CEAPLA, 2011).

Figura 4. Localização da Bacia Hidrográfica do Rio Corumbataí.




Fonte: Modificado de Jardim, Armas e Monteiro (2008).

A bacia hidrográfica do rio Corumbataí localiza-se, geologicamente, na porção nordeste da Bacia Sedimentar do Paraná, na Depressão Periférica Paulista, centro-leste do Estado de São Paulo, representada, no caso, por rochas sedimentares e vulcânicas das eras Paleozóica (Grupo Itararé; formações Tatuí, Irati e Corumbataí),

Mesozóica (formações Pirambóia, Botucatu e Serra Geral) e Cenozóica (Formação Rio Claro)(ZAINE, 1994).

O rio Corumbataí é responsável por todo o abastecimento público da cidade de Piracicaba e é motivo de preocupação devido às cargas poluidoras que recebe em seu trajeto, principalmente esgotos domésticos e industriais oriundos das cidades de Analândia, Corumbataí, Rio Claro e Piracicaba. Entre seus principais afluentes, destacam-se o rio Passa Cinco, drenando uma extensa área de cultivo de cana-de-açúcar, e o Ribeirão Claro, o qual drena a área urbana do município de Santa Gertrudes e parte do município de Rio Claro, bem como uma área de cultivo de cana-de-açúcar. Com respeito a presente qualidade da água, a bacia do rio Corumbataí apresenta em alguns trechos águas enquadradas na classe 4, segundo a Resolução CONAMA/357, entretanto a bacia apresenta um elevado grau de depuração, tornando-se próprio para o abastecimento público da cidade de Piracicaba. (LOLLO; CARMO, 2012). O ponto de amostragem situa-se em um trecho considerado crítico por receber o esgoto doméstico e industrial da cidade de Rio Claro (Estrada Municipal Velha Brotas, na ponte sobre o rio Corumbataí, no município de Rio Claro, Figura 5).

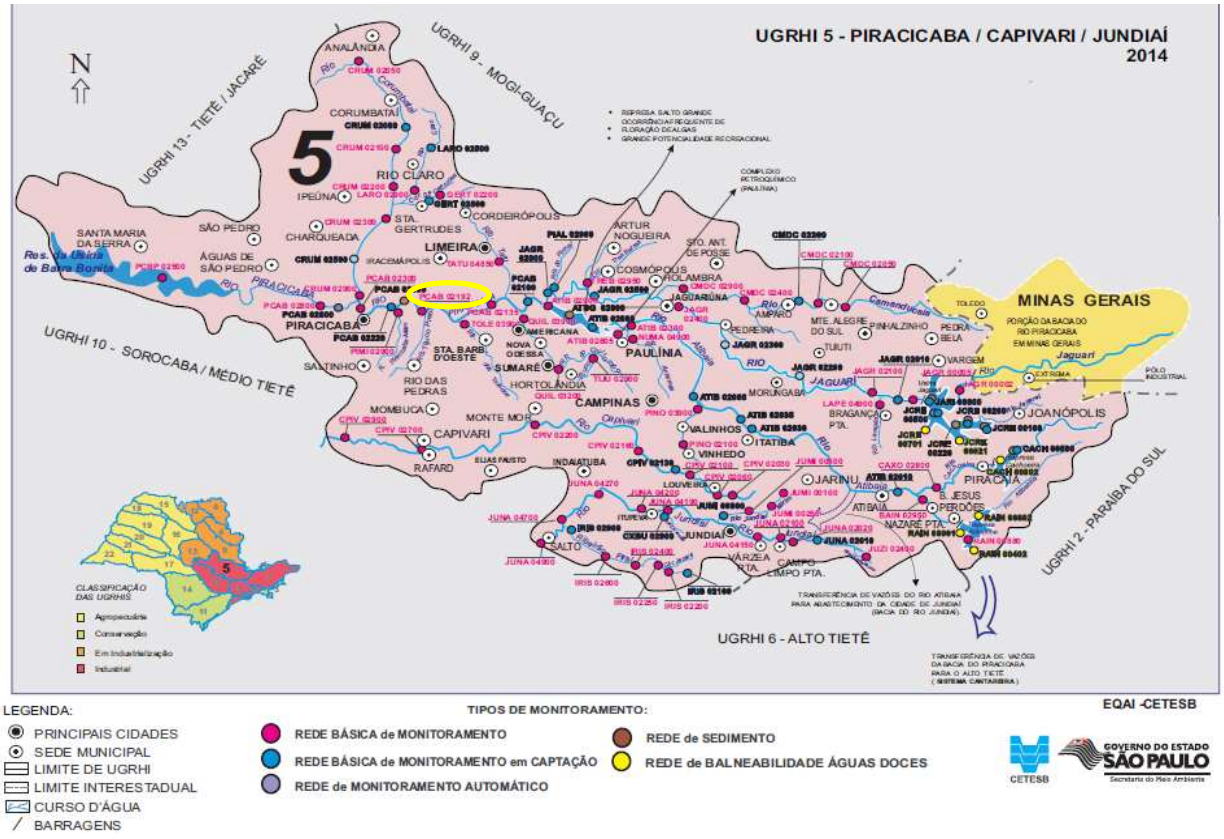
Figura 5. Ponto de amostragem no rio Corumbataí


Fonte: Google Earth

### 4.2. Rio Piracicaba

O rio Piracicaba, formado pela junção dos rios Jaguarí e Atibaia à altura da cidade de Americana e sua foz é junto ao Rio Tietê, no município de Barra Bonita. Sua bacia (formada pelas sub-bacias do Atibaia, Jaguari e Piracicaba), ocupa uma área aproximada de 12.400 km$^2$, sendo 91 % situados no estado de São Paulo e 9 % no estado de Minas Gerais (CESAR NETO, 1998) apresentado na Figura 6 (inserido na unidade hidrográfica de gerenciamento de recursos hídricos do Estado de São Paulo – UGRHI-5).

Figura 6. Localização do ponto de amostragem da UGRHI 05




Fonte: CETESB, 2014

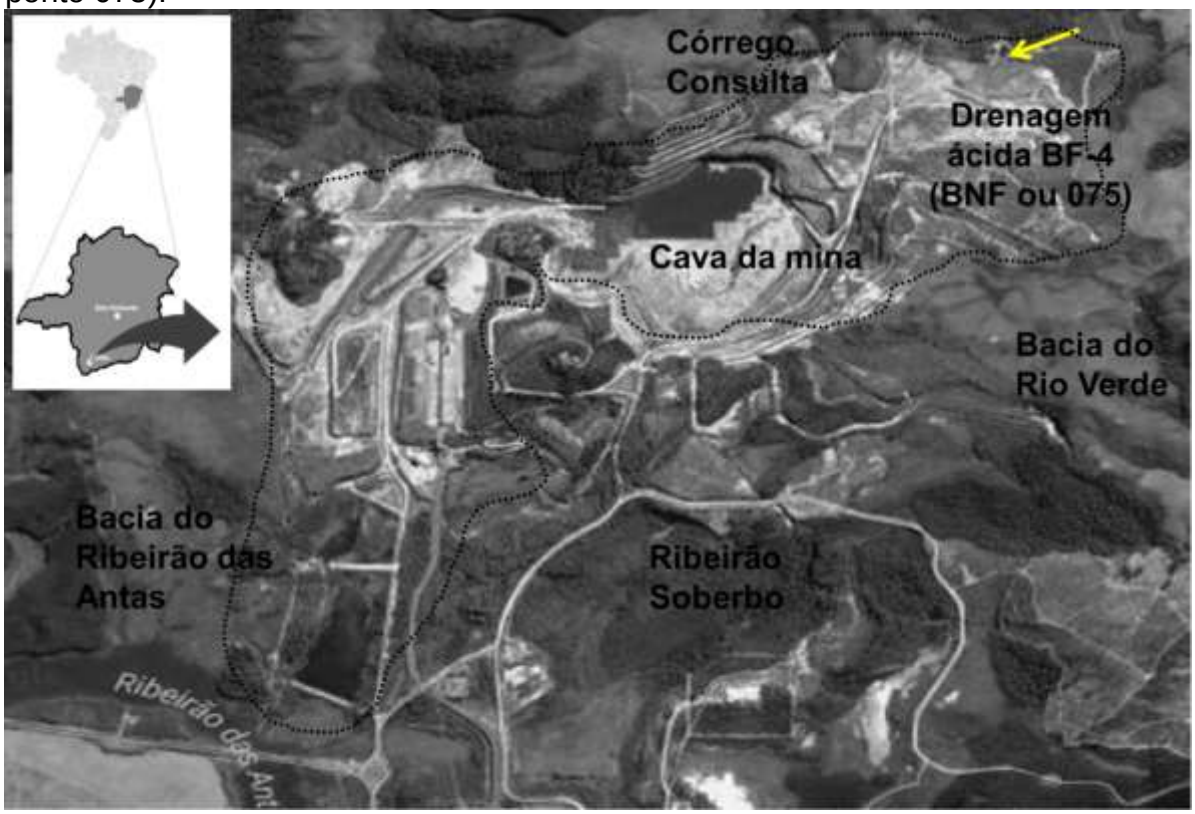
O rio Piracicaba atravessa uma vasta área de plantações de cana-de-açúcar, além de receber águas de um grande numero de córregos e riachos que também atravessam ou nascem em meio destas plantações ou cidades. A amostragem foi

feita em um ponto de amostragem oficial da CETESB, código PCAB 02192, localizado na cidade de Piracicaba, sob a ponte da Avenida Anel viário a 50 metros do km 135,3 leste da rodovia SP 147, perto da Usina Monte Alegre, coordenadas 22°41’20” latitude sul 47°34’58” longitude oeste. Este ponto encontra-se no trecho considerado mais crítico da qualidade das águas do rio Piracicaba, segundo relatórios de águas superficiais da CETESB de 2011 e 2012 (CETESB, 2011; CETESB, 2012).

### *4.3. Complexo Minero-Industrial de Poços de Caldas*

O Complexo Minero-Industrial de Poços de Caldas (CIPC) descoberto em 1970, localiza-se no Planalto de Poços de Caldas, na região sudoeste do estado de Minas Gerais. O planalto corresponde a uma caldeira vulcânica alcalina e abrange municípios de Poços de Caldas, Caldas, Andradas e Águas de Prata. Possui uma área aproximadamente circular, em torno de 800km$^2$, com aproximadamente 35 km de diâmetro (CIPRIANI, 2002). Situado na borda ocidental da Serra da Mantiqueira, em contato com os extremos orientais da bacia sedimentar do Paraná, formando um conjunto morfoestrutural perfeitamente caracterizado entre os estados de São Paulo e Minas Gerais. Geomorfologicamente, a região é caracterizada por um modelado estrutural dômico, com diques anelares individualizados pelas cristas e escarpas abruptas circulares que o delimitam. A área de drenagem abrange as Bacias do Ribeirão das Antas e do Rio Verde com litologia variada, dominando rochas intrusivas (tinguaítos, foiaitos, fonolito, rochas de transição entre estas três e outros tipos de rocha - tufas, lavas ankaratríticas, fenitos chibinitas, laujaritas, biotita-gnáisses, arenitos, caldasitos e bauxita)(LAGE-FILHO, 1996).

Figura 7. Localização da mina Osamu Utsumi (Caldas/MG/Brasil) e o ponto de amostragem na drenagem ácida do bota-fora 4 (Bacia Nestor Figueiredo – BNF ou ponto 075).




Fonte: modificado de Google Earth.

A mina do CIPC foi denominada Mina Osamu Utsumi, em homenagem póstuma a um dos geólogos pioneiros na prospecção de urânio de Poços de Caldas, apresentada na Figura 7 acima. O tipo de lavra adotado foi a céu aberto. A cava da mina, de forma aproximadamente circular, possui um diâmetro de 1200m. Os trabalhos de decapagem tiveram início em 1977 com teor de corte da mina, definido em 170 ppm de concentrado de urânio ($U_3O_8$) solúvel recuperado na usina (foto da cava da mina, ilustrada na Figura 8). Desde 1995, após a paralisação total das atividades de lavra, a área está em fase de descomissionamento (processo de desativação de uma instalação nuclear ao final de sua vida útil, observando-se todos os cuidados para proteger a saúde e a segurança dos trabalhadores, das pessoas em geral e também do meio ambiente) (NÓBREGA, 2007; FRANKLIN, 2007)

Figura 8. Cava da mina de urânio localizada em Caldas, MG

Fonte: Dados da pesquisa.

O material com teor < 170 ppm de $U_3O_8$ era considerado estéril. Parte dos estéreis gerados (cerca de 30%) foi utilizada para a construção de plataformas de estocagem e outras obras civis de utilidade para o CIPC. O restante dos estéreis foi disposto sobre a forma de pilhas de estéril (botas-foras) próximas a cava da mina, identificados como: BF-1A e BF-1B, BF-3 e BF-3A, BF-4A, BF-4B, BF-4C, BF-4D e BF-4E, BF-7, BF-8NA, BF-8NB e BF-8S. (CIPRIANI, 2002; FIGUEIREDO et al., 1995; FRAENKEL et al., 1985). As seis principais pilhas de estéril juntamente com suas origens e volumes respectivos estão listados na Tabela 3.

Tabela 3. Quantidade de estéril e origem das pilhas de estéril

| Bota-fora | Volume (1000 $m^3$) | Origem |
|---|---|---|
| BF-1 | 4400 | Decapagem |
| BF-3 | 9800 | Decapagem |
| BF-4 | 12400 | Decapagem e triagem do corpo B |
| BF-7 | 2400 | Decapagem |
| BF-8 | 15000 | Decapagem e triagem dos corpos A e E |
| Cava da mina | 560 | Triagem do corpo E |

Fonte: Adaptado de WIIKMAN, 1998.

Quando se começou a minerar na região o controle ambiental era ainda mais deficitário, e a drenagem ácida do BF-4 era lançada no córrego da Consulta e do BF-8 era lançada no córrego do Cercado. Após exigências da CNEN em 1983, os leitos dos córregos da Consulta e do Cercado, que estavam sob os bota-foras, foram desviados, respectivamente em 1982 e 1985. Em 1989, foi construída a bacia para captação de águas de drenagem ácida do BF-4 - Bacia Nestor Figueiredo (BNF) mostrada na Figura 9.

Figura 9. Bacia para captação de águas de drenagem ácida do BF-4, BNF (ponto 075)

Fonte: Nóbrega, Lima, Leite (2008) e dados da pesquisa.

Estas águas são bombeadas para uma estação de tratamento. Nesta estação apresentada na Figura 10, as águas ácidas são tratadas com cal (CaO) ou hidróxido de cálcio ($Ca(OH)_2$) para elevação do pH (faixa entre 8 e 11). Nesta faixa de pH, grande parte dos metais e radionuclídeos se precipitam. Os resíduos sólidos resultantes do tratamento dessas drenagens, compostas de diuranato de cálcio (DUCA), sulfato de cálcio, hidróxidos de alumínio e ferro eram enviados para a Bacia de Rejeitos (BR). Em função do esgotamento desta bacia, estes resíduos passaram a ser depositados dentro da cava da mina (Figura 11). O líquido sobrenadante, que possui pH em torno de 10, são lançados a montante das bacias de decantação D3 e D4 (Figura 12) construídas em série para sedimentação e, em seguida liberado para o meio ambiente rumo ao Ribeirão das Antas (CIPRIANI, 2002).

Figura 10. Vista da Estação de Neutralização de Águas Ácidas.

Fonte: Dados da pesquisa

Figura 11. Detalhe do resíduo sólido escuro, oriundo do tratamento de neutralização das águas ácidas.

Fonte: Dados da pesquisa.

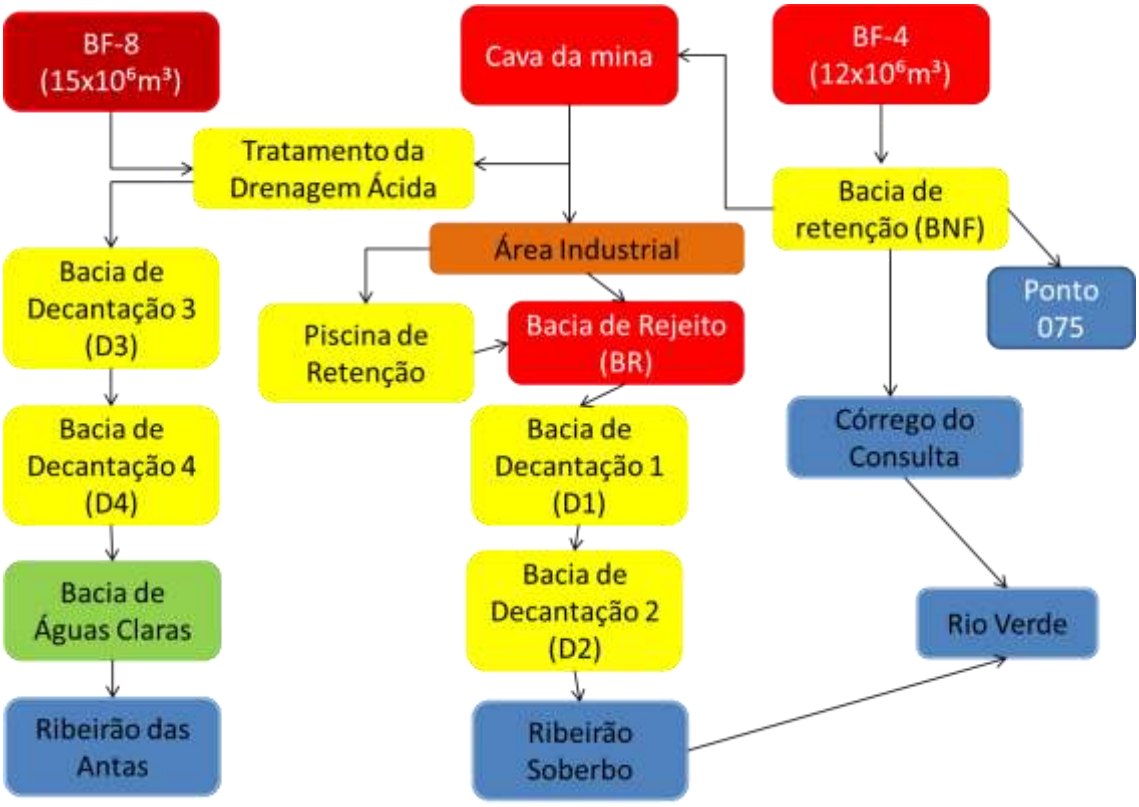
Figura 12. Foto das Bacias de Decantação após o tratamento para neutralização das águas ácidas.

Fonte: Dados da pesquisa.

O esquema de operação do CIPC, apontando as potenciais fontes poluidoras para o meio ambiente e os sistemas de tratamento, são apresentados na Figura 13. Os quadrados em vermelho representam as fontes poluentes (pilhas de estéreis, a

bacia de rejeito e a cava da mina). Em amarelo, são destacados os sistemas de controle, compostos de quatro bacias de decantação (D1, D2, D3 e D4); duas piscinas de retenção, uma (D5 ou BNF) para reter as águas de drenagem da pilha de estéril 4 (BF-4) e outra para reter as águas infiltrantes da Bacia de Rejeitos (BR); e uma unidade de tratamento químico. Em azul denotam-se os corpos d'água receptores dos efluentes (o Ribeirão das Antas), o Ribeirão Soberbo e o Córrego da Consulta, além da Bacia de Águas Claras (em verde), formadora do Ribeirão das Antas e construída com o propósito de retenção dos sólidos oriundos do processo de lavra e servir como suprimento de água de processo para as operações industriais (FERNANDES, 1997).

