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IAN G. DROPPO, STEVEN N. LISS, DECLAN WILLIAMS & GARY G. LEPPARD

Abstract

The transport and fate of pathogenic pollutants are shown to be highly influenced by their strong relationship with suspended and bed sediment particles. As such, pathogen dynamics are strongly linked to the sediment dynamics of a system. Extracellular polymeric substances, although insignificant in terms of organic mass, are shown to be an integral part of the floc and contribute to the retention of microorganisms in the environment and may play an important role in the binding of pathogens. Implications of sediment pathogen interactions on policy development for safe water practices are discussed.

NWRI RESEARCH SUMMARY

Plain language title

Pathogens interact with sediment in aquatic environments which influence their transport, delivery and possible impacts on the aquatic environment and human health.

What is the problem and what do scientists already know about it?

Pathogens (e.g. *E. coli*) are known to affect human health detrimentally. The sources of these pathogens are generally well known (e.g. agriculture and wastewater), however, there is a lack of understanding of their transport and delivery mechanisms within natural aquatic systems. Conventional perspectives most often place pathogens within the environment as transient and freely suspended entities that are not necessarily involved in ecological interactions. Standard microbial tests only evaluate whole water samples and do not view the sediment (suspended and bed sediments) as a separate compartment from the water for pathogen propagation and or storage within the aquatic environment.

Why did NWRI do this study?

National agri-environmental standards are being developed for waterborne pathogens in agricultural watersheds across Canada as part of the National Agri-Environmental Standards Initiative. This study was done to examine the role that sediments play in the mobilization, transport and delivery of pathogens to environmentally sensitive areas and to evaluate policy shortcomings in this regard.

What were the results?

Pathogens are shown to be highly associated with suspended and bed sediment and, as such, the structure of these sediments (flocs) will dictate the erosion, transport and delivery of the associated pathogens. Pathogens settled to the bed of a river or lake can survive for extend periods of time, and if eroded can represent a significant source/reservoir of pathogenic pollutants to downstream water bodies and communities with potential detrimental effects.

How will these results be used?

These results will contribute to the development of standards for waterborne pathogens in agricultural watersheds.

Who were our main partners in the study?

Ryerson University

Interactions sédiments des rivières/agents pathogènes : Importance de l'élaboration d'une politique visant à assurer la salubrité de l'eau

IAN G. DROPPO, STEVEN N. LISS, DECLAN WILLIAMS et GARY G. LEPPARD

Résumé

Il est démontré que le transport et le devenir des agents pathogènes sont fortement influencés par leur lien étroit avec les sédiments en suspension et les sédiments de fond des milieux aquatiques. Ainsi, la dynamique des agents pathogènes est fortement liée à la dynamique des sédiments d'un système. Les substances polymériques extracellulaires, bien qu'elles représentent une masse organique négligeable, s'avèrent faire partie intégrante du floc. Elles contribuent à la rétention des micro-organismes dans l'environnement et peuvent jouer un rôle important dans la liaison des agents pathogènes. L'incidence des interactions des agents pathogènes avec les sédiments sur l'élaboration d'une politique visant à assurer la salubrité de l'eau sont discutées.

Sommaire des recherches de l'INRE

Titre en langage clair

Les agents pathogènes interagissent avec les sédiments des milieux aquatiques. Cette interaction a une incidence sur le transport, la libération et les répercussions possibles de ces derniers avec le milieu aquatique et la santé humaine.

Quel est le problème et que savent les chercheurs à ce sujet?

Il est démontré que les agents pathogènes (p. ex. *E. coli*) ont des effets adverses sur la santé humaine. Les sources de ces agents pathogènes sont généralement bien connues (p. ex. agriculture et eaux usées), mais les mécanismes de transport et de libération de ces derniers sont mal connus dans les réseaux aquatiques naturels. Les agents pathogènes dans l'environnement sont généralement considérés comme des entités en suspension transitoires et libres, qui ne jouent pas nécessairement un rôle dans les interactions écologiques. Les tests microbiens normalisés permettent d'évaluer seulement les échantillons d'eau non filtrée et ne prennent pas en compte les sédiments (sédiments en suspension et de fond) comme un élément séparé de l'eau en ce qui concerne la propagation et/ou le stockage des agents pathogènes dans le milieu aquatique.

Pourquoi l'INRE a-t-il effectué cette étude?