Figura 13. Diagrama de operação do CIPC




Fonte: Adaptado de Fernandes (1997)

# 5. RESULTADOS

## *CAPÍTULO 1*

Agarose-SCG as a binding agent in diffusive gradients in thin-films technique for measurement in acid mine drainage

**Abstract**

A novel binding agent prepared from spent coffee grounds (SGC) for the diffusive gradients in thin films (DGT) technique is proposed for determination of Cd, Cu, Ni, Pb and Zn in acid mine drainage water (AMD) since the Chelex resin has a limited capacity to low pH. DGT samplers were assembled with binding agent made from SGC immobilized in agarose gel (25-mm disk containing 10 % m/v SGC and 3.0 % m/v agarose) and a diffusive layer of polyacrylamide hydrogel. The effects of some DGT parameters (e.g., immersion time, ionic strength, and pH) were evaluated. Elution of analytes from the binding agent was effectively done with 2 mol $L^{-1}$ HCl. The experimental results are in excellent agreement with the predicted theoretical curve for mass uptake. Consistent results were found for solutions with ionic strengths between 0.005 mol $L^{-1}$ and 0.1 mol $L^{-1}$ and within a pH range of 3.5 - 8.0. Determination of metal ions in spiked river water sample (from the Corumbataí River) performed using the proposed device was in agreement with total and dissolved metal concentrations. The uptake of labile metals *in situ* performed at acid mine drainage water (AMD) of Osamu Utsumi mine (Caldas/MG/Brazil) showed agreement with the theoretical values of chemical speciation vMINTEQ.

### 1. Introduction

Acid Mine Drainage (AMD) is produced when sulfide minerals are exposed to oxygen and water. Although this process occurs naturally, mining can promote AMD generation simply through increasing the quantity of sulfides exposed. Naturally-occurring bacteria can accelerate AMD production by assisting in the breakdown of sulfide minerals [1], creating environmental problems associated with a diversity of dissolved metals (such as iron, aluminum, manganese, lead, copper and zinc) and low pH values. To prevention and minimization of AMD is necessary the

management from areas of waste and/or sterile containing sulfide minerals avoiding oxidizing conditions in the presence of water [2].

Information of trace element speciation in waters is necessary to an understanding of aquatic toxicity and bioaccumulation, as well as to the partitioning of elements between water and colloidal and particulate phase [3]. Alteration in the speciation of trace elements can dramatically change their toxicity. Techniques which combine high sensitivity with speciation capabilities are significantly needed for trace metal analysis in waters [4].

A capable analytical speciation method is diffusive gradients in thin films technique (DGT) [5]. This technique is based on 1° Fick's Law in which occurs molecular diffusion of hydrated metal ions, dissolved inorganic complexes and small labile organic complexes through a hydrogel layer of known thickness and pore size with a subsequent immobilization of the metal ions on a chelating resin. This resin only adsorbs free and kinetically labile metal ions, therefore this technique provides a superior measure of biologically available metals than analysis of dissolved or total metal concentrations.

A major advantage of the DGT technique is its *in situ* deployment whereas in conventional sampling and storage have been shown to induce changes in the nature and distribution of metal species in freshwaters. In addition, the DGT device provides a time-integrated measure of metal concentrations, furthermore is capable of multi-element measurement. It pre-concentrates metal solutes, improving the problems of contamination and low detection at trace levels [6].

DGT have been considered suitable for evaluating the speciation of Cd, Co, Cu, Ni, Mn, Pb, U and Zn, in mine effluents [7–9]. A comparison of the DGT results in acid drainage waters (AMD) with computer chemical speciation software CHEAQS, Visual MINTEQ and WHAM VI showed good similarities to Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn [8,10–12].

A limitation to the DGT technique is the reduced performance of the conventional Chelex-resin at both high and low pH ranges, probably due to competition between metals and hydrogen ions for the binding agent. In literature, DGT accurately measured Cd concentrations in the pH range 5–10; Co, Mn and Zn concentrations, between pH 3.5 and 10 and Cu was measured correctly between pH 2 and 10 [13]. This pH operational range is a challenge for the request of DGT on acid mine drainage where the pH is usually between 2 and 5.

The rising awareness of the accumulation of metals in the environment has favored to a demand for “green” technologies. The biomass has a great advantage for being amply available as waste or spinoff of other materials and therefore has been a focus of many studies in recent years. It was found that several functional groups present in the cell wall of biomass provide some attractions forces to the metal ions providing a high efficiency its removal [14,15]. Biomasses as peanut hulls [16], apple [17] and watermelon [18]; bagasse from sugarcane [19]; *Saccharomyces cerevisiae* yeast [20] among others, have been investigated and providing a significant removal efficiency of several metal ions. The use of low cost adsorbents such as coffee grounds was evaluated for the removal of copper ions in aqueous media [21]. Coals produced from coffee grounds are also being developed [22]. The present study uses residual biomass as a binding agent for the DGT technique for the first time. Previously, only some studies were used a yeast *Saccharomyces cerevisiae* immobilized in agarose gel as a binding agent for the DGT technique to determination of Ba, Cd and Pb in sea water and river water finding results in agreement with the conventional technique [23–25].

In the present study, the DGT device was applied on acid mine drainage (AMD) from a uranium mine waste rock pile in Caldas, Brazil. The purpose of the study was to (1) investigate the pH and ionic strength dependence of adsorption of analites on the proposed binding layer under controlled conditions in the laboratory; (2) perform *in situ* measurements with DGT device in the acid mine drainage to evaluate the labile concentrations of analites; and (3) compare observed labile concentrations with predictions using the speciation program Visual MINTEQ ver. 3.0.

## 2. Experimental

### 2.1. Equipment and accessories

Elemental analysis of aqueous eluates was carried out by inductively coupled plasma optical emission spectrometry (ICP-OES) using an iCAP™ 6000Series (ThermoScientific™, Germany) equipped with a V-groove nebuliser (Glass Expansion, AUS) and a cyclonic spray chamber (Glass Expansion, AUS). The ICP-OES operating conditions are listed in Table 1.

**Table 1.** ICP-OES operating conditions

| | |
|---|---|
| RF power (W) | 1150 W |
| Plasma gas flow rate | 10 L $min^{-1}$ |
| Auxiliary gas flow rate | 0.5 L $min^{-1}$ |
| Nebulizer gas flow rate | 0.75 L $min^{-1}$ |
| Sample introduction flow rate | 3.0 ml $min^{-1}$ |
| Emission line Cd | 228.8 nm |
| Emission line Cu | 324.7 nm |
| Emission line Ni | 221.6 nm |
| Emission line Pb | 220.3 nm |
| Emission line Zn | 213.8 nm |

For DGT experiments in laboratory and/or *in situ*, a pH meter (Model 3505, Jenway, UK), a conductivity meter (Model 470, Jenway, UK), a magnetic stirrer (Model 752, Fisatom, BR), an orbital shaker (PR70 Red Rotor, Hoefer, US) and refrigerator incubator BOD (TE-390, Tecnal, BR) were used. For centrifugation, Falcon tubes (15 ml) and a centrifuge (Model B4i, Jouan, FR, angle rotor AB-15.4; max. speed 4000 rpm; angle degree 37; radius 135 mm; max. RCF 2415 x g) were used.

The total organic carbon (TOC) was determined by a General Electric Carbon Analyser (GE Sievers InnovOx, USA). The major anions, sodium and potassium, in acid mine drainage and river water samples were determined by ion chromatography (Metrohm 930 Compact IC Flex, unit of cation model 2.761.0010 with a column for cation Metrosep C 4-150/4.0 packing silica gel with carboxylic groups, and unit of anion model 2.761.0020 with a column for anion Metrosep A Supp 5-150/4.0 packing polyvinyl alcohol with quaternary ammonium compounds groups).

## 2.2. Reagents, materials and solutions

All reagents were of analytical grade or better and all solutions were prepared with ultra-pure water (Milli-Q, with resistance >18.2 $M\Omega cm^{-1}$, Millipore, USA). DGT polyacrylamide hydrogel diffusion layers (0.80mm thickness), DGT Chelex-100 resin

layer and nylon DGT holders were purchased from DGT Research Ltd., UK. The binding gel was prepared with agarose NA (Amresco LLC, USA). Cellulose acetate membranes (Membrane Filter Supplies, 25 mm diameter, 0.45 mm pore size and 0.13 mm thickness, Sartorius Stedim Biotech, USA) were used as the covering diffusive gel (soaked in 1 mol $L^{-1}$ $HNO_3$ for 24 h, rinsed and stored in 0.025 mol $L^{-1}$ $NaNO_3$ at 4 °C before use). Analytical grade nitric and hydrochloric acid were purified by a subboiling distillation (DistillAcid, Berghof, DE) prior to use. All vessels (glass, DGT devices, PE, HDPE) used for experiments were prepared by acid washing (cleaned by keeping in 20% $HNO_3$ for at least 4 hours), rinsed at least 7 times with deionized water, 3 times with ultrapure water and dried before use.

The deployment solutions were prepared from a stock multi standard solution 1000 mg $L^{-1}$ (obtained from SpecSol, BR). The ionic strength was adjusted using a 1 mol $L^{-1}$ $NaNO_3$ stock solution (Synth, BR).

### 2.3. Biosorbent and characterization

The experiments were performed using SGC (Pilão™, BR) from espresso coffee machine (pressure 9 bar; temperature 92 °C, Cafemaq model La Spaziale S2, BR). Dried SGC (35 - 40°C, 12 hours, forced air laboratory oven (Fanem, Model 320 SE, BR) were first ground in a stainless-steel beater mill (IKA™ A11 Basic Mill, BR) three times for 30 seconds. After pulverization, the coffee dreg's particles were sieved and the fraction 250 µm was selected for work.

Specific surface area and pore size distribution of SGC surface were determined by nitrogen adsorption in Surface Area and Porosimetry Analyzer (ASAP 2020, Micromeritics, US) at temperature 77 K using 0.5 g of minced SGC. The Brunauer–Emmett–Teller (BET) method was used for determination of surface area.

Scanning Electron Microscope (SEM) with Energy Dispersive X-ray spectrometer (EDX) (Hitachi Tabletop Microscope TM3000, JP) (20 kV) operated at low vacuum chamber (1–15 Pa), was used for characterizing the morphology and structure of the agarose-SGC gel disks surface. The sample was not dried or covered with gold in order not to lose the gel structure.

### 2.4. Preparation of agarose–SGC binding gel

Agarose (0.9 g - 3 % m/v) was dissolved in 30 mL of water (80 °C) in a hot plate and dried SGC (3.0 g - 10 % m/v) were added to the solution with stirring. The mixture was then transferred to a preheated gel-casting (a homemade gel-casting support was built with two plates of glass, spaced by 0.6 mm plastic) (80 °C) and remained on the support until it reached room temperature. The gel (≈0.6 mm thick) was cut into 2.5 cm diameter disks, washed, and preserved in purified water (4 °C). After some tests involving different amounts of agarose and SGC had been executed, it was determined that the ideal amount of the compound needed to make the binding gel more consistent and easier to handle are 3 % m/v of agarose and 10 % m/v of SGC.

### 2.5. Assembly of DGT devices and elution procedure of agarose-SGC gel disks

DGT samplers were assembled by placing an agarose–SGC gel disk on the piston (25 mm diameter) with a diffusive layer of polyacrylamide and a cellulose acetate membrane to protect the device.

After deployment, the DGT devices were removed from the solution and disassembled. The agarose-SGC gel disks were rinsed with water and the ligand was placed in a plastic tube containing 4 mL of HCl 2 mol $L^{-1}$ for elution.

For elution procedure, agarose-SGC gel disks were removed from the solution, washed with water and placed in a plastic tube containing 4 mL of HCl 2 mol $L^{-1}$. The tubes were shaken for a period of 24 hours. After that, liquid and solid phases were separated by centrifugation for 4,000 rpm in 20 minutes. The liquid phase was then introduced into the ICP OES.

The elution factor ($f_e$) was calculated according to Eq. 1:

$$f_e = \frac{m_{elu}}{m_{ret}} \qquad (1)$$

where $m_{elu}$ is the mass eluted and the $m_{ret}$ is the mass retained in the ligand (agarose-SGC gel disks).

To evaluate the elution procedure, two agarose-SGC gel disks were placed into each solution (50 mL, duplicate) containing 500 µg $L^{-1}$ of analytes. The pH of the

solutions was adjusted to 5.5 with 1 mol $L^{-1}$ HCl, and the ionic strength was adjusted to 0.025 mol $L^{-1}$ with $NaNO_3$. The solutions were maintained under constant stirring for 6 h.

### 2.6. Diffusion coefficient of metal ions

The test standard solution were used with two litres of a solution containing 500 µg $L^{-1}$ of analytes at pH 5.5, ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$ and temperature of 23 ± 1 °C, DGT devices and clamps was stabilized for 24 h (without the binding, diffusive agent and cellulose acetate membrane), under stirring, to avoid decrease of concentration of solution during deployment. After that the DGT devices were washed with ultra-pure water, assembled with the agarose-SGC gel disk, polyacrylamide diffusive gel and cellulose acetate membrane and deployed in the solution at 4, 12, 24, 48 h, the analytes was eluted from the disks.

The basic equation of DGT theory is presented as Eq. 2 [26]:

$$C_s = M\Delta g(DtA)^{-1} \qquad (2)$$

where $A$ ($cm^2$) is the exposed area of ligand; $\Delta g$ the ligand + diffusive gel thickness (cm); $M$ is the mass of analyte in each disc; $C_s$ is the analyte concentration of deployment solution and $t$ is the deployment time. Therefore, the diffusion coefficient, D ($cm^2$ $s^{-1}$), was calculated according Eq. 3:

$$D = M\Delta g(C_s tA)^{-1} \qquad (3)$$

For a specific solution, if analytes and the other DGT parameters are maintained constant, Eq. 2 implies that there is a linear relationship between *M* and *t*. For correlation of diffusion coefficients performed in different temperatures was used Stokes-Einstein relation (Eq. 4)[27]:

$$(D_1\eta_1)/T_1 = (D_2\eta_2)/T_2 \qquad (4)$$

where $D_1$ and $D_2$ are diffusion coefficients, $\eta_1$ and $\eta_2$ are viscosities of water at absolute temperature $T_1$ and $T_2$, respectively.

### 2.7. Effect of ionic strength and pH on the retention of analytes

Four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C and pH 5.5, were prepared with ionic strength values of 0.005 mol $L^{-1}$, 0.01 mol $L^{-1}$, 0.05 mol $L^{-1}$ and 0.1 mol $L^{-1}$ $NaNO_3$ was used to evaluate of effect ionic strength on the retention of

analytes. To test of pH, four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C and 0.025 mol $L^{-1}$ $NaNO_3$ ionic strength were prepared with pH values of 3.5, 5.0, 6.5 and 8.0. The devices were then retrieved from the solutions after 6 h for both tests.

### 2.8. Deployment of the DGT devices in synthetic samples and spiked river water

After the DGT devices had been assembled, they were deployed in polyethylene containers containing well stirred test standard solutions (2 L), 500 µg $L^{-1}$of analytes at pH 5.5 and ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$. Deployments in the laboratory were done at constant temperature (23 °C) to avoid changing the diffusion coefficient of analytes. The solutions were stirred constantly to reduce the diffusive boundary layer (DBL). At the surface of the DGT device where the tangential velocity of the water decreases, creating a diffusive boundary layer (DBL) that increases the actual thickness of the diffusion layer. In well-stirred solutions, the diffusive boundary layer thickness (δ) has long been supposed to be insignificant compared to defined hydrogel thickness Δg [28].

DGT samplers were deployed in spiked river water (2L, collected from the Corumbataí River – Rio Claro/SP, Brazil) for six hours to evaluate the performance of the binding gels. Sample was spiked with 20 µg $L^{-1}$ of metal ions of this study. This river was chosen for this study because it is an important agricultural area of sugar cane and also receives effluents from industries such as textile, ethanol, and chemistry. Table 3 shows the physical and chemical parameters of the spiked Corumbataí river.

### 2.9. Field Evaluation

DGT measurements were performed *in situ* in acid mine drainage water coming from a uranium mine waste rock pile situated in Osamu Utsumi mine (Caldas/MG, Brazil) with a volume of 12.4 x $10^6$ $m^3$ covering an area 569 x $10^3$ $m^2$, where the acid drainage generated was collected (retention basin Nestor Figueiredo – BNF, point 075), with pH around 3 to 3.5 that is continuously pumped to the cava exhausted mine [29].

The deployment period varied from 4 to 20 h. Temperature, conductivity and pH were measured before and after deployment of DGT device. The DGT device was then removed from the water, thoroughly rinsed with MilliQ water, and stored in clean plastic Zip-lock bags until disassembled in the laboratory. Water samples were collected in acid-washed Falcon tubes for determination of total and dissolved metal concentrations, dissolved organic carbon and major anions. Samples for dissolved metal analysis were filtered by membranes of pore size 0.45 µm. All samples for metal analysis were preserved by acidification to pH 1-2 by addition of HNO3 and stored in the dark at room temperature. Physical and chemical parameters of this *in situ* location are given in Table 3.

### 2.10. Modeling Speciation

Speciation modeling was undertaken using Visual MINTEQ ver. 3.0 [30]. This is software based on NICA-Donnan model for the prediction of equilibrium-speciation of free metal ions, and those bound to humic and fulvic substances.

## 3. Results and Discussions

### Characterization of biosorbent

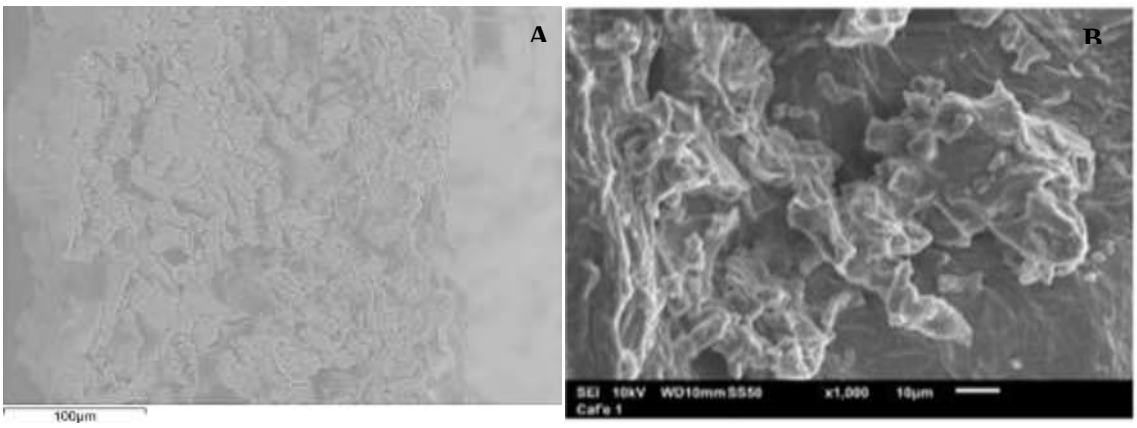



Fig. 1 SEM images (A and B) of spent coffee grounds at two different magnifications: (A) x50 and (B) x1000.

The morphology of SCG were observed with Scanning Electron Microscope (SEM). Fig. 1A has low magnification in order to present the surface of agarose-SGC gel and Fig. 1B is a high-magnification (1000x) image taken on the surface of spent coffee ground in order to show the particle size. These SEM images showed that

nature of the biomass was substantial with lots of pores, making it possible for biosorption of several metal ions.

The agarose-SCG gel surface before metal biosorption was characterized by X-ray Energy Dispersive Spectroscopy coupled with Scanning Electron Microscopy (SEM-EDX) from various spots on the surface of agarose-SCG gel. Fig. 2 illustrates a typical spectrum.

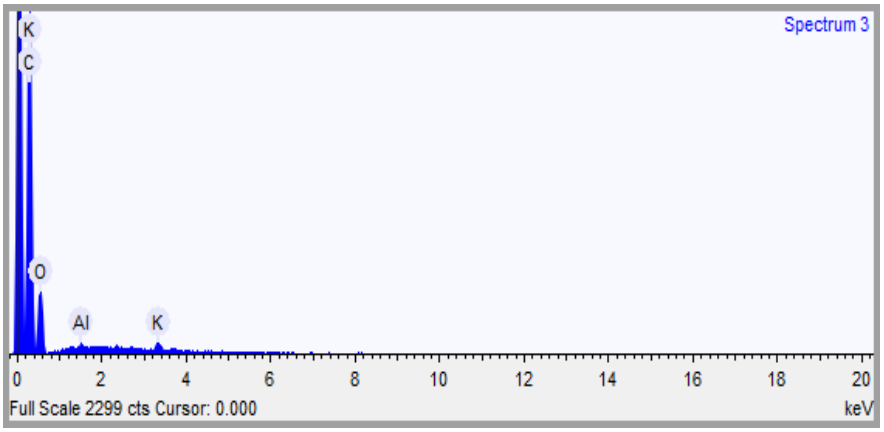



**Fig. 2** EDX spectrum of agarose-coffee gel disk surface without plating Au.

The results of the elemental analysis of agarose-SCG before biosorption were carried out using EDX. This EDX spectrum revealed a particularly intense peak of potassium (reported in previous studies of Zarrinbakhsh et al. [31]) and a presence of C (64%), O (35%), K (1%) and Al (0.3%). The contents of agarose-SCG are similar to work of Dávila-Guzmán et al. [30] where oxygenated groups are related to carboxylic, hydroxyl and phenolic groups, which are functional groups capable to adsorb metals from aqueous solutions. Table 2 presents the average results of the nitrogen adsorption experiments for surface area $S_P$ ($m^2\ g^{-1}$) and pore volume $V_P$ ($cm^3\ g^{-1}$).