Les normes agroenvironnementales nationales relatives aux agents pathogènes d'origine hydrique présents dans les bassins hydrographiques agricoles au Canada sont élaborées dans le cadre de l'Initiative sur les normes agroenvironnementales nationales. La présente étude visait à examiner le rôle des sédiments dans la mobilisation, le transport et la libération des agents pathogènes dans des zones écologiquement sensibles et à évaluer les lacunes sur le plan des politiques à cet égard.

Quels sont les résultats?

Il est démontré que les agents pathogènes sont fortement associés aux sédiments en suspension et aux sédiments de fond; par conséquent, la structure de ces sédiments (flocs) a une incidence sur l'érosion, le transport et la libération des agents pathogènes associés. Les agents pathogènes déposés au fond des lacs ou des rivières peuvent survivre pendant une longue période et, si celui-ci est érodé, il peut représenter une source/un réservoir important d'agents pathogènes susceptibles d'avoir des effets néfastes sur la qualité des plans d'eau et sur la santé des collectivités situés en aval.

Comment ces résultats seront-ils utilisés?

Ces résultats serviront à l'élaboration de normes concernant les agents pathogènes d'origine hydrique dans les bassins hydrographiques agricoles.

Quels étaient nos principaux partenaires dans cette étude?

Ryerson University

River sediment/pathogen interactions: Importance for policy development on safe water practices

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Abstract The transport and fate of pathogenic pollutants are shown to be highly influenced by their strong relationship with suspended and bed sediment particles. As such, pathogen dynamics are strongly linked to the sediment dynamics of a system. Extracellular polymeric substances, although insignificant in terms of organic mass, are shown to be an integral part of the floc and contribute to the retention of microorganisms in the environment and may play an important role in the binding of pathogens. Implications of sediment pathogen interactions on policy development for safe water practices are discussed.

Key Words bed sediment; floc; deposition; EPS; erosion; pathogens; policy implications; suspended sediment; transport

Introduction

Pathogenic organisms can represent a significant health risk if exposure is above an infectious dose. Drinking water warnings and beach closures are consistently in the news and are reactive measures to pathogenic problems. Standard microbial tests only evaluate whole water samples and do not view the sediment (suspended and bed sediments) as a separate

compartment from the water for pathogen propagation and or storage within the aquatic environment. While it is recognized that pathogens are associated with sediments in the aquatic environment (Jamieson *et al.*, 2004), our understanding of the implications of this relationship to public health is poor. This paper examines the role that sediment (floc) structure plays on the deposition, erosion, transport and fate of pathogens within fluvial systems and examines the implications of this on policy development for safe water practices.

Methods

Samples of suspended sediment and bed sediment were collected from the South Nation River near Ottawa, Ontario, Canada. The river is very slow moving and is primarily a depositional environment until velocities increase and bottom scour can occur. The river transports primarily fine-grained cohesive sediment with a d_{50} of 10 μm. Further details on the South Nation River can be found in Chapman and Putnam (1966). Suspended sediment was collected using a continuous flow centrifuge, while bed sediment was collected using a Ponar bed sediment sampler.

All sediment structural features (i.e. size, shape, density and porosity) were measured following the methods of Droppo *et al.* (1997). These methods are based on an optical image analysis approach and the readers are referred to this publication for more information. When required, ultrastructural features were examined by transmission electron microscopy according to Liss *et al.* (1996).

Confocal laser scanning microscopy (CLSM) was employed to view the association of bacteria within the flocculated material. Molecular probes were used to visualize Eubacteria and *E. coli*. The probe EUB338I, labelled with the fluorophore BODIPY 493/510 (Sigma Genosys, The Woodlands, TX, USA) was used for the detection of Eubacteria. For the detection of *E. coli*, the probe ECOII labelled with CY3 (Sigma Genosys, The

Woodlands, TX, USA) was used. The DNA sequences of these probes were obtained from probeBase (Loy *et al.*, 2003). Hybridization of the probes to the target bacteria was performed using the method of Amann et al. (1990). Fluorescence-labelled lectins were used to visualize carbohydrates associated with the sediment particles. Sediment samples were treated with tetramethylrhodamine-labelled wheat germ agglutinin, Alexafluor 633-labelled concanavalin A, and Alexafluor 488-labelled soy bean agglutinin. These lectins bind N-acetylglucoseamine, α-D-glucose and α-D-mannose residues, and α-β-N-acetylgalactoseamine and galactopyranosyl residues respectively. All probes from Molecular Probes, Eugene, OR, USA). More information on the CLSM methods can be found in Droppo *et al.* (1997).