**Table 2.** Physical parameters obtained by porosimetry analysis by $N_2$ adsorption for SCG.

| | $S_P$ ($m^2\ g^{-1}$) | $V_P$ ($cm^3\ g^{-1}$) |
|---|---|---|
| SCG | 0.30 ± 0.03 | 0.00088 ± 0.00002 |

For comparative purposes, in the work of Liu et al. [32] were presented results of the analysis of nitrogen adsorption porosimetry for dried banana peel with values

of $S_p$ and $V_p$ 0.41, 0.000108, respectively, indicating that values of specific area and volume of SGC studied and this alternative adsorbent were similar.

### Measurements by Different Techniques

The conductivity, ionic strength, solid total dissolved (TDS), dissolved organic carbon (DOC), pH, temperature and major ion composition of spiked river water and acid mine drainage water used as field sites are given in Table 3.

**Table 3.** Physical and chemical parameters measured *in situ* and in spiked river water.

| | 075 jul/14 | 075 feb/15 | 075 dec/15 | Corumbataí river |
|---|---|---|---|---|
| Conductivity (μS $cm^{-1}$) | 1308 | 1290 | 1375 | 94 |
| Ionic strength[a] (mol $L^{-1}$) | 0.0163 | 0.0160 | 0.0175 | 0.0009 |
| TDS[b] (mg $L^{-1}$) | 837 | 826 | 880 | 49 |
| DOC (mg $L^{-1}$) | < 1 | < 1 | < 1 | - |
| pH | 3.48 | 3.42 | 3.80 | 7.3 |
| T (°C) | 21.0 | 21.5 | 20.7 | 25.0 |
| $HCO_3^-$ (mg $L^{-1}$) | - | - | - | 27.2 |
| $F^-$ (mg $L^{-1}$) | 117 | 129 | 117 | 0.075 |
| $Cl^-$ (mg $L^{-1}$) | < 0.01 | 0.39 | < 0.01 | 4.76 |
| $NO_2^-$ (mg $L^{-1}$) | < 0.02 | < 0.02 | < 0.02 | 0.055 |
| $NO_3^-$ (mg $L^{-1}$) | 3.14 | 2.13 | 2.16 | 7.45 |
| $PO_4^-$ (mg $L^{-1}$) | < 0.040 | < 0.080 | < 0.080 | < 0.040 |
| $SO_4^{2-}$ (mg $L^{-1}$) | 917 | 1052 | 873 | 1.83 |
| $Li^+$ (mg $L^{-1}$) | < 0.010 | < 0.010 | < 0.010 | < 0.010 |
| $Na^+$ (mg $L^{-1}$) | 1.622 | 1.034 | 1.139 | 4.56 |
| $K^+$ (mg $L^{-1}$) | 4.72 | 8.14 | 6.78 | 1.59 |
| $Mg^{2+}$ (mg $L^{-1}$) | 6.37 | 5.45 | 5.67 | 3.30 |
| $Ca^{2+}$ (mg $L^{-1}$) | 72.63 | 64.93 | 73.93 | - |
| $NH_4^+$ (mg $L^{-1}$) | 3.68 | 4.53 | 3.90 | < 0.050 |

[a] ionic strength, values calculated according to Griffin and Jurinak [33].
[b] TDS total dissolved solids, relationship between TDS and electrical conductivity proposed by APHA, AWWA and WPCF [34].

### Elution efficiency of the agarose-coffee grounds gel disks

The process of elution is an important step in the DGT measurement. In order to confirm the accuracy of the measurement, high elution efficiency is essential for the removal of metal ions from the binding phase. Table 4 shows elution efficiencies of 60 to 91 % for all metals with standard deviations between 1 and 10 %. The values of Cu and Pb are higher than the established value of 80% using 1 mol $L^{-1}$ $HNO_3$ with a binding layer of Chelex [6] suggesting that elution is effective also with HCl. Mason

et al. [35] also conducted their studies with the use of HCl in elution of Cd, Cu, Mn, Mo, P and Zn with elution efficiency of 92 % for all elements excluding Mo (79%). The values of Cd, Ni and Zn close to 60 % could be concentrations of such metals coming from the biomass itself.

**Table 4.** Elution efficiency of metal ions. Metal ± sd.

| | $Cd^{2+}$ | $Cu^{2+}$ | $Ni^{2+}$ | $Pb^{2+}$ | $Zn^{2+}$ |
|---|---|---|---|---|---|
| Elution efficiency (%) | 63 ± 3 | 91 ± 10 | 60 ± 5 | 90 ± 9 | 60 ± 7 |

### Diffusion coefficient measurements using deployment curve

The linear relationship between accumulated mass (*M*) and deployment time (*t*) has been used as a requirement for the viability of novel DGT devices. In order to avoid high values of white that can come from own biomass, the mass of accumulated metal ion is normalized by the solution concentration of metal ion. In this study, mass of metal ion accumulated in the proposed agarose-SGC gel disk is proportional to the exposure time, as shown in Fig. 3 with good precision (correlation coefficients $R^2$ = 0.992 – 0.999).

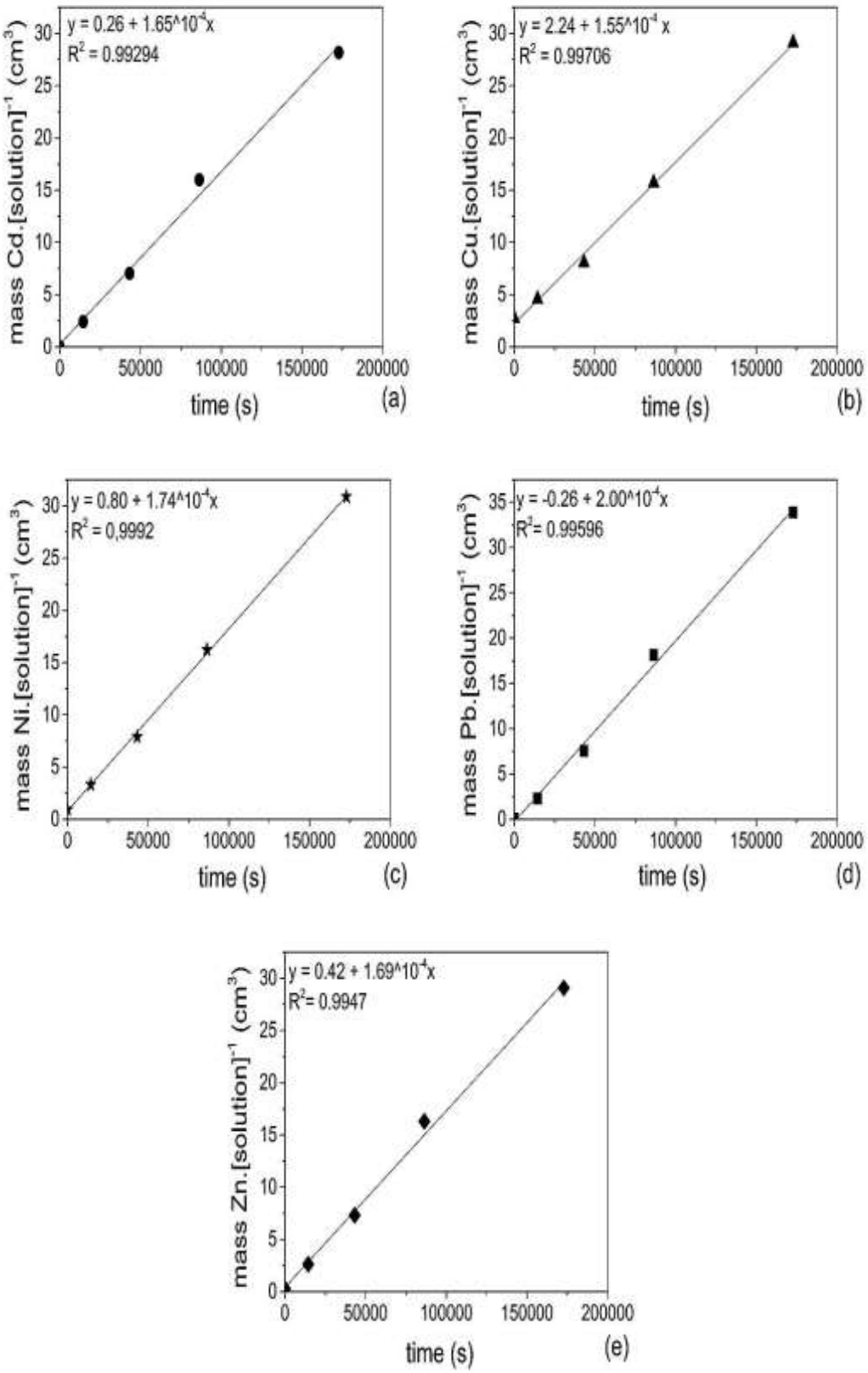



**Fig. 3** Mass of ion: (a) $Cd^{2+}$, (b) $Cu^{2+}$, (c) $Ni^{2+}$, (d) $Pb^{2+}$ and (e) $Zn^{2+}$ accumulated in the resin normalized by the ion concentration in the solution for various periods of time, following parameters: ionic strength = 0.025 mol $L^{-1}$ $NaNO_3$; pH = 5.5; temperature = 23°C; $\Delta g$ = 0.093 cm; A = 3.14 $cm^2$.

The diffusion coefficients (D) of metal ions in the diffusive layer can be calculated from the slope (s) of the relationship between the amount of metal ion accumulated by the DGT units (normalized for metal ion concentration in solution) and the deployment time Eq. 5:

$$s = DA(\Delta g)^{-1} \qquad (5)$$

Thus, the diffusion coefficient can be calculated with the Eq. 6:

$$D = s\Delta g(A)^{-1} \qquad (6)$$

Diffusion coefficients of metal ions measured in deployment curve are presented in Table 5. The diffusion coefficients found are in convenient agreement with previously reported values (81 – 96%) [27,36]. The diffusion coefficients founded were also agreed with those measured by Panther et al. using a mixed binding layer (Chelex-100 and the titanium dioxide based adsorbent Metsorb) (78 – 101 %). The diffusion coefficient reflect the mass transport (diffusion) and the binding capacity of analytes, therefore the proposed material made of agarose-SGC can be used as a binding agent for DGT technique.

**Table 5.** Diffusion coefficients ($10^{-6}$ $cm^2$ $s^{-1}$) of metal ions in polyacrylamide gel by deployment curve at pH 5.5 and temperature 23°C. Mean ± sd.

| | Found (x $10^{-6}$ $cm^2$ $s^{-1}$) | Reference (x $10^{-6}$ $cm^2$ $s^{-1}$) |
|---|---|---|
| Cd | 4.61 ± 0.02 | 5.21 (88 %)[a] |
| Cu | 4.79 ± 0.03 | 5.90 (81 %)[b] |
| Ni | 5.17 ± 0.01 | 5.47 (95 %) [b] |
| Pb | 6.26 ± 0.02 | 7.61 (82 %) [b] |
| Zn | 5.52 ± 0.02 | 5.76 (96 %)[b] |

[a] Data from Garmo et al. [36]
[b] Data from DGT Research [37]

### DGT method detection limit

The DGT method detection limits (MDL, µg $L^{-1}$) were calculated for each analyte for proposed agarose-SGC binding layer as the ratio between an instrumental limit of

detection (ICP OES) and a preconcentration factor (calculated from equation reported by Zhang and Davison [6]. These values are presented in Table 6.

**Table 6.** DGT method detection limits (MDL, µg $L^{-1}$) for both binding layers

| | LD ICP OES (µg $L^{-1}$) | Pre concentration factor (48 hours) | DGT-SCG MDL (µg $L^{-1}$) |
|---|---|---|---|
| Cd | 0.20 | 4.40 | 0.05 |
| Cu | 0.36 | 6.63 | 0.05 |
| Ni | 0.82 | 4.42 | 0.19 |
| Pb | 1.65 | 7.73 | 0.21 |
| Zn | 0.17 | 3.09 | 0.05 |

Mason et al. [35] and using a mixed binding layer of ferrihydrite and Chelex-100 for evaluation of P, Mn, Cu, Mo, Zn and Cd reported values of MBL ranged from 0.02 to 4.76 µg $L^{-1}$ for these analytes. Compared to present approach, the levels detected are similar. Another DGT method detection limit reported by Shiva et al. using a mixed binding layer of Metsorb and Chelex was ranged from 0.001 to 2.05 µg $L^{-1}$[38].

### Effect of pH and ionic strength on the retention of metal ions

Effect of ionic strength on the binding of metal ions to agarose-SGC gel disks is shown in Fig. 4. For all the range of strength ionic studied, Zn concentration obtained by DGT-SGC was showed a little variation, suggesting that the strength ionic range did not affect the Zn accumulation. The results obtained showed that the Cd and Ni uptake by SGC were slightly overestimated in the range of strength ionic studied with recoveries between 111% and 141%. This increase in low ionic strength was earlier reported for the same ions in polyacrylamide diffusive gel [39,40] and 17 Chr chromatography paper diffusion [41].

For Cu and Pb, the concentrations determined by the DGT technique decreased slightly with the reduction of ionic strength but this effect was small than 20 % so the proposed ligand can be used in range of ionic strength studied. The same behavior at low ionic strength for Pb was reported in work of Pescim et al. [23] whose effects more significant (more or equal than 25 %) were encountered at ionic

strength 0.01 - 0.0005. The authors were related this effect to diffusion layer (cellulose layer) due to small interactions of Pb with the cellulose.

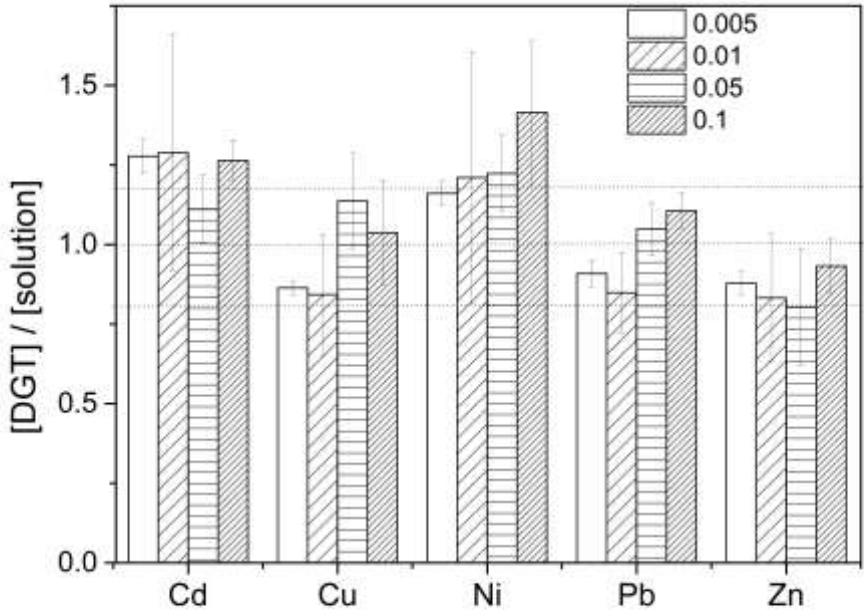



**Fig. 4** Effect of ionic strength (0.005 – 0.1 mol $L^{-1}$ $NaNO_3$) on uptake of $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ by agarose-spent coffee grounds gel disks. Deployment solution: 500 µg $L^{-1}$; temperature: 23°C; pH: 5.5; deployment time: 6 h; DGT devices: 3. Mean ± sd.

The pH dependence on the uptake of metal ions by the proposed binding gel was evaluated with DGT devices immersed in $NaNO_3$ solutions (500 ug $L^{-1}$ of analytes) in pH range 3.5 – 8.0. The ratios of metal ion concentrations measured by DGT to the concentration obtained by direct ICP OES measurement were plotted against pH (Fig. 5). Cu and Zn were measured suitably in whole pH range studied. The same pH dependence of Cu to Chelex-100 in studies of sorption was achieved by Pesavento and Biesuz [42] in range of pH 2- 5. The similar uptake of Zn was published by Gimpel et al. [13] where DGT measurements of Zn agreed well with the bulk solution concentration. For Cd, Ni and Pb there were a decrease (around 25 %) at pH 3.5, probably due to lower selectivity below pH 5. These results are consistent with another studies where the retention of Cd was also decreased in pH below 5 due to binding properties of Chelex-100 were reduced at low pH [6,13].

As the pH of most acid drainage waters are around 3.5, the agarose-SGC gel disk is suitable mainly for the application of Cu and Zn in AMD. The pH of natural waters is usually in the range of 4 - 9 which makes the agarose-SGC gel disks applicable in these types of water to the other ions.

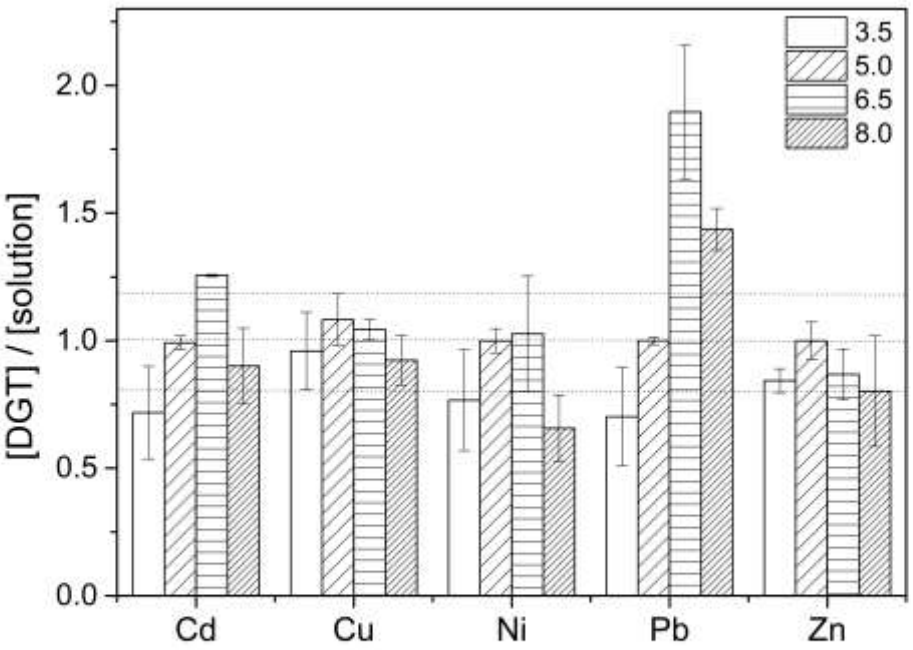



**Fig. 5** Effect of pH on uptake of $Cd^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ by agarose-spent coffee grounds gel disks. Deployment solution: 500 μg $L^{-1}$; temperature: 23 °C; ionic strength: 0.025 mol $L^{-1}$ $NaNO_3$; deployment time: 6 h; DGT devices: 3. Mean ± sd.

### Analysis of spiked river water sample

The proposed binding agent agarose-SGC was corroborated in spiked sample of river water (Cd, Cu, Ni, Pb and Zn 20 μg $L^{-1}$, Corumbataí river). For this analysis, three devices of proposed DGT-SGC were deployed for 6 h and temperature was maintained at 23 °C. The concentrations determined by the proposed DGT-SGC and ICP OES are given in Table 7. Determinations of Ni, Pb and Zn using the proposed method were in agreement with the total dissolved concentration of these ions determined directly by ICP OES. These results suggest that the DGT labile concentrations of Ni, Pb and Zn correspond to all (or almost all) the soluble fraction of these elements in the sample researched.