Sediment samples were diluted in pH buffered water and plated onto bismuth sulphite and MacConkey agars (Becton, Dickinson and Company, Sparks, MD, USA). MacConkey agar plates were incubated at 37°C overnight. The number of lactose-fermenting colonies, those appearing pink, was counted and three representative colonies were selected from each plate. These colonies were used to inoculate tubes of EC medium containing 4-methylumbelliferyl-β-D-glucuronide (MUG) (Becton, Dickinson and Company, Sparks, MD, USA) to confirm the presence of *E. coli*. Replicate sets of tubes were incubated at 37 °C and 44 °C for 24 hours, and examined for gas production and fluorescence under UV light using a LKB 2011 Macrovue transilluminator (LKB, Bromma, Sweeden). Colonies that induced fluorescence in this medium were presumptively identified as *E. coli*.

Bismuth sulfite plates were incubated at 37°C for up to 48 hours to allow all strains of *Salmonella* to grow. Colonies were counted and three representative colonies from each plate were streaked onto Oxoid *Salmonella* Chromogenic Agar (Oxoid, Nepean, ON, Canada) and incubated at 37 °C for 18 hours. Purple colonies were presumptively identified as *Salmonella*.

These culture experiments were used to assess presence or absence of *E. coli* and *Salmonella* and their general significance within the sediment populations relative to water. Actual counts of these pathogens are being determined within accredited labs for the National Agri-Environmental Standards Initiative and are not available for this publication.

Extracellular polymeric substances (EPS) were extracted from sediment samples using the cation exchange method (Frolund *et al.* 1996) and the EPS constituents quantified. Carbohydrates were measured using the anthrone method (Gaudy, 1962). Acid polysaccharides, measured as uronic acid residue, were measured using the *m*-hydoxydiphenyl sulphuric acid method (Filisetti-Cozzi & Carpita, 1991). Protein and humic acids were measured using the Lowry method (Lowry *et al.* 1951). A Spectronic 20 (Thermo Electron Corporation, Madison WI, USA) was used to measure absorbance of the samples for all of these colorimetric methods. DNA was quantified using a BioRad Fluorescent DNA Quantitation Kit (BioRad Laboratories, Hercules, CA, USA) and a Shimadzu RF-Mini 150 Recording Fluorometer (Shimadzu, Columbia, MD, USA).

Results and Discussion

While preliminary, our results demonstrate that pathogens are strongly associated with sediment particles. This strong sediment/pathogen association is related to the beneficial environment that the sediment provides in terms of a nutrient/food source (DOC) and protection from environmental stress (Gerba & Mcleod, 1976). Our results show that *E. coli* and *Salmonella* are particularly elevated within the suspended sediment relative to the bed sediment and water. These observations are consistent with other studies (e.g. Obiri-Danso & Jones, 2000; Schendel *et al.*, 2004). Crabill *et al.* (1999) and Obiri-Danso & Jones (2000) observed that counts within bed sediments can be orders of magnitude higher than for overlying water, with pathogen survival rates well above those in the water column. Jamieson

et al. (2005) found that enteric bacteria (*E.coli*) could survive for up to six weeks within bed sediment. As such, bed and suspended sediment can represent significant reservoirs of infectious pathogens with concomitant downstream detrimental effects for beneficial water use.

Fig. 1a illustrates this strong association of bacteria (species unknown) within the porous extracellular and clay matrix of a riverine suspended floc. Such consortia are typical of all cohesive sediment dominated aquatic environments and have been demonstrated to be strong and effective transporters/harbourers of bacterial pollution within natural aquatic environments (Droppo, 2004). Using CLSM combined with fluorescent in situ hybridization, *E. coli* were found to be present in association with sediment particles of the South Nation River. The association of pathogens with suspended sediments changes their hydrodynamic properties by increasing their downward flux. Individually suspended pathogens, because of their small size and low density, will generally remain in suspension until they become associated with a solid substrate (floc or biofilm). As such, the suspended sediment provides a sink for pathogens within a system. Given the flow dynamics of rivers, however, deposited pathogens may be eroded and transported further down stream in association with sediment when the critical bed shear stress for erosion is surpassed. Such erosion events can represent an important and, as of yet, unconsidered source of pathogens to downstream waters for water quality models and effective management decisions.