**Table 7.** Concentrations of metal ions in spiked river water (µg $L^{-1}$) (Corumbataí river) using the proposed approach.

| | Reference[a] | Found[b] | Recovery (%)[c] |
|---|---|---|---|
| Cd | 13 ± 4 | 23 ± 5 | 177 |
| Cu | 8 ± 3 | 13 ± 4 | 162 |
| Ni | 17 ± 2 | 14 ± 5 | 82 |
| Pb | 3 ± 1 | 3 ± 1 | 100 |
| Zn | 40 ± 22 | 54 ± 38 | 135 |

[a]Direct determination by ICP OES after spike of 20 µg $L^{-1}$ of analytes.

[b]Labile concentrations using the proposed device DGT-SGC.

[c]Recovery is defined as the labile metal concentration obtained by proposed DGT-SGC (Found) divided by concentration obtained by direct determination (Reference).

### ***In situ*** **analysis**

The *in situ* analysis were performed in dry and rainy seasons from DGT samplers assembled using conventional material (Chelex-100 as the binding agent and polyacrylamide gel as the diffusive layer) and the suggested material (agarose-SCG as binding agent and polyacrylamide gel as the diffusive layer). For each sampling, water samples for analysis of total and dissolved metals were also collected. The total, dissolved, DGT-labile and predicted speciation performed by vMINTEQ are shown in Table 8.

**Table 8.** Total, dissolved and labile concentrations (conventional-Chelex and proposed-SCG) compared to predictions by chemical speciation

| | | total | dissolved | labile Chelex | labile agarose-SCG | speciation vMINTEQ (%) | |
|---|---|---|---|---|---|---|---|
| **jul/14** | **Cd** | 23 ± 1 | 20.2 ± 0.2 | 2.6 ± 2,1 | 4.1 ± 2.8 | 64.5 | $Cd^{2+}$ |
| | | | | | | 32.3 | $CdSO_4(aq)$ |
| | | | | | | 3.2 | $Cd(SO_4)_2^{2-}$ |
| | **Cu** | 15 ± 1 | 13.8 ± 0.1 | 5.5 ± 0.9 | * | 67.1 | $Cu^{+2}$ |
| | | | | | | 32.9 | $CuSO_4$ (aq) |
| | **Ni** | 96 ± 2 | 95 ± 1 | 83 ± 7 | 31 ± 12 | 70.1 | $Ni^{2+}$ |
| | | | | | | 29.9 | $NiSO_4(aq)$ |
| | **Pb** | 75 ± 1 | 68 ± 1 | 55 ± 3 | 36 ± 1 | 47.7 | $Pb^{2+}$ |
| | | | | | | 50.0 | $PbSO_4(aq)$ |
| | | | | | | 2.2 | $Pb(SO_4)_2^{2-}$ |
| | **Zn** | 10066 ± 266 | 9389 ± 85 | 3386 ± 293 | 2310 ± 931 | 66.8 | $Zn^{2+}$ |
| | | | | | | 31.2 | $ZnSO_4(aq)$ |
| | | | | | | 2.0 | $Zn(SO_4)_2^{2-}$ |
| **feb/15** | **Cd** | 12.88 ± 0.02 | 12.13 ± 0.05 | 2.0 ± 0.6 | 4.7 ± 0.4 | 58.6 | $Cd^{2+}$ |
| | | | | | | 36.7 | $CdSO_4(aq)$ |
| | | | | | | 4.7 | $Cd(SO_4)_2^{2-}$ |
| | **Cu** | < 1.3 | < 1.3 | < 1.3 | < 1.3 | 62.0 | $Cu^{+2}$ |
| | | | | | | 38.0 | $CuSO_4$ (aq) |
| | **Ni** | 77.7 ± 0.2 | 77.6 ± 0.1 | 42.9 ± 0.4 | 51 ± 13 | 65.2 | $Ni^{2+}$ |
| | | | | | | 34.8 | $NiSO_4(aq)$ |
| | **Pb** | 76.6 ± 0.2 | 74.9 ± 0.3 | 30 ± 1 | 36 ± 3 | 41.9 | $Pb^{2+}$ |
| | | | | | | 54.9 | $PbSO_4(aq)$ |
| | | | | | | 3.1 | $Pb(SO_4)_2^{2-}$ |
| | **Zn** | 10979.15 ± 0.03 | 10775 ± 20 | 2090 ± 226 | 1959 ± 208 | 65.2 | $Zn^{2+}$ |
| | | | | | | 35.8 | $ZnSO_4(aq)$ |
| | | | | | | 2.9 | $Zn(SO_4)_2^{2-}$ |
| **dec/15** | **Cd** | 28.1 ± 0.3 | 26.3 ± 0.3 | 0.9 ± 0.1 | 1.9 ± 0.5 | 65.0 | $Cd^{2+}$ |
| | | | | | | 32.0 | $CdSO_4(aq)$ |
| | | | | | | 3.05 | $Cd(SO_4)_2^{2-}$ |
| | **Cu** | 8 ± 1 | 8 ± 2 | 4.9 ± 0.2 | < 1.15 | 67.5 | $Cu^{+2}$ |
| | | | | | | 32.5 | $CuSO_4$ (aq) |
| | **Ni** | 94 ± 1 | 89 ± 1 | 52 ± 3 | 22 ± 2 | 70.5 | $Ni^{2+}$ |
| | | | | | | 29.5 | $NiSO_4(aq)$ |
| | **Pb** | 108 ± 8 | 103 ± 3 | 20 ± 1 | 24 ± 1 | 48.2 | $Pb^{2+}$ |
| | | | | | | 0.02 | $PbF^{+}$ |
| | | | | | | 0.01 | $PbNO_3^{+}$ |
| | | | | | | 49.6 | $PbSO_{4(}aq)$ |
| | | | | | | 2.1 | $Pb(SO_4)_2^{2-}$ |
| | **Zn** | 10120 ± 122 | 9559 ± 69 | 986 ± 115 | 639 ± 203 | 67.2 | $Zn^{2+}$ |
| | | | | | | 30.9 | $ZnSO_4(aq)$ |
| | | | | | | 1.9 | $Zn(SO_4)_2^{2-}$ |

For all sampled sites, there was no significant difference in concentrations between total and dissolved samples of all metal ions, suggesting that these elements were mainly present in their dissolved forms. This was commonly reliable with other study reported for Cd, Ni and other ions [9]. Concentrations of dissolved metals, collected over the course of the DGT deployments, were considerably

greater than the DGT-labile concentrations measured by conventional DGT-Chelex and proposed DGT-SCG for all metal ions.

Cu concentrations in feb/15 were below detection limits (<1.3 µg $L^{-1}$) for total, dissolved and labile determinations. The proposed DGT-SCG cannot bind ions Cu in any sampling (suggesting a higher complexation of Cu with organic matter and suspended particles, although the conventional DGT-Chelex sampled a certain amount of labile fraction). These lower Cu DGT labile concentrations were expected because of high complexation of Cu by dissolved organic matter and suspended particles related in some researches [7,43,44].

The ratios between conventional DGT labile and dissolved concentrations ([DGT]/[dissolved] in percentage) were 3-17% of Cd, 40-61% of Cu, 55-87% of Ni, 19-87% of Pb and 10-36% of Zn (dissolved concentrations). And the proposed DGT-labile sampled 7-39% of Cd, 25-66% of Ni, 23-53% of Pb and 7-25% of Zn dissolved concentrations measured at the field sites.

Concentrations of total dissolved collected were considerably greater than the DGT labile concentrations measured by Chelex and SGC for all cations at sampling sites. Lower labile concentrations of Cd and Zn (3 – 39 % of dissolved concentrations) suggesting the complexation with non-labile complexes and/or colloids. This observation has also been reported in some articles discussed below. The works of Twiss and Moffett [43] comparing Cu speciation with analytical voltammetry and DGT; in the studies of Liu, Lead and Zhang have demonstrated that the proportions of DGT labile metal species were lower than those of ultrafiltered fraction for Cd, Cu, Ni, Pb, Zn and other ions [45] and Shiva et al.. [38] about studies of binding layers made of Chelex, PAMPAA (polyacrylamide- polyacrylic acid), Metsorb and a mixed binding layer (MBL, containing both Chelex and Metsorb) with DGT labile concentrations representing 24 – 41 % of Cd, 17 – 24% of Cu, 27 – 41 % of Ni, 17 – 37% of Pb and 23 – 31 % of Zn filterable concentrations.

For Cd, the DGT-labile concentrations measured by proposed DGT-SGC were higher than conventional DGT-Chelex, although the results obtained using both DGT devices were considerably lower compared than other studies where almost all dissolved fraction of Cd is considerate labile [46,47].

The conventional DGT-Chelex concentrations for Ni were higher than proposed DGT-SCG (except for feb/15 sample where the DGT labile concentrations for proposed device were a little higher than conventional device).

The vMINTEQ predictions were compared with metal speciation determined by DGT technique. This comparison showed good similarities between the vMINTEQ predictions and DGT results of labile metal species sampled for proposed DGT-SGC for Ni and Pb. This good concordance between predictions made of chemical speciation modelling and DGT results was similarly investigated by other authors [8–12,23].

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## 3. Conclusions

A new DGT style based on the use of biomass had been proposed. With the agarose-SCG gel as the binding phase, DGT can selectively sample and measure Cu, Cd, Ni, Pb and Zn ions with a satisfactory recovery in synthetic solution. It was also presented that the new DGT-SCG could successfully measure these ions in spiked river water and *in situ*. The DGT-SCG probably can become a very reliable tool for sampling ions in different kinds of water samples. Also, the ions rate of accumulation rate was independent of pH for all the studied range which includes the possibility to sample these ions in AMD areas. It was satisfactory for ionic strength values from 0.005 mol $L^{-1}$ to 0.1 mol $L^{-1}$, which allows its use in freshwaters of a wide variety of conditions. Therefore, the agarose-SCG is an effective binding layer to sample Cd, Cu, Ni, Pb and Zn in DGT technique.

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## *CAPÍTULO 2*

## *Agarose-banana peel as a binding agent in diffusive gradients in thin-films technique for measurement in freshwater*

**Abstract**

The diffusive gradients in thin films (DGT) technique for measurement of Cd, Cu, Pb and Zn is evaluated using, for the first time, a binding layer made of biomass from *Musa cavendishi* banana peel (BP) immobilized in agarose gel (25-mm disk containing 10 % m/v banana peel and 3 % m/v agarose). DGT-BP had a significant binding capacity at drainage acid water pH. The DGT-BP devices were successfully validated for Cd, Cu, Pb and Zn (accumulated mass vs. time $R^2$ between 0.969 and 0.998).Validation was also undertaken for these ions in spiked river water (Corumbataí and Piracicaba river) with recoveries between 62 % and 166 %. The accumulation rate of metal ions discussed was not significantly affected by ionic strength of solutions up to 0.005 mol $L^{-1}$ and for the entire studied pH range (from 3.5 to 8.0). Field deployments of the DGT devices were successful at measuring Cd, Pb and Zn concentrations in river water and acid mine drainage.

### 1. Introduction

The fate, transport and pollution of trace metals in the environment especially the aquatic ecosystems are becoming a serious environmental problem over the years. The bioavailability of trace metals in the environment is a function of the chemical species in which they occur and several works have been demonstrated that the bioavailability of dissolved metals to aquatic organisms normally varies as a function of the free metal ion concentration in solution [1,2]. Techniques which combine high sensitivity with speciation capabilities are significantly needed for trace metal analysis in waters [3].

A capable analytical speciation method is diffusive gradients in thin films technique (DGT) developed by Davison and Zhang in 1994 [4] is a passive sampler method which has widely been used as an *in situ* sampler for labile determination of trace metals in waters, sediments and soil. Traditional DGT devices comprise a

polyacrylamide hydrogel (PAM) as a diffusion layer and a Chelex-100 resin as a binding agent. Since then, a lot of adsorbents have been developed as the binding agent for the measurement of different analytes, e.g. cellulose phosphate ion exchange membrane (P81 Whatman) for Mn, Co, Ni, Cu, Zn, Pb, Hg, Ba, Cu and Cd [5–8], Spheron-thiol resin for Hg[9], Dowex resin adsorbed with methylthymolblue [10,11], among others. These resins only adsorb free and kinetically labile metal ions, therefore this technique provides a superior measure of biologically available metals than analysis of dissolved or total metal concentrations[12].

A major advantage of the DGT technique is its *in situ* deployment whereas in conventional sampling and storage have been shown to induce changes in the nature and distribution of metal species in freshwaters. In addition, the DGT device provides a time-integrated measure of metal concentrations, furthermore is capable of multi-element measurement. It pre-concentrates metal solutes, improving the problems of contamination and low detection at trace levels [12].

DGT have been considered suitable for evaluating the speciation of Cd, Co, Cu, Ni, Mn, Pb, U and Zn, in mine effluents [13–15]. A comparison of the DGT results in acid drainage waters (AMD) with computer speciation software CHEAQS, Visual MINTEQ and WHAM VI [16] showed good similarities to Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn [14,17–19].

The rising awareness of the accumulation of metals in the environment has favored to a demand for “green” technologies. Based on the considerable capacity of metal binding, the search for new sustainable technologies involving the removal of toxic metals from water and wastewaters has directly focused on biosorption using various biological materials [20]. The biomass has a great advantage for being amply available as waste or spinoff of other materials and therefore has been a focus of many studies in recent years. It was found that several functional groups present in the cell wall of biomass provide some attractions forces to the metal ions providing a high efficiency its removal [12,13].

Biomasses as peanut peel [21], apple peel [22] and watermelon peel [20]; bagasse from sugarcane [23]; *Saccharomyces cerevisiae* yeast [24] among others, have been investigated and providing a significant removal efficiency of several metal ions. Some studies used *Saccharomyces cerevisiae* immobilized in agarose gel as a binding agent for the DGT technique to determination of Ba, Cd and Pb in sea water

and river water finding results in agreement with the conventional technique [7,25,26].

Banana is a very common fruit (the fruit most consumed in the world) and therefore several tons of banana peels are discarded daily [27]. The chemical groups that exist in the banana peel surface are numerous, including carboxyl, hydroxyl and amide groups, which have been improving biosorption capacity [28].

In the present study, the DGT device was applied on acid mine drainage (AMD) from a uranium mine waste rock pile in Caldas, Brazil and river water (Piracicaba river). The purpose of the study was to (1) investigate the pH and ionic strength dependence of adsorption of analites on the proposed resin layer of the DGT under controlled conditions in the laboratory; (2) perform *in situ* measurements with DGT device in the acid mine drainage to evaluate the labile concentrations of analites; and (3) compare observed labile concentrations with predictions using the speciation program Visual MINTEQ ver. 3.0.

## 4. Experimental

### Equipment and accessories

Elemental analysis of aqueous eluates was performed using an iCAP 6000 Series ICP-OES spectrometer (ThermoScientific, USA) equipped with a V-groove nebuliser (Glass Expansion, AUS) and a cyclonic spray chamber (Glass Expansion, AUS). The spectrometer was operated under the following conditions: forward power = 1.2 kW; plasma gas flow rate = 10 L $min^{-1}$; auxiliary gas flow rate = 0.5 L $min^{-1}$; nebulizer gas flow rate = 0.75 L $min^{-1}$; sample introduction flow rate = 3.0 mL $min^{-1}$. Measurements of Cd, Cu, Pb and Zn were carried out at lines 228.8 nm, 324.7 nm, 220.3 nm and 213.8 nm, respectively.

For DGT experiments in laboratory and/or *in situ*, a pH meter (Model 3505, Jenway, UK), a conductivity meter (Model 470, Jenway, UK), a magnetic stirrer (Model 752, Fisatom, BR), an orbital shaker (PR70 Red Rotor, Hoefer, US) and refrigerator incubator BOD (TE-390, Tecnal, BR) were used. For centrifugation, Falcon tubes (15 ml) and a centrifuge (Model B4i, Jouan, FR, angle rotor AB-15.4; max. speed 4000 rpm; angle degree 37; radius 135 mm; max. RCF 2415 x g) were used.

The total organic carbon (TOC) was determined by a General Electric Carbon Analyser (GE Sievers InnovOx, USA). The major anions, sodium and potassium, in river water sample were determined by ion chromatography (Metrohm 930 Compact IC Flex, unit of cation model 2.761.0010 with a column for cation Metrosep C 4-150/4.0 packing silica gel with carboxylic groups, and unit of anion model 2.761.0020 with a column for anion Metrosep A Supp 5-150/4.0 packing polyvinyl alcohol with quaternary ammonium compounds groups).

### Reagents, materials and solutions

All reagents were of analytical grade or better and all solutions were prepared with ultra-pure water (Milli-Q, with resistance >18.2 $M\Omega cm^{-1}$, Millipore, USA). DGT polyacrylamide hydrogel diffusion layers (0.80mm thickness), DGT Chelex-100 resin layer and nylon DGT holders were purchased from DGT Research Ltd., UK. The binding gel was prepared with agarose NA (Amresco LLC, USA). Cellulose acetate membranes (Membrane Filter Supplies, 25 mm diameter, 0.45 mm pore size and 0.13 mm thickness, Sartorius Stedim Biotech, USA) were used as the covering diffusive gel (soaked in 1 mol $L^{-1}$ $HNO_3$ for 24 h, rinsed and stored in 0.025 mol $L^{-1}$ $NaNO_3$ at 4 °C before use). Analytical grade nitric and hydrochloric acid were purified by a subboiling distillation (DistillAcid, Berghof, DE) prior to use. All vessels (glass, DGT devices, PE, HDPE) used for experiments were prepared by acid washing (cleaned by keeping in 20% $HNO_3$ for at least 4 hours), rinsed at least 7 times with deionized water, 3 times with ultrapure water and dried before use.
The deployment solutions were prepared from a stock multi standard solution 1000 mg $L^{-1}$ (obtained from SpecSol, BR). The ionic strength was adjusted using a 1 mol $L^{-1}$ $NaNO_3$ stock solution (Synth, BR).

### Biosorbent characterization

The experiments were performed using dried banana peel which was first minced in a stainless-steel beater mill (IKA™ A11 Basic Mill, BR) for 30 seconds with maximum velocity 28000 rpm (3 times). After pulverization, the banana peel's particles were sieved and the fraction 250 µm was selected for work.

Specific surface area and pore size distribution of banana peel surface were determined by $N_2$(g) adsorption in Surface Area and Porosimetry Analyzer (ASAP 2020, Micromeritics, US) at temperature 77 K using 0.5 g of minced banana peel. The Brunauer–Emmett–Teller (BET) method was used for determination of surface area.

**Preparation of agarose–banana peel binding gel**

Agarose (0.9 g - 3 % m/v) was dissolved in 30 mL of water (80 °C) in a hot plate and dried banana peel (3.0 g - 10 % m/v) was added to the solution with stirring. The mixture was then transferred to a preheated gel-casting (a homemade gel-casting support was built with two plates of glass, spaced by 0.6 mm plastic) and remained on the support until it reached room temperature. The gel (≈0.6 mm thick) was cut into 2.5 cm diameter disks, washed, and preserved in purified water (4 °C). After some tests involving different amounts of agarose and banana peel had been executed, it was determined that the ideal amount of the compound needed to make the binding gel more consistent and easier to handle are 3 % m/v of agarose and 10 % m/v of banana peel.

Scanning electron microscopy (SEM) (Hitachi TableTop TM3000, JP) (20 kV) with a low vacuum chamber (1–15 Pa) was used for characterizing the morphology and structure of the agarose-banana peel gel disks surface. The sample was not dried or covered with gold in order not to lose the gel structure.

**Assembly of DGT devices and elution procedure of agarose-banana peel gel disks**

DGT samplers were assembled by placing an agarose–banana peel gel disk on the piston (25 mm diameter) with a diffusive layer of polyacrylamide and a cellulose acetate membrane to protect the device.

After deployment, the DGT devices were removed from the solution and disassembled. The agarose-banana peel gel disks were rinsed with water and the ligand was placed in a plastic tube containing 4 mL of HCl 2 mol $L^{-1}$ for elution.

For elution procedure, agarose-banana peel gel disks were removed from the solution, washed with water and placed in a plastic tube containing 4 mL of HCl 2 mol

$L^{-1}$. The tubes were shaken for a period of 24 hours. After that, liquid and solid phases were separated by centrifugation for 4,000 rpm in 20 minutes. The liquid phase was then introduced into the ICP OES.

The elution factor ($f_e$) of XX was calculated according to Eq. 1:

$$f_e = \frac{m_{elu}}{m_{ret}} \qquad (1)$$

where $m_{elu}$ is the mass eluted and the $m_{ret}$ is the mass retained in the ligand (agarose-banana peel gel disks).

To evaluate the elution procedure, two agarose-banana peel gel disks were placed into each solution (50 mL, duplicate) containing 500 µg $L^{-1}$ of analytes. The pH of the solutions was adjusted to 5.5 with 1 mol $L^{-1}$ HCl, and the ionic strength was adjusted to 0.025 mol $L^{-1}$ with $NaNO_3$. The solutions were maintained under constant stirring for 6 h.

The removal capacity of metal ions by biosorbent is calculated using the following equation, according to Chabani and Bensmaili [29], the concentration of the retained ions ($q_t$) by biosorbent can be found by Eq. 2:

$$q_t = (Ci - Cf)V\, m^{-1} \qquad (2)$$

where:

$q_t$ = removal capacity of metal ions by biosorbent (ng $g^{-1}$)

$C_i$ = concentration of initial solution (ng $mL^{-1}$)

$C_f$ = concentration of final solution (ng $mL^{-1}$)

V = solution volume (mL)

m = resin mass (g)

### Diffusion coefficient of metal ions

The test standard solution were used with two litres of a solution containing 500 µg $L^{-1}$ of analytes at pH 5.5, ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$ and temperature of 23 ± 1 °C, DGT devices and clamps was stabilized for 24 h (without the binding, diffusive agent and cellulose acetate membrane), under stirring, to avoid decrease of concentration of solution during deployment. After that the DGT devices were washed with ultra-pure water, assembled with the agarose-banana peel gel disk, polyacrylamide diffusive gel and cellulose acetate membrane and deployed in the solution at 4, 12, 24 and 48 h, the analytes were eluted from the disks.

### Effect of ionic strength and pH on the retention of analytes

Four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C and pH 5.5, were prepared with ionic strength values of 0.005 mol $L^{-1}$, 0.01 mol $L^{-1}$, 0.05 mol $L^{-1}$ and 0.1 mol $L^{-1}$ $NaNO_3$ was used to evaluate of effect ionic strength on the retention of analytes. To test of pH, four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C and 0.025 mol $L^{-1}$ $NaNO_3$ ionic strength were prepared with pH values of 3.5, 5.0, 6.5 and 8.0. The devices were then retrieved from the solutions after 6 h for both tests.

### Deployment of the DGT devices in synthetic samples and spiked river water

After the DGT devices had been assembled, they were deployed in polyethylene containers containing well stirred test standard solutions (2 L), 500 µg $L^{-1}$of analytes at pH 5.5 and ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$. Deployments in the laboratory were done at constant temperature (23 °C) to avoid changing the diffusion coefficient of analytes. The solutions were stirred constantly to reduce the diffusive boundary layer (DBL).

DGT samplers were deployed in spiked river water (2L, collected from the Corumbataí River – Rio Claro/SP, Brazil and Piracicaba River – Piracicaba/SP, Brazil) for six hours to evaluate the performance of the binding gels. Sample was spiked with 20 µg $L^{-1}$ and 50 µg $L^{-1}$ of metal ions of this study. These rivers were chosen for this study because it is an important agricultural area of sugar cane and also receives effluents from industries such as textile, ethanol, and chemistry.

### Modeling Speciation

The present study was employed a chemical speciation modeling Visual MINTEQ (vMINTEQ) [30]. This is a freeware chemical equilibrium model for the calculation of metal speciation for natural waters based on NICA-Donnan model (for the prediction of equilibrium-speciation of free metal ions) and Stockholm Humic model (SHM) to describe the formation of metal and humic and fulvic complexes.

## 5. Results and Discussions

### Characterization of biosorbent

SEM micrograph of agarose-banana peel gel disk surface before metal biosorption is presented in Fig. 1. This micrograph showed that nature of the biomass was substantial with lots of pores, making it possible for biosorption of several metal ions.

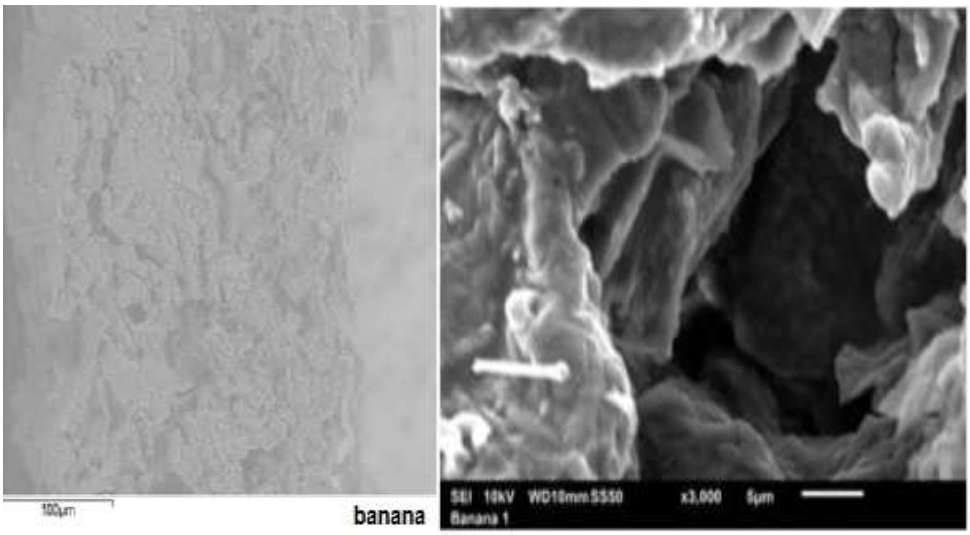


**Fig. 1** Scanning Electron Micrographs of banana peel gel disks surface (100x and 3000x)

The agarose-banana peel gel disk before metal biosorption was characterized by energy dispersive X-ray spectroscopy coupled with scanning electron microscopy (SEM-EDX). SEM micrograph of banana peel indicated that the nature of the biomass was irregular and heterogeneous with lots of pores, making it possible for the biosorption of many trace metals in different parts of the biosorbent. This irregular nature and an existence of several pores in banana peel was also reported by Liu et al. [20].

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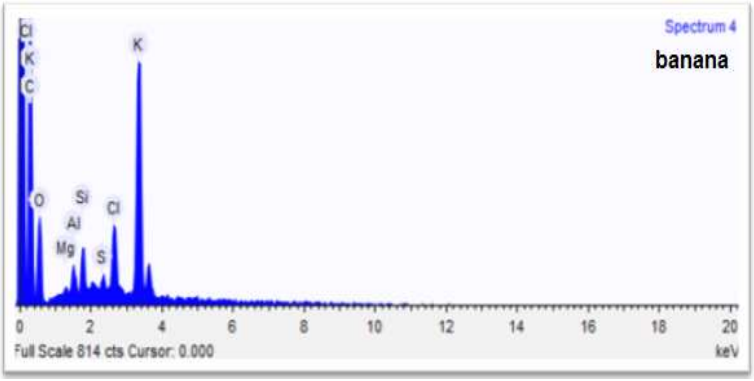


**Fig. 2** EDX spectrum of agarose-coffee gel disks surface without plating Au.

Fig. 2 illustrates a typical spectrum obtained to agarose-banana peel gel disks. In the EDX technique employed, the detection range were detected as major elements C (46 %), O (29 %), K (17%), Cl (4 %), Si (2 %) and Al (0.9 %), S (0.9 %) and Mg (0.3 %). This EDX spectrum is similar to study of banana peel for removal of Cr (VI) where the elemental analysis of banana peel before chromium treatment revealed the presence of C, O, Si, P, S, Cl, Ca and a particularly intense peak of potassium [31].

Table 1 presents the average results of the nitrogen adsorption experiments for surface area $S_P$ ($m^2\ g^{-1}$), pore volume $V_P$ ($cm^3\ g^{-1}$). For comparative purposes the values of specific area and volume of banana peel were very similar with previous work of Liu et al. that presented results of the analysis of nitrogen adsorption porosimetry for dried banana peel with values of $S_p$ and $V_p$ 0.41, 0.00011, respectively [32].

**Table 1.** Physical parameters obtained by porosimetry analysis for banana peel.

| | $S_P$ ($m^2\ g^{-1}$) | $V_P$ ($cm^3\ g^{-1}$) |
|---|---|---|
| Banana peel | 0.17 ± 0.04 | 0.00037 ± 0.00003 |

Several biosorbents have been considered for Cd, Cu, Pb and Zn removal, such as peanut peel, *Saccharomyces cerevisiae*, orange peel, apple peel, watermelon peel, bagasse from sugarcane and orange, among others. Table 2 gives a removal capacity of metal ions by banana peel in present study. These values indicated that the biosorption capacity of banana peel for Cd and Cu is better than Pb and Zn. The Cu biosorption capacity calculated in this present work was higher than previous

reported by Liu et al. (8.24 mg $g^{-1}$) [20]. The values for Cd and Zn were also higher then reported article (5.71 and 2.18 for Cd and Pb, respectively) [33]. Such a comparison of various biosorbents mentioned, this banana peel presented a suitable performance on the removal of metal ions.

**Table 2**. Biosorption capacity for Cd, Cu, Pb and Zn removal

| Analyte | Removal capacity (mg $g^{-1}$) |
|---|---|
| Cd | 18.6 |
| Cu | 13.5 |
| Pb | 6.43 |
| Zn | 6.57 |

### Measurements by Different Techniques

The conductivity, ionic strength, total dissolved solids (TDS), pH, temperature and major ion composition measured in situ and in spiked river water (Corumbataí River and Piracicaba River) are given in Table 3.

**Table 3.** Physical-Chemical parameters measured in situ and in spiked river water

| | 075 jul/14 | 075 dec/15 | Corumbataí River | Piracicaba River |
|---|---|---|---|---|
| Conductivity (µS $cm^{-1}$) | 1308 | 1375 | 94 | 330 |
| Ionic strength[a] (mol $L^{-1}$) | 0.0163 | 0.0175 | 0.0009 | 0.004 |
| TDS[b] (mg $L^{-1}$) | 837 | 880 | 49 | 211 |
| DOC[c] (mg $L^{-1}$) | < 1 | < 1 | - | 7.13 ±0.167.5 |
| pH | 3.48 | 3.80 | 6.75 | 7.5 |
| T (°C) | 21.0 | 20.7 | 25.0 ±1.0 | 22.5 ±0.6 |
| $HCO_3^-$ (mg $L^{-1}$) | - | - | 27.2 | - |
| $F^-$ (mg $L^{-1}$) | 117 | 117 | 0.075 | 0.29 |
| $Cl^-$ (mg $L^{-1}$) | < 0.01 | < 0.01 | 4.76 | 47 |
| $NO_2^-$ (mg $L^{-1}$) | < 0.02 | < 0.02 | 0.055 | 0.40 |
| $NO_3^-$ (mg $L^{-1}$) | 3.14 | 2.16 | 7.45 | 30 |
| $PO_4^-$ (mg $L^{-1}$) | < 0.040 | < 0.080 | < 0.040 | 0.94 |
| $SO_4^{2-}$ (mg $L^{-1}$) | 917 | 873 | 1.83 | 56 |
| $Li^+$ (mg $L^{-1}$) | < 0.010 | < 0.010 | < 0.010 | - |
| $Na^+$ (mg $L^{-1}$) | 1.622 | 1.139 | 4.56 | - |
| $K^+$ (mg $L^{-1}$) | 4.72 | 6.78 | 1.59 | - |
| $Mg^{2+}$ (mg $L^{-1}$) | 6.37 | 5.67 | 3.30 | - |
| $NH_4^+$ (mg $L^{-1}$) | 72.63 | 73.93 | < 0.050 | - |

[a] ionic strength, values calculated according Griffin and Jurinak [34]
[b]TDS total dissolved solids, relationship between TDS and electrical conductivity proposed by APHA, AWWA and WPCF [35]
[c]dissolved organic carbon

### Elution efficiency of the agarose-banana peel gel disks

The binding agent agarose-banana peel proposed was immersed in 50 mL solution containing 500 mg $L^{-1}$ Cd, Cu, Pb and Zn. After the sorption time (6h), the metal ions concentration remaining in solution was measured by ICP OES. The Cu and Zn retained by the ligand agent was eluted with HCl 2mol $L^{-1}$ and measured by ICP

OES. By analyzing the ratio between mass retained and eluted from the binding agent, an elution factor of 79 ± 2, 95 ± 12, 86 ± 9, 79 ± 4 for Cd, Cu, Pb and Zn, respectively, was achieved.

The values of elution factor are equal or higher than the established value of 80% using 1 mol $L^{-1}$ $HNO_3$ with a binding layer of Chelex [12] suggesting that elution is effective also with HCl. Mason et al. [36] also conducted their studies with the use of HCl in elution of Cd, Cu, Mn, Mo, P and Zn with elution efficiency of 92 % for all elements excluding Mo (79%).

### Diffusion coefficient measurements using deployment curve

In this study, six DGT devices were deployed in 6L of a solution containing 500 mg $L^{-1}$ Cd, Cu, Pb and Zn (pH = 5.4, T= 23 °C and ionic strength = 0.025 mol $L^{-1}NaNO_3$) for 0, 4, 12, 24 and 48h. Fig. 3 shows the relationship between the mass of these ions (retained by the proposed binding agent) and the deployment time (deployment curve).

The linear relationship between the amount of metal ion accumulated by the DGT devices (normalized for metal ion concentration in deployment solution, in order to avoid high values of blank that can come from own biomass) and the deployment time has been used as a requirement for the viability of novel DGT devices. A linear relationships (correlation coefficient - $R^2$ between 0.969 and 0.998) were observed and there is no suggestion about the saturation of ligand until 48 h, indicating that the interaction of these ions with the diffusive agent is insignificant and that retention of these analytes by the binding agent is satisfactory. Even though the deployment curve is made under environmental non-realistic concentrations, the diffusion coefficient is independent of the concentrations (according to Fick's Law).

Consequently, the new DGT device is effective for the sampling of Cu and Zn in aqueous solutions under the evaluated conditions cited.

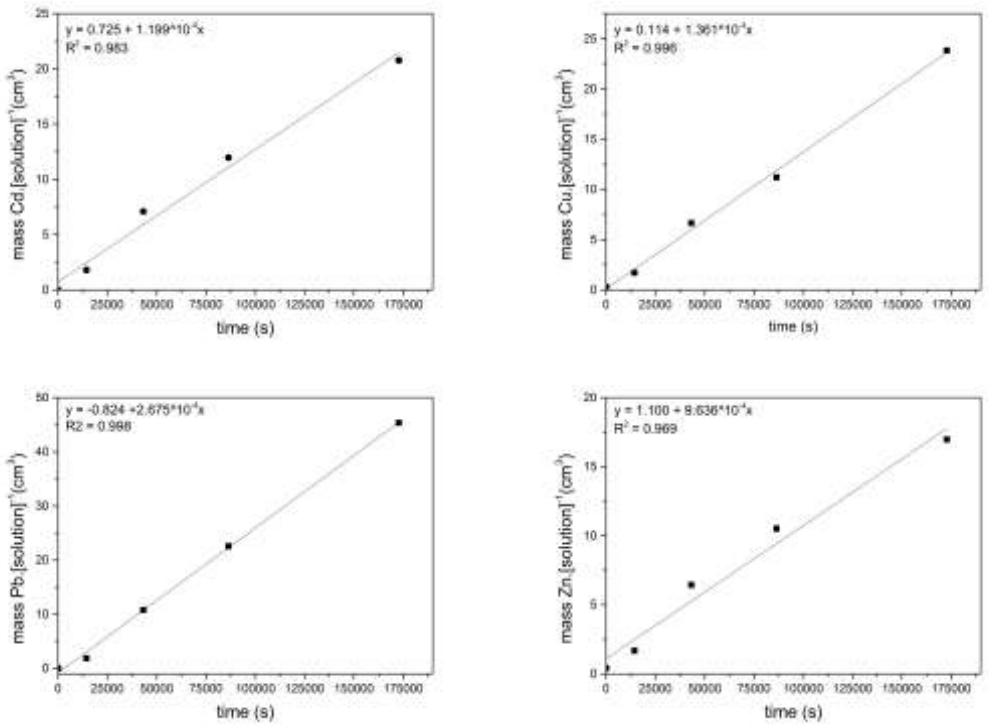


**Fig. 3** Mass of ion: Cd, Cu, Pb and Zn accumulated in agarose-BP normalized by the ion concentration in the solution for various periods of time, following parameters: ionic strength = 0.025 mol $L^{-1}$ $NaNO_3$; pH = 5.4; temperature = 23°C; Δg = 0.093 cm; A = 3.14 $cm^2$.

The diffusion coefficient (*D*) of metal ions on polyacrylamide diffusive layer can be calculated applying the theory of the DGT technique and a slope of the relationship between the amount of metal ions accumulated by the DGT devices and the deployment time, in according to Eq. 3:

$$s = DA(\Delta g)^{-1} \qquad (3)$$

Thus, the diffusion coefficient can be calculated with the Eq. 4:

$$D = s\Delta g(A)^{-1} \qquad (4)$$

Where *s* is the slope of the deployment curve, *A* is the cross-sectional area (3.14 $cm^2$) and *Δg* is the thickness of the agarose-banana peel gel disks plus cellulose acetate membrane (0.093 cm).

Table 4 compares the diffusion coefficient of Cd, Cu, Pb and Zn obtained for polyacrylamide hydrogel with diffusion coefficient obtained in studies of DGT Research. [37] and Garmo et al. [38]. Corrections for measurements prepared at

different temperatures, were made using the Stokes–Einstein relation related by [39] (Eq. 5):

$$(D_1\eta_1)/T_1 = (D_2\eta_2)/T_2 \qquad (5)$$

**Table 4.** Diffusion coefficients (x $10^{-6}$ $cm^2$ $s^{-1}$) of metal ions in polyacrylamide gel by deployment curve (500 µg $L^{-1}$ Cd, Cu, Pb and Zn; T = 23 °C; pH = 5.5; DGT devices = 3; Mean ± sd)

| | Found values (x $10^{-6}$ $cm^2$ $s^{-1}$) | Reference values (x $10^{-6}$ $cm^2$ $s^{-1}$) |
|---|---|---|
| Cd | 3.55 ±0.23 | 5.21 (68 %)[a] |
| Cu | 4.03 ± 0.13 | 5.90 (68 %)[b] |
| Pb | 7.92 ± 0.18 | 7.61 (104 %)[b] |
| Zn | 2.86 ± 0.25 | 4.40 (65 %)[a] |

[a] Data from DGT Research[37]
[b] Data from Garmo et al. [38]

The diffusion coefficients founded are in reasonable agreement with previously reported values (65 – 104 %) [37,38]. It proposes that metal ions were quantitatively retained by the agarose–BP gel disks and that the proposed material can be used as a binding agent for the DGT technique.

The Pb diffusion coefficient founded is in excellent agreement with previously reported value (104 %) [38]. The diffusion coefficient of Cd in polyacrylamide in this present study is very similar to that published by the dialysis membrane with the Py–PEI as the binding agent (3.32 ± 0.29 × $10^{-6}$ $cm^2$ $s^{-1}$ ) [40]

**DGT method detection limit**

The DGT method detection limit (MDL) was calculated from a limit of detection (LOD) using ICP OES in conditions cited in present text and a theoretical preconcentration factor obtained for deployment time of 48 hours. The theoretical preconcentration factor was considered as the ratio of the predicted analyte concentration in the eluent to the analyte concentration in the bulk solution [12]. Thus, from these data, the values of MDL are presented in Table 5.

**Table 5.** DGT method detection limits (MDL, µg $L^{-1}$) for proposed approach

| | LD ICP OES (µg $L^{-1}$) | Preconcentration factor (48 hours) | DGT-BP MDL (µg $L^{-1}$) |
|---|---|---|---|
| Cd | 0.20 | 3.57 | 0.06 |
| Cu | 0.36 | 4.90 | 0.07 |
| Pb | 1.65 | 6.94 | 0.24 |
| Zn | 0.17 | 3.20 | 0.05 |

All detection limits in our study (except for Pb, with value of 0.24) were below 0.05 µg L-1. The assessed detection limits in present study are concordant with previously reported values for DGT-MBL [36,41,42] DGT-Chelex [42], DGT-PAMPAA [43]. The detection limit for Pb was just a little more higher compared to others. However, the calculated MDL is generally acceptable for these elements.

### Effect of pH and ionic strength on the retention of metal ions

The effect of ionic strength on the Cd, Cu, Pb and Zn binding capacity of DGT-banana peel was considered in Fig. 4 indicated by the ratio of concentration measured by DGT to concentration in solution ($C_{DGT}/C_{sol}$). The concentration of these ions measured by DGT-banana peel showed good agreement with the solution concentration over the range of ionic strength studied.

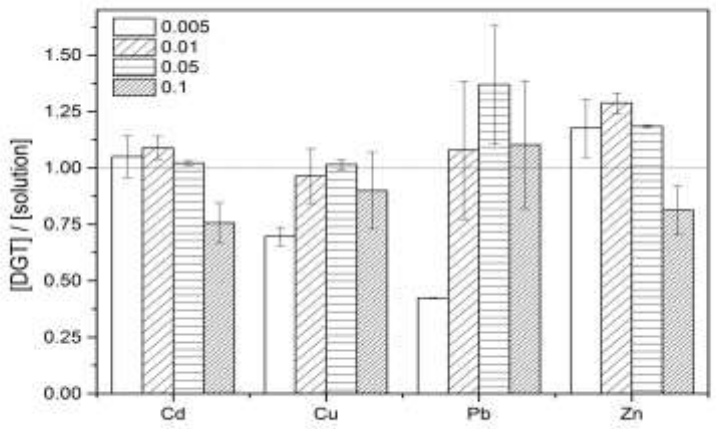


**Fig. 4** Effect of ionic strength (0.005 – 0.1 mol $L^{-1}$ $NaNO_3$) on uptake of Cd, cu, Pb and Zn by agarose-banana peel. Deployment solution: 500 µg $L^{-1}$; temperature = 23°C; pH = 5.5; deployment time = 6 h; DGT devices = 3; Mean ± sd.

The concentration of Cd and Cu measured by DGT-banana peel showed good agreement with the solution concentration over the ionic strength range performed (0.005 – 0.1 mol $L^{-1}$ $NaNO_3$). In a work of Zhang and Davison [12], the range acceptable for DGT application was $C_{DGT}/C_{sol}$ ratio = 0.90 – 1.1. For ionic strength 0.005 mol $L^{-1}$, the $C_{DGT}/C_{sol}$ ratio for Cu was lower than 0.90 (0.70) possibly attributed to electrostatic interactions between trace metals and fixed charged groups in APA hydrogel in solutions with low ionic strength reported in literature [44,45]. The $C_{DGT}/C_{sol}$ ratio for Zn was also lower than 0.90 at the ionic strength of 0.1 mol $L^{-1}$, probably due to mass effect of $NO_3^-$more expressive in these solutions due to higher concentration of $NaNO_3$. This same behavior was related in recent work of V determination by Amberlite IRA-410 [46]

For the range from 0.01 mol $L^{-1}$ to 0.1 mol $L^{-1}$, Pb concentration in each solution obtained by agarose-BP-DGT devices ($C_{DGT}$) showed slight variation compared to the values obtained by direct measurement of the aliquots ($C_{solution}$), suggesting that the range of ionic strength did not affect the Pb accumulation rate. Lower Pb accumulation can be observed for solution with ionic strength equal to 0.005 mol $L^{-1}$ $NaNO_3$ (Fig. 3), which can be attributed to modification of the diffusion of ions through the gel layer. Several studies have reported inaccurate measurements of elements by DGT at ionic strengths less than 0.001 mol $L^{-1}$[47–49]. The same behavior at low ionic strength for Pb was reported in work of Pescim et al. [25] whose effects more significant (more or equal than 25 %) were encountered at ionic strength 0.01 - 0.0005.

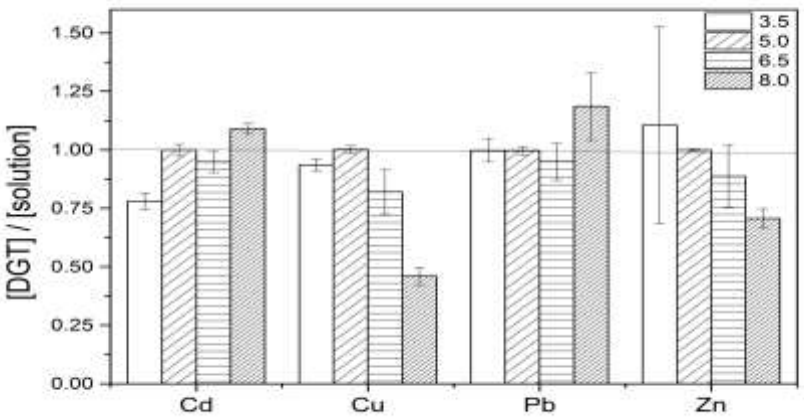


**Fig. 5** Effect of pH (3.5 – 8.0) on uptake of Cd, Cu, Pb and Zn by agarose-banana peel. Deployment solution: 500 µg $L^{-1}$; ionic strength = 0.025 mol $L^{-1}$ $NaNO_3$; temperature = 23°C; deployment time = 6 h; DGT devices = 3; Mean ± sd.

The effect of pH on the retention of Cd, Cu, Pb and Zn is shown in Fig. 5. For all pH values evaluated, the ratios $C_{DGT}/C_{sol}$ for these ions were calculated by dividing the DGT-BP concentration by direct concentrations in the test solution. Cu concentration determined by DGT-banana peel between pH 3.5 and 6.5 are in practical agreement ($C_{DGT}/C_{sol}$ 0.82 – 1.00), except for lower Cu uptake at pH 8.0 ($C_{DGT}/C_{sol}$ = 0.46) , where a small amount of metal were bound at pH = 8 probably due to the formation of insoluble hydroxide forms of Cu reported previously by Li et al [50]. However at natural water pH range, the Cu ions were bound efficiently to DGT-banana peel.
The suitable retention of Cd, Pb and Zn was reproducible throughout pH range studied (3.5 – 8.0), indicated by ratios $C_{DGT}/C_{sol}$ between 0.71 and 1.18.

### Analysis of spiked river water sample

The proposed binding agent DGT-banana peel was validated in spiked river water by deployment of the proposed device in two spiked samples of river water (Cd, Cu, Pb and Zn 20 µg $L^{-1}$, Corumbataí river; Cd, Cu, Pb and Zn 50 µg $L^{-1}$, Piracicaba river). For these analysis, three devices of each one (proposed and original devices) were deployed for 6 h and temperature was maintained at 23 °C and these results were presented in Table 6.

**Table 6.** Concentrations of metal ions in spiked river water (µg $L^{-1}$) (Corumbataí river) using the proposed approach.

| | Analyte | Reference[a] | Found[b] | Recovery (%)[c] |
|---|---|---|---|---|
| Corumbataí river | Cd | 13 ± 4 | 16 ± 7 | 123 |
| | Cu | 8 ± 3 | 9 ± 2 | 112 |
| | Pb | 3 ± 1 | 7 ± 2 | 233 |
| | Zn | 40 ± 22 | 86 ± 23 | 215 |
| Piracicaba river | Cd | 50 ± 8 | 31 ± 2 | 62 |
| | Cu | 58 ± 10 | 45 ± 4 | 78 |
| | Pb | 33 ± 9 | 1.7 ± 0.6 | 5 |
| | Zn | 75± 5 | 125 ± 24 | 166 |

[a]Direct determination by ICP OES after spike of 20 µg $L^{-1}$ or 50 µg $L^{-1}$ of analytes.

[b]Labile concentrations using the proposed device DGT-BP.

[c]Recovery is defined as the labile metal concentration obtained by proposed DGT-BP (Found) divided by concentration obtained by direct determination (Reference).

Determinations of Cd and Cu using the proposed approach were in agreement with the total dissolved concentration of these ions determined directly by ICP OES. These results suggest that the DGT labile concentrations of Cd and Cu correspond to all (or almost all) the soluble fraction of these elements in the samples researched. DGT labile concentrations of Zn were overestimated in both field deployments. In the Piracicaba River, the recovery of proposed DGT-BP for labile concentrations of Pb (recovery = 5 %) was very low probably due to the presence of natural organic matter which complexed a significant fraction of the spiked Pb. This presence of organic matter complexing almost all fraction of Cd and Cu species was related by Li et al. [50].

### *In situ* analysis

The *in situ* analysis were performed in two locals (a river water – Piracicaba river and an acid mine drainage in dry and rainy seasons) from DGT devices assembled using conventional material (Chelex-100 as the binding agent and polyacrylamide gel as the diffusive layer) and the suggested material (agarose-BP as binding agent and polyacrylamide gel as the diffusive layer). For each sampling, water samples for analysis of total and dissolved metals were also collected. The total, dissolved and proposed DGT-labile concentrations in Piracicaba river are shown in Table 7 and total, dissolved, labile concentrations and predicted speciation performed by vMINTEQ are shown in Table 8.

**Table 7.** Total, dissolved and labile *in situ* concentrations (µg $L^{-1}$) in Piracicaba river (Piracicaba/SP/Brazil)

| | Total | Dissolved | Labile agarose-BP | $C_{DGT}/C_{dissolved}$ % |
|---|---|---|---|---|
| Cd | 0.3 ± 0.1 | 0.17 ± 0.04 | 0.15 ± 0.01 | 88 ± 5 |
| Cu | 4.3 ± 0.5 | 3.5 ± 0.1 | 20 ± 5 | 571 |
| Pb | 2.5 ± 0.9 | 1.0 ± 0.4 | 1.6 ± 0.4 | 160 ± 40 |

Table 7 shows Cd, Cu and Pb values for *in situ* determination performed in Piracicaba River. A Cd and Pb recoveries by DGT-BP between 88 and 160 % of the dissolved concentration was obtained and a very higher overestimation for labile Cu concentration was achivied.

**Table 8.** Total, dissolved and labile concentrations (conventional-Chelex and proposed-BP) (μg $L^{-1}$) compared to predictions by chemical speciation

| | | total | dissolved | labile Chelex | labile agarose-BP | speciation vMINTEQ (%) | |
|---|---|---|---|---|---|---|---|
| jul/14 | Cd | 23 ± 1 | 20.2 ± 0.2 | 2.6 ± 2.1 | 5.9 ± 3.6 | 64.5 | $Cd^{2+}$ |
| | | | | | | 32.3 | $CdSO_4(aq)$ |
| | | | | | | 3.2 | $Cd(SO_4)_2^{2-}$ |
| | Cu | 15 ± 1 | 13.8 ± 0.1 | 5.5 ± 0.9 | < 1.3 | 67.1 | $Cu^{+2}$ |
| | | | | | | 32.9 | $CuSO_4$ (aq) |
| | Pb | 75 ± 1 | 68 ± 1 | 55 ± 3 | 45 ± 13 | 47.7 | $Pb^{2+}$ |
| | | | | | | 50.0 | $PbSO_4(aq)$ |
| | | | | | | 2.2 | $Pb(SO_4)_2^{2-}$ |
| | Zn | 10066 ± 266 | 9389 ± 85 | 3386 ± 293 | 3519 ± 1631 | 66.8 | $Zn^{2+}$ |
| | | | | | | 31.2 | $ZnSO_4(aq)$ |
| | | | | | | 2.0 | $Zn(SO_4)_2^{2-}$ |
| dec/15 | Cd | 28.1 ± 0.3 | 26.3 ± 0.3 | 0.9 ± 0.1 | 2.6 ± 0.6 | 65.0 | $Cd^{2+}$ |
| | | | | | | 32.0 | $CdSO_4(aq)$ |
| | | | | | | 3.05 | $Cd(SO_4)_2^{2-}$ |
| | Cu | 8 ± 1 | 8 ± 2 | 4.9 ± 0.2 | 4.4 ± 0.6 | 67.5 | $Cu^{+2}$ |
| | | | | | | 32.5 | $CuSO_4$ (aq) |
| | Pb | 108 ± 8 | 103 ± 3 | 20 ± 1 | 30 ± 3 | 48.2 | $Pb^{2+}$ |
| | | | | | | 0.02 | $PbF^{+}$ |
| | | | | | | 0.01 | $PbNO_3^{+}$ |
| | | | | | | 49.6 | $PbSO_{4(}aq)$ |
| | | | | | | 2.1 | $Pb(SO_4)_2^{2-}$ |
| | Zn | 10120 ± 122 | 9559 ± 69 | 986 ± 115 | 1076 ± 307 | 67.2 | $Zn^{2+}$ |
| | | | | | | 30.9 | $ZnSO_4(aq)$ |
| | | | | | | 1.9 | $Zn(SO_4)_2^{2-}$ |

For all sampled sites, there was no significant difference in concentrations between total and dissolved samples of all metal ions, suggesting that these elements were mainly present in their dissolved forms. This was commonly reliable with other study reported for Cd, Ni and other ions [15]. Concentrations of dissolved metals, collected over the course of the DGT deployments, were considerably greater than the DGT-labile concentrations measured by conventional DGT-Chelex and proposed DGT-SGC for all metal ions.

The ratios between conventional DGT labile and dissolved concentrations ($C_{DGT}/C_{dissolved}$ in percentage) were 3 - 13 % of Cd, 40 - 61 % of Cu, 19 – 81 % of Pb and 10 – 36 % of Zn. And the proposed DGT-labile sampled10 – 29 % of Cd, 55 % of Cu, 29 – 66 % of Pb and 11 – 38 % of Zn dissolved concentrations measured at the field sites.

The lower labile concentrations for Cd and Zn (only 3 - 38 % of dissolved concentrations) suggests the greater part of these metals existed as colloids and/or non-labile complexes which are generally not measured by DGT, also reported previously in some studies [41,51,52].

The labile concentrations measured by conventional DGT devices and proposed DGT devices were similar (no significant differences) for all ions studied in present work. These values show that both DGT devices measured related ranges of metal species. Shiva et al. and Panther et al. mentioned an analogous association between measurements of metals by conventional DGT-Chelex and their proposed DGT devices [41,42].

The average concentration of total inorganic Cd, Cu, Pb and Zn species as predicted by vMINTEQ ranged about 68 % of dissolved Cd, 67 % of dissolved Cu, 50 % of dissolved Pb and 68 % of dissolved Zn and results for Cu and Pb were concordant with predictions of the chemical speciation modeling.

## 4. Conclusions

For the first time, biomass of banana peel was employed as a binding layer for DGT technique for effective retention of Cd, Cu, Pb and Zn. The proposed DGT-BP can be successfully applied *in situ* for Cd, Pb and Zn measurements in acid mine drainage water (with recoveries of up to 80 % for Pb, as example), in river water (Piracaba River) for *in situ* Cd and Zn determinations with recoveries suitable (88 – 160 %) and in synthetic solutions with pH range from 3.5 to 8.0 and ionic strength from 0.005 to 0.1 mol $L^{-1}NaNO_3$. Laboratory tests performed in both spiked river water showed quantitative recoveries. Overall, the DGT technique has been establish to be effective for chemical speciation of trace metals *in situ* in acid mine drainage water of uranium mine of the Caldas/MG, Brazil. The chemical speciation vMINTEQ was presented reasonable predictions; however, more studies are necessary on effect of kinetics on uptake of metal ions in complexes matrices.

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## *CAPÍTULO 3*

Agarose-moringa seeds as a binding agent in diffusive gradients in thin-films technique for measurement in freshwater

**Abstract**

A novel diffusive gradients in thin films (DGT) binding agent, using shelled *Moringa Oleifera* seeds (SMOS) immobilised in agarose gel has been proposed for sampling and determination of labile Cd, Cu, Ni, Pb and Zn. The SMOS-DGT devices were effectively applied in synthetic solutions, spiked river water and *in situ* at acid mine drainage water (AMD, Osamu Utsumi mine, Caldas/MG/Brazil). The SMOS-DGT provides consistent results over pH range of 3.5 – 8.0 and a range of ionic strength from 0.005 to 0.1 mol $L^{-1}$. The labile concentrations of ions studied in spiked river water measured by DGT-SMOS showed good recoveries. Labile concentrations *in situ* for DGT-SMOS were also concordant with the values measured by conventional Chelex-DGT and predictions measured by chemical speciation modelling vMINTEQ.

**Keywords:** moringa seeds. Diffusive gradients in thin films (DGT). Passive sampler. Speciation. Fractionation

### 1. Introduction

Trace metals may be introduced into aquatic ecosystems generally through natural and anthropogenic sources. The information about a determination of total concentration of a specific metal is incomplete to evaluate the geochemistry, bioavailability and toxicity of trace metals in environmental systems. For that reason, speciation analysis is a substantial analytical tool used for the interpretation qualitative and quantitative of the chemical species [1]. Techniques which combine high sensitivity with speciation competences are expressively required for trace metal analysis in waters [2].

The diffusive gradients in thin films technique (DGT) is a significant tool, once it provides *in situ* of labile metal species. Additional features of DGT include

preconcentration and multielement capability, among others. This technique was proposed by Davison and Zhang [3] based on 1° Fick's Law. The traditional DGT device was mounted with resin Chelex-100 (binding layer) followed by a diffusive layer of polyacrylamide hydrogel and a 0.45 μm thick pore size Millipore cellulose acetate membrane to protect this device. Other binding layers have been applied to this technique such as diethylaminoethyl acetate anion exchange membrane (DE81) [4,5], liquid binding phases such as aqueous solution of sodium polyacrylate (PA) [6], activated carbon [7,8]. DGT have been considered suitable for evaluating the speciation of Cd, Co, Cu, Ni, Mn, Pb, U and Zn, in mine effluents [9–11]. A comparison of the DGT results in acid drainage waters (AMD) with computer speciation software CHEAQS, Visual MINTEQ and WHAM VI [12] showed good similarities to Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn [4,10,13,14].

The search for new, effective and economical "green" technologies has intended attention to biosorption based on metal binding capacities of diverse biological material [15,16]. The biomass has a great advantage for being amply available as waste or spinoff of other materials and therefore has been a focus of many studies in recent years. It was found that several functional groups present in the cell wall of biomass provide some attractions forces to the metal ions providing a high efficiency its removal [17,18]. Biomasses as peanut hulls [19], apple [20] and watermelon [21]; bagasse from sugarcane [22]; *Saccharomyces cerevisiae* yeast [23] among others, have been investigated and providing a significant removal efficiency of several metal ions. Some studies used *Saccharomyces cerevisiae* immobilized in agarose gel as a binding agent for the DGT technique to determination of Ba, Cd and Pb in sea water and river water finding results in agreement with the conventional technique [24–26].

*Moringa oleifera* is a plant native to India, growing mainly in tropical and subtropical semi-arid areas. It prefers dry, sandy soils and tolerates poor soils. At moment, its cultivation is spread throughout Africa, Central America, South America, Sri Lanka, India, Mexico, Malaysia and Philippines [27]. Various parts of the plant are identified for innumerable pharmacological properties [28,29]. *M. oleifera* seeds have been also employed for treating turbid water due to their flocculating properties [30,31]. A shelled *Moringa oleifera* seeds (SMOS) is one of the some attractive seed biomass for the removal of As, Cd, Cr, Ni and Pb as evidenced in earlier works [32–35]

In the present study, the DGT device was applied on acid mine drainage (AMD) from a uranium mine waste rock pile in Caldas (UTM-Caldas, Minas Gerais, Brazil)

currently in decommissioning process. The cut-off grade of the mine was set at 170 mg $kg^{-1}$ of uranium, and all materials mined with less than this level were considered sterile cipriani. The main pile (sterile pile, BF-4), which covers an area of 56.9 ha and contains $12.4\times10^3$ $m^3$ of sterile. In construction of BF-4, the boulders of sterile were unloaded on the valley and on the bed of Consulta Creek without any control. At present, the acid drainage of BF-4 is launched in the Nestor Figueiredo retention basin (BNF, approximate area of 2170.5 $m^2$) with a purpose of capture the water that percolates through BF-4, and also receives part of the superficial runoff from the same pile [36]. The pH of this acid drainage water is about 3.0, and contains high concentrations of elements such as aluminum, manganese, iron, fluoride, among others, and radioactive elements such as uranium, radium, and thorium [37,38].

The purpose of the study was to (1) investigate the pH and ionic strength dependence of adsorption of analites on the proposed resin layer of the DGT under controlled conditions in the laboratory; (2) perform *in situ* measurements with DGT device in the acid mine drainage to evaluate the labile concentrations of analites; and (3) compare observed labile concentrations with predictions using the speciation program Visual MINTEQ ver. 3.0.

## 2. Materials and Methods

### 2.1. Equipment and accessories

Elemental analysis of aqueous eluates was performed using an iCAP 6000 Series ICP-OES spectrometer (ThermoScientific, USA) equipped with a V-groove nebuliser (Glass Expansion, AUS) and a cyclonic spray chamber (Glass Expansion, AUS). The spectrometer was operated under the following conditions: RF power = 1150 W; plasma gas flow rate = 10 L $min^{-1}$; auxiliary gas flow rate = 0.5 L $min^{-1}$; nebulizer gas flow rate = 0.75 L $min^{-1}$; sample introduction flow rate = 3.0 mL $min^{-1}$. Measurements of Cd, Cu, Pb and Zn were carried out at lines 228.8, 324.7, 220.3 and 213.8 nm, respectively.

For DGT experiments in laboratory and/or *in situ*, a pH meter (Model 3505, Jenway, UK), a conductivity meter (Model 470, Jenway, UK), a magnetic stirrer (Model 752, Fisatom, BR), an orbital shaker (PR70 Red Rotor, Hoefer, US) and refrigerator incubator BOD (TE-390, Tecnal, BR) were used. For centrifugation, Falcon tubes (15

ml) and a centrifuge (Model B4i, Jouan, FR, angle rotor AB-15.4; max. speed 4000 rpm; angle degree 37; radius 135 mm; max. RCF 2415 x g) were used.
The total organic carbon (TOC) was determined by a General Electric Carbon Analyser (GE Sievers InnovOx, USA). The major anions, sodium and potassium, in river water sample were determined by ion chromatography (Metrohm 930 Compact IC Flex, unit of cation model 2.761.0010 with a column for cation Metrosep C 4-150/4.0 packing silica gel with carboxylic groups, and unit of anion model 2.761.0020 with a column for anion Metrosep A Supp 5-150/4.0 packing polyvinyl alcohol with quaternary ammonium compounds groups).

### 2.2. Reagents, materials and solutions

All reagents were of analytical grade or better and all solutions were prepared with ultra-pure water (Milli-Q, with resistance >18.2 $M\Omega cm^{-1}$, Millipore, USA). DGT polyacrylamide hydrogel diffusion layers (0.80mm thickness), DGT Chelex-100 resin layer and nylon DGT holders were purchased from DGT Research Ltd., UK. The binding gel was prepared with agarose NA (Amresco LLC, USA). Cellulose acetate membranes (Membrane Filter Supplies, 25 mm diameter, 0.45 mm pore size and 0.13 mm thickness, Sartorius Stedim Biotech, USA) were used as the covering diffusive gel (soaked in 1 mol $L^{-1}$ $HNO_3$ for 24 h, rinsed and stored in 0.025 mol $L^{-1}$ $NaNO_3$ at 4 °C before use). Analytical grade nitric and hydrochloric acid were purified by a subboiling distillation (DistillAcid, Berghof, DE) prior to use. All vessels (glass, DGT devices, PE, HDPE) used for experiments were prepared by acid washing (cleaned by keeping in 20% $HNO_3$ for at least 4 hours), rinsed at least 7 times with deionized water, 3 times with ultrapure water and dried before use.
The deployment solutions were prepared from a stock multi standard solution 1000 mg $L^{-1}$ (obtained from SpecSol, BR). The ionic strength was adjusted using a 1 mol $L^{-1}$ $NaNO_3$ stock solution (Synth, BR).

### 2.3. Biosorbent and characterization

The experiments were executed using shelled *M. oleifera* seeds acquired from a seedling nursery located in Narandiba/SP/Brazil. The seeds were dried in a forced air laboratory oven (Fanem, Model 320 SE, BR) at 35 – 40°C for 12 hours. Posteriorly,

SMOS were minced in a stainless-steel beater mill (IKA™ A11 Basic Mill, BR) for 30 seconds (3 times). After pulverization, the SMOS's particles were sieved and the fraction 250 µm was selected for work.

Specific surface area and pore size distribution of SMOS surface were determined by $N_2$(g) adsorption in Surface Area and Porosimetry Analyzer (ASAP 2020, Micromeritics, US) at temperature 77 K using 0.5 g of minced SMOS. The Brunauer–Emmett–Teller (BET) method was used for determination of surface area.

Scanning electron microscopy (SEM) (Hitachi TableTop TM3000, JP) (20 kV) with a low vacuum chamber (1–15 Pa) was used for characterizing the morphology and structure of the agarose-SMOS gel disks surface. The sample was not dried or covered with gold in order not to lose the gel structure.

### 2.4. Preparation of agarose–moringa seeds binding gel

Agarose (0.9 g - 3 % m/v) was dissolved in 30 mL of water (80 °C) in a hot plate and SMOS (3.0 g - 10 % m/v) were added to the solution with stirring. The mixture was then transferred to a preheated gel-casting (a homemade gel-casting support was built with two plates of glass, spaced by 0.6 mm plastic) (80 °C) and remained on the support until it reached room temperature. The gel (≈0.6 mm thick) was cut into 2.5 cm diameter disks, washed, and preserved in purified water (4 °C). After some tests involving different amounts of agarose and moringa seeds had been executed, it was determined that the ideal amount of the compound needed to make the binding gel more consistent and easier to handle are 3 % m/v of agarose and 10 % m/v of SMOS.

### 2.5. Assembly of DGT devices and elution procedure of agarose-moringa seeds gel disks

DGT samplers were assembled by placing an agarose–SMOS gel disk on a piston (25 mm diameter) with a diffusive layer of polyacrylamide and a cellulose acetate membrane to protect the device.

After deployment, the DGT devices were removed from the solution and disassembled. The agarose-moringa seeds gel disks were rinsed with water and the ligand was placed in a plastic tube containing 4 mL of HCl 2 mol $L^{-1}$ for elution.

For elution procedure, agarose-moringa seeds gel disks were removed from the solution, washed with water and placed in a plastic tube containing 4 mL of HCl 2 mol $L^{-1}$. The tubes were shaken for a period of 24 hours. After that, liquid and solid phases were separated by centrifugation for 4,000 rpm in 20 minutes. The liquid phase was then introduced into the ICP OES.

The elution factor ($f_e$) of XX was calculated according to Eq. 1:

$$f_e = m_{elu}\, m_{ret}^{-1} \qquad (1)$$

where:

$m_{elu}$ = mass eluted

$m_{ret}$ = mass retained in the ligand (agarose-SMOS).

To evaluate the elution procedure, an agarose-SMOS gel disks were placed into each solution (50 mL, duplicate) containing 500 µg $L^{-1}$ of analytes. The pH of the solutions was adjusted to 5.5 with 1 mol $L^{-1}$ HCl, and the ionic strength was adjusted to 0.025 mol $L^{-1}$ with $NaNO_3$. The solutions were maintained under constant stirring for 6 h.

### 2.6. Diffusion coefficient of metal ions

The test standard solution were used with two litres of a solution containing 500 µg $L^{-1}$ of analytes at pH 5.5, ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$ and temperature of 23 ± 1 °C, DGT devices and clamps was stabilized for 24 h (without the binding, diffusive agent and cellulose acetate membrane), under stirring, to avoid decrease of concentration of solution during deployment. After that the DGT devices were washed with ultra-pure water, assembled with the agarose-SMOS binding gel, polyacrylamide diffusive gel and cellulose acetate membrane and deployed in the solution at 4, 12, 24, 48 h, the analytes were eluted from the disks.

### 2.7. Effect of ionic strength and pH on the retention of analytes

Four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C and pH 5.5, were prepared with ionic strength values of 0.005 mol $L^{-1}$, 0.01 mol $L^{-1}$, 0.05 mol $L^{-1}$ and 0.1 mol $L^{-1}$ $NaNO_3$ was used to evaluate of effect ionic strength on the retention of analytes. To test of pH, four solutions (1 L) containing 500 µg $L^{-1}$ of analytes at 23 °C

and 0.025 mol $L^{-1}$ $NaNO_3$ ionic strength were prepared with pH values of 3.5, 5.0, 6.5 and 8.0. The devices were then retrieved from the solutions after 6 h for both tests.

### 2.8. Deployment of the DGT devices in synthetic samples and spiked river water

After the DGT devices had been assembled, they were deployed in polyethylene containers containing well stirred test standard solutions (2 L), 500 µg $L^{-1}$of analytes at pH 5.5 and ionic strength of 0.025 mol $L^{-1}$ $NaNO_3$. Deployments in the laboratory were done at constant temperature (23 °C) to avoid changing the diffusion coefficient of analytes. The solutions were stirred constantly to reduce the diffusive boundary layer (DBL).

DGT devices were deployed in spiked river water (2L, collected from the Corumbataí River – Rio Claro/SP, Brazil) for six hours to evaluate the performance of the binding gels. Sample was spiked with 20 µg $L^{-1}$ of metal ions of this study. This river was chosen for this study because it is an important agricultural area of sugar cane and also receives effluents from industries such as textile, ethanol, and chemistry. Composition of this river water is given in Table 1.

### 2.9. Field Evaluation

DGT measurements were performed *in situ* in acid mine drainage water coming from a uranium mine waste rock pile situated in Osamu Utsumi mine (Caldas/MG, Brazil) with a volume of 12.4 x $10^6$ $m^3$ covering an area 569 x $10^3$ $m^2$, where the acid drainage generated was collected at its base (BNF retention basin – point 075), with pH around 3 to 3.5 that is continuously pumped to the cava exhausted mine [39].

The deployment period varied from 4 to 6 h. Temperature, conductivity and pH were measured before and after deployment of DGT device. The DGT device was then removed from the water, thoroughly rinsed with MilliQ water, and stored in clean plastic Zip-lock bags until disassembled in the laboratory. Water samples were collected in acid-washed Falcon tubes for determination of total and dissolved metal concentrations, dissolved organic carbon and major anions. Samples for dissolved metal analysis were filtered by membranes of pore size 0.45 µm. All samples for metal analysis were preserved by acidification to pH 1-2 by addition of $HNO_3$ and

stored in the dark at room temperature. Composition of this *in situ* location is given in Table 1.

### 2.10. Modeling Speciation

Speciation modeling was undertaken using Visual MINTEQ ver. 3.0 (GUSTAFSSON, 2013). This software is based on NICA-Donnan model for the prediction of equilibrium-speciation of free metal ions, and those bound to humic and fulvic substances.

## 3. Results and Discussions

### 3.1. Measurements by Different Techniques

The major ion composition, pH, DOC content of spiked river water and acid mine drainage water used as field site are given in Table 1.

**Table 1.** Physical and chemical parameters measured *in situ* and in spiked river water.

| | 075 jul/14 | 075 dec/15 | Corumbataí river |
|---|---|---|---|
| Conductivity (μS $cm^{-1}$) | 1308 | 1375 | 94 |
| Ionic strength[a] (mol $L^{-1}$) | 0.0163 | 0.0175 | 0.0009 |
| TDS[b] (mg $L^{-1}$) | 837 | 880 | 49 |
| DOC (mg $L^{-1}$) | < 1 | < 1 | - |
| pH | 3.48 | 3.80 | 7.3 |
| T (°C) | 21.0 | 20.7 | 25.0 |
| $HCO_3^-$ (mg $L^{-1}$) | - | - | 27.2 |
| $F^-$ (mg $L^{-1}$) | 117 | 117 | 0.075 |
| $Cl^-$ (mg $L^{-1}$) | < 0.01 | < 0.01 | 4.76 |
| $NO_2^-$ (mg $L^{-1}$) | < 0.02 | < 0.02 | 0.055 |
| $NO_3^-$ (mg $L^{-1}$) | 3.14 | 2.16 | 7.45 |
| $PO_4^-$ (mg $L^{-1}$) | < 0.040 | < 0.080 | < 0.040 |
| $SO_4^{2-}$ (mg $L^{-1}$) | 917 | 873 | 1.83 |
| $Li^+$ (mg $L^{-1}$) | < 0.010 | < 0.010 | < 0.010 |
| $Na^+$ (mg $L^{-1}$) | 1.622 | 1.139 | 4.56 |
| $K^+$ (mg $L^{-1}$) | 4.72 | 6.78 | 1.59 |
| $Mg^{2+}$ (mg $L^{-1}$) | 6.37 | 5.67 | 3.30 |
| $Ca^{2+}$ (mg $L^{-1}$) | 72.63 | 73.93 | - |
| $NH_4^+$ (mg $L^{-1}$) | 3.68 | 3.90 | < 0.050 |

[a] ionic strength, values calculated according Griffin and Jurinak [41]
[b] TDS total dissolved solids, relationship between TDS and electrical conductivity proposed by APHA, AWWA and WPCF [42]

### 3.2. Characterization of biosorbent

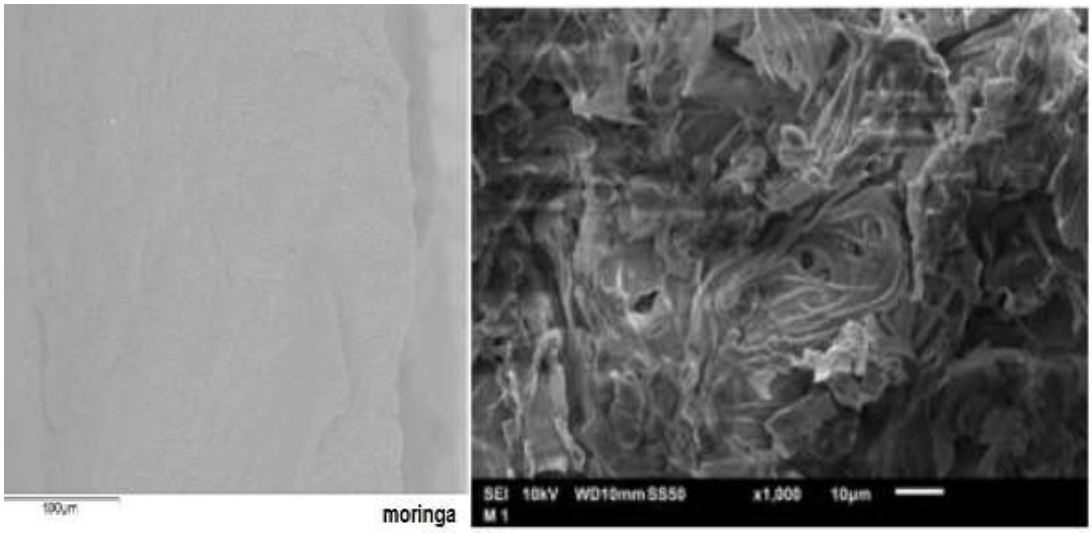


**Fig. 1** Scanning Electron Micrographs of moringa seeds gel disks surface

SEM micrograph of agarose-SMOS gel disk surface before metal biosorption is presented in Fig.1. In general, the SMOS micrograph present surface irregularity, exhibiting a greater amount of branches and pores, was making it possible for biosorption of several metal ions.

The agarose-SMOS gel disk before metal biosorption was characterized by energy dispersive X-ray spectroscopy coupled with scanning electron microscopy (SEM-EDX). Fig. 2 illustrates a typical spectrum.

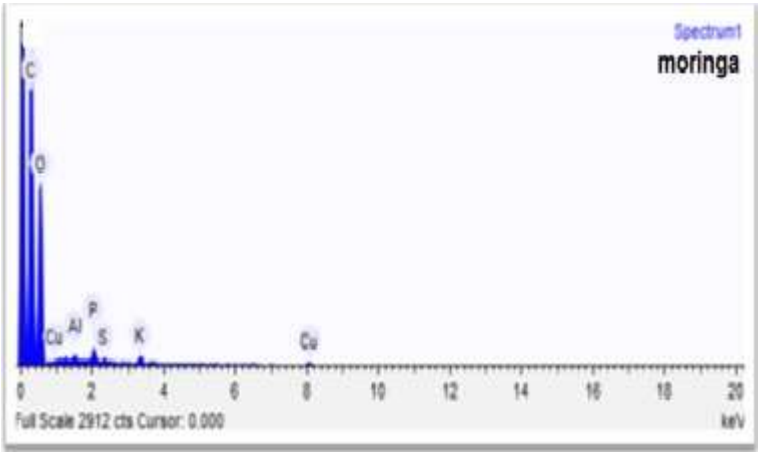


**Fig. 2** EDX spectrum of agarose-coffee gel disks surface without plating Au.

In the technique employed, the detection range were detected as major elements C (48 %), O (50 %), P (0.847 %), K (0.636 %), S (0.253 %), Al (0.250 %) and Cu (0.134 %).

Table 2 present the average results of the nitrogen adsorption experiments for BET surface area $S_P$ ($m^2$ $g^{-1}$) and pore volume $V_P$ ($cm^3$ $g^{-1}$).

**Table 2.** Physical parameters obtained by porosimetry analysis by $N_2$ adsorption for moringa seeds.

| | $S_P$ ($m^2$ $g^{-1}$) | $V_P$ ($cm^3$ $g^{-1}$) |
|---|---|---|
| Moringa seed | 0.55 ± 0.08 | 0.0015 ± 0.0003 |

The BET surface area of SMOS in present study was higher than earlier work reported by Acheampong et al. (reported value of Moringa seeds was 0.1 $m^2$ $g^{-1}$) and similar surface area to coconut and sawdust about studies of coconut materials, Moringa oleifera seed powder and sawdust [43].

**Elution efficiency of the agarose-coffee grounds gel disks**

The process of elution is an important step in the DGT measurement. In order to confirm the accuracy of the measurement, high elution efficiency is essential for the removal of metal ions from the binding phase. Table 3 shows elution efficiencies from 58 to 86 % for all metals with standard deviations between 1 and 8 %. The values of Pb and Zn are equal or higher than the established value of 80% using 1 mol $L^{-1}$ $HNO_3$ with a binding layer of Chelex [44] suggesting that elution is effective also with HCl. Mason et al. [45] also conducted their studies with the use of HCl in elution of Cd, Cu, Mn, Mo, P and Zn with elution efficiency of 92 % for all elements excluding Mo (79%). The values of Cd and Cu close to 60 % could be concentrations of such metals coming from the biomass itself.

**Table 3.** Elution efficiency of metal ions. Metal ± sd.

| | Cd | Cu | Pb | Zn |
|---|---|---|---|---|
| Elution efficiency (%) | 58 ± 6 | 69 ± 6 | 86 ± 8 | 77 ± 1 |

**Diffusion coefficient measurements using deployment curve**

The basic equation of DGT (Eq. (2)) is followed above:

$$C_b = (M\Delta g)\,(DtA)^{-1} \qquad (2)$$

where $C_b$ is the analyte concentration of bulk solution (ng $mL^{-1}$); M is the mass of analyte in each disk (ng); Δg is the ligand + diffusive gel thickness (cm); D is the diffusion coefficient of the analyte ($cm^2$ $s^{-1}$); t is the deployment time (s) and A ($cm^2$) is the exposed area of ligand.

From this equation mentioned, a linear relationship between *M* and *t* is expected for any given solution, keeping constant the other parameters. This relationship has been used as a requirement for the viability of novel DGT devices. In order to avoid high values of white that can come from own biomass, the mass of accumulated metal ion is normalized by the solution concentration of metal ion. In this study, mass of metal ion accumulated in the proposed agarose-moringa seeds gel disk is proportional to the exposure time, as shown in Fig. 3 with good precision (correlation coefficients $R^2$ = 0.985 – 0.997).

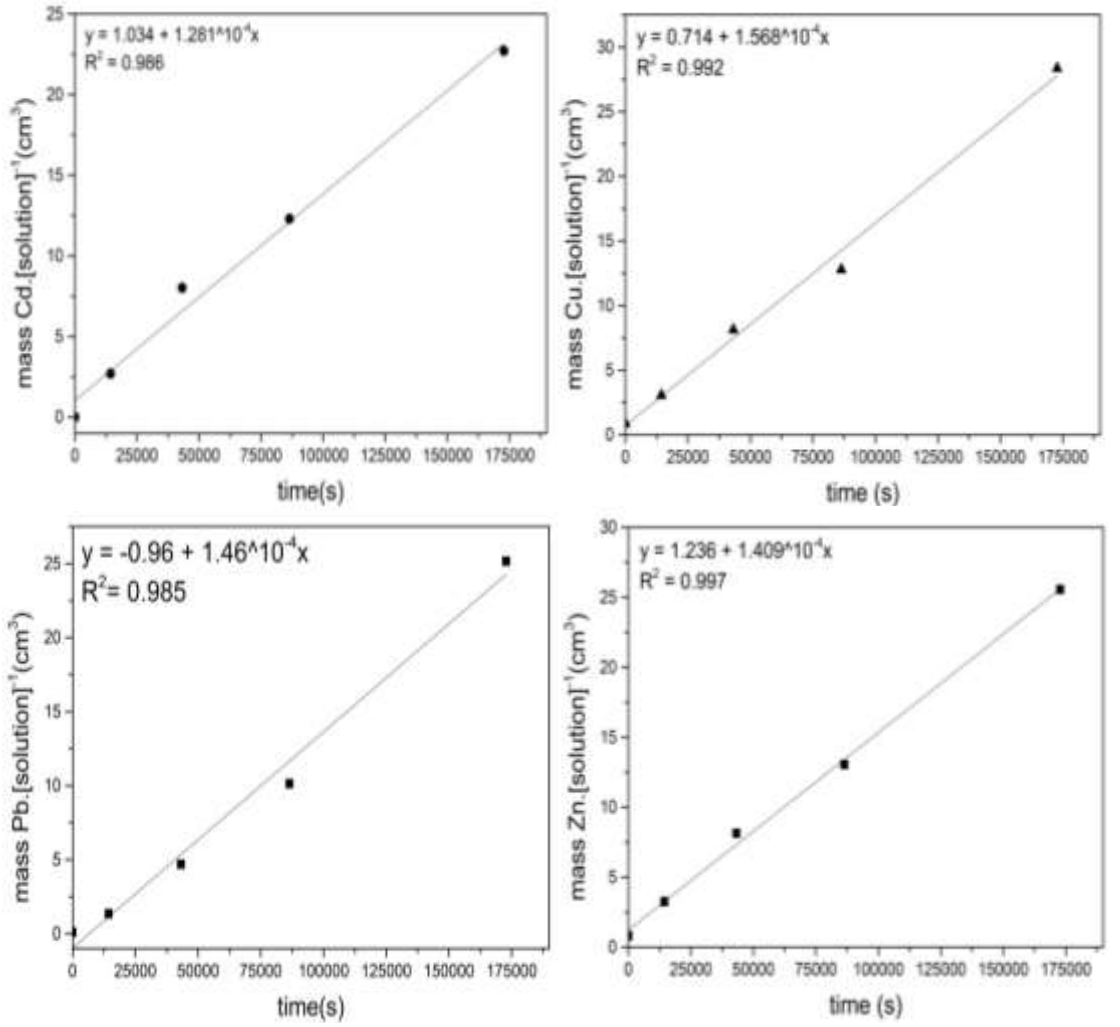


**Fig. 3** Mass of ion: Cd, Cu, Pb and Zn accumulated in the resin normalized by the ion concentration in solution for various periods of time, following parameters: ionic strength = 0.025 mol $L^{-1}$ $NaNO_3$; pH = 5.5; temperature = 23°C; Δg = 0.093 cm; A = 3.14 $cm^2$

Diffusion coefficients of metal ions measured in deployment curve are presented in Table 4. Diffusion coefficients is dependent of temperature (T) and viscosity (η) and adjustments of D are done by the Stokes-Einstein equation related by Zhang and Davison (ZHANG; DAVISON, 1999)(Eq. (3)):

$$(D_1\eta_1)/T_1 = (D_2\eta_2)/T_2 \qquad (3)$$

where $D_1$ and $D_2$ are diffusion coefficients, $\eta_1$ and $\eta_2$ are viscosities of water at absolute temperature $T_1$ and $T_2$, respectively.

**Table 4.** Diffusion coefficients ($10^{-6}$ $cm^2$ $s^{-1}$) of metal ions in polyacrylamide gel by deployment curve at pH 5.2 and temperature 23°C. Mean ± sd.

| | Found values ($x10^{-6}$ $cm^2$ $s^{-1}$) | Reference values ($x10^{-6}$ $cm^2$ $s^{-1}$) |
|---|---|---|
| Cd | 3.79 ± 0.23 | 5.21 (73%)[a] |
| Cu | 4.64 ± 0.21 | 5.90 (79 %)[b] |
| Pb | 4.32 ± 0.27 | 7.61 (57%)[b] |
| Zn | 4.17 ± 0.12 | 4.40 (95 %)[a] |

[a] Data from Garmo et al. pH 5.4 [46]

[b] Data from DGT Research [47]

The diffusion coefficients found are in reasonable agreement with previously reported values (57 – 95 %) [46,48]. It proposes that metal ions were quantitatively retained by the agarose–SMOS gel disks and that the proposed material can be used as a binding agent for the DGT technique.

**DGT method detection limit**

The DGT method detection limit (MDL) was calculated from a limit of detection (LOD) using ICP OES in conditions cited in present text and a theoretical preconcentration factor obtained for deployment time of 48 hours. The theoretical preconcentration factor was considered as the ratio of the predicted analyte concentration in the eluent to the analyte concentration in the bulk solution [44]. Thus, from these data, the values of MDL are presented in Table 5.

**Table 5.** DGT method detection limits (MDL, µg $L^{-1}$) for proposed approach

| | LD ICP OES (µg $L^{-1}$) | Preconcentration factor (48 hours) | DGT-SMOS MDL (µg $L^{-1}$) |
|---|---|---|---|
| Cd | 0.20 | 3.15 | 0.06 |
| Cu | 0.36 | 4.70 | 0.08 |
| Pb | 1.65 | 5.17 | 0.32 |
| Zn | 0.17 | 4.70 | 0.04 |

The assessed detection limits in present study are concordant with previously reported values for DGT-MBL [45,49,50] DGT-Chelex [50], DGT-PAMPAA [51]. The detection limit for Pb was just a little more higher compared to others. However, the calculated MDL is generally acceptable for these elements.

**Effect of ionic strength and pH on the retention of metal ions**

To visualize the effect of ionic strength on the uptake of metal ions by agarose-SMOS gel disks, the ratio $C_{DGT\text{-}SMOS}/C_{sol}$ is shown in Table 6. Cd, Pb and Zn concentrations determined by DGT-SMOS were concordant with bulk solution measurements in all range of ionic strength studied with recoveries between 97 % and 156 % (considering their standard deviations from 0.05 to 0.46 %, mainly to higher Cd ratios. Therefore, there is no dependence on the uptake of these ions by the DGT-SMOS on the ionic strength studied. It is consistent with the previous literature for other alternative types of DGT devices [45,52,53].
Lower Cu accumulation rate can be observed for solutions with ionic strength inferior or equal to 0.01 mol $L^{-1}$ $NaNO_3$ with recoveries of 75%. Several studies have described imprecise measurements of elements by DGT at low ionic strengths which have been attributed to modification of the diffusion of ions through the gel layer or significant residual charge in the diffusive gel [45,54–58]

**Table 6.** Effect of ionic strength on uptake of Cd, Cu, Pb and Zn by DGT-SMOS.

| Ionic strength (mol $L^{-1}$ $NaNO_3$) | $C_{DGT\text{-}SMOS}/C_{sol}$ Cd | $C_{DGT\text{-}SMOS}/C_{sol}$ Cu | $C_{DGT\text{-}SMOS}/C_{sol}$ Pb | $C_{DGT\text{-}SMOS}/C_{sol}$ Zn |
|---|---|---|---|---|
| 0.005 | 1.48 ± 0.22 | 0.78 ± 0.03 | 1.15 ± 0.07 | 1.08 ± 0.20 |
| 0.01 | 1.53 ± 0.20 | 0.76 ± 0.10 | 0.97 ± 0.05 | 1.14 ± 0.22 |
| 0.05 | 1.51 ± 0.46 | 0.94 ± 0.29 | 1.10 ± 0.43 | 1.20 ± 0.45 |
| 0.1 | 1.31 ± 0.21 | 0.86 ± 0.20 | 1.34 ± 0.37 | 1.08 ± 0.21 |

Deployment solution: 500 µg $L^{-1}$of Cd, Cu, Pb and Zn; T = 23 °C; pH = 5.5; time of deployment = 6 h; DGT devices = 3; Mean ± sd.

In the pH test, the proposed DGT devices (triplicate) were deployed in solution with a pH range between 3.5 and 8.0 containing 500 µg $L^{-1}$Cd, Cu, Pb and Zn for 6 h. Table 7 shows the retention of these ions by the agarose-SMOS gel disk.

**Table 7.** Effect of pH on uptake of Cd, Cu, Pb and Zn by DGT-SMOS.

| pH | $C_{DGT\text{-}SMOS}/C_{sol}$ Cd | $C_{DGT\text{-}SMOS}/C_{sol}$ Cu | $C_{DGT\text{-}SMOS}/C_{sol}$ Pb | $C_{DGT\text{-}SMOS}/C_{sol}$ Zn |
|---|---|---|---|---|
| 3.5 | 0.713 ± 0.005 | 0.94 ± 0.03 | 1.28 ± 0.06 | 0.67 ± 0.03 |
| 5.0 | 1.00 ± 0.15 | 1.00 ± 0.17 | 1.00 ± 0.14 | 0.99 ± 0.20 |
| 6.5 | 1.18 ± 0.04 | 1.19 ± 0.11 | 0.96 ± 0.11 | 1.09 ± 0.05 |
| 8.0 | 0.74 ± 0.07 | 0.54 ± 0.03 | 3.16 ± 0.25 | 0.54 ± 0.03 |

Deployment solution: 500 µg $L^{-1}$of Cd, Cu, Pb and Zn; T = 23 °C; ionic strength = 0.025 mol $L^{-1}$ $NaNO_3$; time of deployment = 6 h; DGT devices = 3; Mean ± sd.

The ratio between $C_{DGT}$ and $C_{sol}$ was achieved for Cu and Pb within pH range 3.5 – 6.5. At higher pH (pH = 8.0), the recoveries of Cd, Cu and Zn become lower (recovery for Cu and Zn were about 50 %) as a result of the formation of the insoluble metal-hydroxide species reported in some articles [52,59].
At very low pH (pH = 3.5), the recoveries of Cd and Zn decreased below to 75 %, probably due to reduced capacity of binding metal ions in acid solutions. Previous studies were also related which there was a reduction of the Cd (and other ions) binding capability at pH < 5 [44,59–61]. As the pH of most natural waters is in the range of 4–9, the proposed DGT-SMOS is appropriate for the application of the metals measurements in natural waters.

**Analysis of spiked river water sample**

The validation of the DGT measurement with the proposed DGT-SMOS device is also assessed by deploying the proposed devices and original devices in spiked sample of river water (20 µg $L^{-1}$, Corumbataí river). For this analysis, three devices were immersed for 6 h and temperature was maintained at 23 °C and these results were presented in Table 8.

**Table 8.** Concentrations of metal ions in spiked river water (µg $L^{-1}$) (Corumbataí river) using the proposed approach.

| | Reference[a] | Found[b] | Recovery (%)[c] |
|---|---|---|---|
| Cd | 13 ± 4 | 21 ± 2 | 161 |
| Cu | 8 ± 3 | 4 ± 2 | 50 |
| Pb | 3 ± 1 | 6 ± 2 | 200 |
| Zn | 40 ± 22 | 114 ± 83 | 137 |

[a]Direct determination by ICP OES after spike of 20 µg $L^{-1}$ of analytes.

[b]Labile concentrations using the proposed device DGT-SMOS.

[c]Recovery is defined as the labile metal concentration obtained by proposed DGT-SMOS (Found) divided by concentration obtained by direct determination (Reference).

The values obtained by the proposed approach agreed partially with the value obtained from the direct determination, showing recoveries between 50 % and 200 %. Taking into account the high standard deviation (probably due to some contamination), the agreement between the both measurements was considered more reasonable.

### ***In situ*** **analysis**

Table 9 presents the results in two periods of year (winter and summer) from analysis *in situ* with DGT samplers assembled using conventional material (Chelex-100 as the binding agent and polyacrylamide gel as the diffusive layer) and the suggested material (agarose-SMOS as the binding agent and polyacrylamide gel as the diffusive layer).

**Table 9.** Total, dissolved and labile concentrations (conventional-Chelex and proposed-SCG) (µg $L^{-1}$) compared to predictions by chemical speciation

| | | total | dissolved | labile Chelex | labile agarose-SCG | speciation vMINTEQ (%) | |
|---|---|---|---|---|---|---|---|
| jul/14 | Cd | 23 ± 1 | 20.2 ± 0.2 | 2.6 ± 2.1 | 6.1 ± 0.3 | 64.5 | $Cd^{2+}$ |
| | | | | | | 32.3 | $CdSO_4(aq)$ |
| | | | | | | 3.2 | $Cd(SO_4)_2^{2-}$ |
| | Cu | 15 ± 1 | 13.8 ± 0.1 | 5.5 ± 0.9 | < 1.3 | 67.1 | $Cu^{+2}$ |
| | | | | | | 32.9 | $CuSO_4$ (aq) |
| | Pb | 75 ± 1 | 68 ± 1 | 55 ± 3 | 55 ± 8 | 47.7 | $Pb^{2+}$ |
| | | | | | | 50.0 | $PbSO_4(aq)$ |
| | | | | | | 2.2 | $Pb(SO_4)_2^{2-}$ |
| | Zn | 10066 ± 266 | 9389 ± 85 | 3386 ± 293 | 2330 ± 58 | 66.8 | $Zn^{2+}$ |
| | | | | | | 31.2 | $ZnSO_4(aq)$ |
| | | | | | | 2.0 | $Zn(SO_4)_2^{2-}$ |
| dec/15 | Cd | 28.1 ± 0.3 | 26.3 ± 0.3 | 0.9 ± 0.1 | 2.5 ± 0.8 | 65.0 | $Cd^{2+}$ |
| | | | | | | 32.0 | $CdSO_4(aq)$ |
| | | | | | | 3.05 | $Cd(SO_4)_2^{2-}$ |
| | Cu | 8 ± 1 | 8 ± 2 | 4.9 ± 0.2 | < 1.15 | 67.5 | $Cu^{+2}$ |
| | | | | | | 32.5 | $CuSO_4$ (aq) |
| | Pb | 108 ± 8 | 103 ± 3 | 20 ± 1 | 52 ± 15 | 48.2 | $Pb^{2+}$ |
| | | | | | | 0.02 | $PbF^{+}$ |
| | | | | | | 0.01 | $PbNO_3^{+}$ |
| | | | | | | 49.6 | $PbSO_{4(}aq)$ |
| | | | | | | 2.1 | $Pb(SO_4)_2^{2-}$ |
| | Zn | 10120 ± 122 | 9559 ± 69 | 986 ± 115 | 623 ± 201 | 67.2 | $Zn^{2+}$ |
| | | | | | | 30.9 | $ZnSO_4(aq)$ |
| | | | | | | 1.9 | $Zn(SO_4)_2^{2-}$ |

For all samplings, total and dissolved concentrations of all metal ions were significant similar, indicating that these elements were mainly present in their dissolved forms. Othe work were also reported the same similarity to total and dissolved concentrations for Cd, Ni and other ions [11]. Moreover, all metal concentrations determined by DGT were in agreement with the total and dissolved concentrations.

The proposed DGT-SMOS cannot bind ions Cu in any sampling (although the conventional DGT-Chelex sampled a certain amount of labile fraction). These lower Cu DGT labile concentrations were expected because of high complexation of Cu by dissolved organic matter and suspended particles related in some researches [9,62,63]. The ratios between conventional DGT-Chelex labile and dissolved concentrations ([DGT]/[dissolved] in percentage) were 3 - 13 % for Cd, 40 - 61 % for Cu, 19 - 81 % for Pb and 10 - 36 % for Zn. And the proposed DGT-SMOS labile sampled 9 - 30% of Cd, 50 – 81 % of Pb and 7 – 25 % of Zn dissolved concentrations measured at the field sites.

Concentrations of dissolved metals, collected over the course of the DGT

deployments, were considerably greater than the DGT-labile concentrations measured by conventional DGT-Chelex and proposed DGT-SMOS for all metal ions. This statement has also been described in some articles discussed below. In studies of Liu, Lead and Zhang have demonstrated that the proportions of DGT labile metal species were lower than those of ultrafiltered fraction for Cd, Cu, Ni, Pb, Zn and other ions [64] and Shiva et al. [49] about studies of binding layers made of Chelex, PAMPAA (polyacrylamide- polyacrylic acid), Metsorb and a mixed binding layer (MBL, containing both Chelex and Metsorb) with DGT labile concentrations representing 24 – 41 % of Cd, 17 – 24% of Cu, 27 – 41 % of Ni, 17 – 37% of Pb and 23 – 31 % of Zn filterable concentrations.

For Cd and Zn, the DGT-labile concentrations measured by conventional DGT-Chelex and proposed DGT-SMOS are relatively limited. The results obtained using both DGT devices were considerably lower compared than other studies where almost all dissolved fraction of Cd is considerate labile [65,66] since Cd(II) is usually weakly bound to humic substances in aquatic system. The difference between DGT-labile concentrations and dissolved concentration was also related by Odzak et al. [67] which attributed this difference by incomplete exchange of very strong complexes, binding a large fraction of metal ions (Cu and Ni in their work)

A recovery of Pb by DGT-SMOS was equal or higher than conventional DGT-Chelex. These results indicate that conventional DGT-Chelex and proposed DGT-SMOS measured similar ranges of metal species. Panther et al. and Shiva et al. were reported a similar correspondence between measurements by DGT-Chelex and DGT-MBL in synthetic seawater [49,50].

There was no significant difference between conventional DGT and DGT-SMOS for the measurement of ions studied in present work, demonstrating that the measurements of Cd, Pb and Zn with DGT-Chelex and DGT-SMOS were equivalent. Therefore, the proposed DGT-SMOS was suitable for the selective measurement of these ions in water samples.

Only the speciation of Pb was concordant with the predictions of chemical modelling speciation vMINTEQ. The same concordance was indicated in work of Balistrieri and Blank [68] where predicted dynamic Pb concentrations using NICA-Donnan and WHAM VI were similar and lower than measured labile concentrations. In general, comparison of chemical speciation modelling with DGT results are not simple because these predictions take into account equilibrium systems, which does not

happen in real samples [63,69].

## 4. Conclusions

For the first time, biomass of moringa seeds as employed as a binding layer for DGT technique for effective retention of Cd, Pb and Zn. The proposed DGT-SMOS can be successfully applied *in situ* for Cd, Pb and Zn measurements in acid mine drainage water (with recoveries of up to 80 % for Pb, as example) and synthetic solutions with pH range from 3.5 to 8.0 and ionic strength from 0.005 to 0.1 mol $L^{-1}NaNO_3$. Laboratory tests performed in both natural and synthetic freshwater showed quantitative recoveries. Overall, the DGT technique has been establish to be effective for chemical speciation of trace metals *in situ* in acid mine drainage water of uranium mine of the Caldas/MG, Brazil. The chemical speciation vMINTEQ was presented reasonable predictions; however, more studies are necessary on effect of kinetics on uptake of metal ions in complexes matrices.

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## 6. CONSIDERAÇÕES FINAIS

Por meio da revisão da literatura foi ressaltado que não há nenhum agente ligante para a técnica DGT, feito a partir de biomassas e estudos sobre o emprego desta técnica em áreas de drenagem ácida com pH em torno de 3,0 estão começando a ser relatados. O presente trabalho apresentou certa viabilidade do emprego destas biomassas como agente ligante em águas de drenagem ácida, com melhores resultados para os agentes ligantes feitos de borra de café. Em amostras de água naturais (Rio Corumbataí e Rio Piracicaba) os agentes ligantes pesquisados apresentaram um desempenho superior frente as estudos feitos em águas de drenagem ácida, indicando que estes agentes ligantes também podem ser empregados em matrizes de água diferentes daquelas características de drenagem ácida.

A técnica DGT mede a fração de metal lábil que de acordo com diversos estudos, vem ser uma melhor medida da quantidade de metal biodisponível do que os estudos determinando somente a concentração total ou dissolvida (preconizada pela Resolução CONAMA, por exemplo) e determina as concentrações de metais integradas no tempo durante longos períodos de implementação (até semanas / meses). Os resultados deste estudo mostram que esta técnica pode ser uma alternativa viável em detrimento das técnicas convencionais para monitoramento da DAM

Uma investigação mais detalhada da dependência do pH na adsorção dos metais pela técnica DGT, bem como estudos de desempenho desta técnica em diferentes matrizes reais, são necessárias para posteriores e futuras aplicações em DAM.

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