Fig. 1b illustrates the typical structure of the flocs from the South Nation River, while Fig. 2 provides typical distributions for the suspended sediment of the South Nation River. The river's effective floc size was relatively small ranging from 11 to 63 μm (d_{50}), while sonicated distributions range from 5 to 13 μm. While sonication does not provide the absolute primary particle distribution, it is evident that the sediment is composed of floc material. This small size is likely related to the very slow moving water of this dammed river (<10cm s^{-1})

limiting particle interactions. Regardless of their small size, multiple bed samples at 18 cross sections over a 10 km reach of the river confirms significant deposition. The determination of the settling velocity of these particles was not possible due to their small size (below resolution of the analytical technique) and because suspended solid concentrations were very low. As such, the surficial fine grain laminae (SFGL) recently formed on the river bed was sampled by gently resuspending this fluff layer and sampling it within a 4 l container. This resulted in a much larger floc size distribution, but one which may be more representative of particles eroded from the bed during a storm event. It has been documented that this SFGL contains the majority of pathogens with a decrease in counts with depth (Obiri-Danso & Jones, 2000). Fig. 3 illustrates an example settling velocity, density and porosity distribution of this eroded sediment. Mean settling velocity from 30 samples ranged from 1 to 5.3 mm s^{-1}. These rates fall within those of other aquatic environments (Droppo, 2004) and suggest that transient deposition and erosion sequences are occurring. The eroded flocs were also found to generally have porosities above 70% and densities below 1.2 g cm^{-3} (Fig. 3b) suggesting that the floc matrix is relatively open with high water content (see also Fig. 1a). This open matrix along with biostabilization may explain the very large size of the eroded flocs.

Using a 13 year average discharge of 29.4 m^3 s^{-1} and the average suspended solid concentration for the period of sampling 07/05 – 10/05 of 26.8 mg l^{-1} it was estimated that the average daily solid load for the period of sampling was 68 tonnes d^{-1}. Of this 6.6 tonnes d^{-1} was organic matter with only 0.02, 0.01 and 0.01 tonnes d^{-1} contributed by EPS and the dominant constituents humic acids, acid polysaccharides and proteins respectively (note that the DNA component of the EPS was insignificant and that measures of total polysaccharides indicates that this fraction were largely acidic polysaccharides). While insignificant in terms of mass of solids transported, these EPS colloidal particles are a primary mechanism of attachment for pathogens to sediment particles and influence floc building and sediment

stabilization (Droppo, 2001). This is primarily related to their enormous surface area and general sticky nature (Liss *et al.*, 1996). Bacteria may produce different quantities and components of EPS depending on species and on local environmental stresses (Wingender *et al.*, 1999). Using lectin stains, Fig. 4 illustrates that there are multiple congeners of the EPS polysaccharide associated with the sediment particles. Fig. 4e provides a composite image of all three stains showing the complex configuration of the EPS and the fact that these polymers are integral to the floc matrix as further illustrated in Fig. 1a.

Interestingly within a sub watershed of the river (Little Castor Ck), EPS components were found to increase down river for 3 of 3 bed samples suggesting a possible increase in EPS production and possible pathogen attachment through the watershed. This is partially substantiated by 5 out of 8 downstream bed samples exhibiting higher *E. coli* and *Salmonella* counts than the upstream site. These differences may also reflect a change in pathogen source down stream.

Policy Implications

While there are a number of best management practices which are designed to minimize or eliminate the impact of pathogen source areas on the aquatic environment (e.g. buffer strips, manure containment areas, and various municipal and agricultural treatment systems, among others), the issue of pathogen pollution is still of significant concern for drinking water and recreational water management. Standard microbial tests for indicator microorganisms do not allow for the understanding of the mechanisms that control their transport, storage and mobilization within aquatic environments. These tests only evaluate whole water samples and do not view the sediment (suspended and bed sediments) as a separate compartment from the water for pathogen propagation and or storage. Conventional perspectives most often place pathogens within the environment as transient and freely suspended entities that are not

necessarily involved in ecological interactions. There is increasing evidence of pathogen interactions with microbial aggregates/sediments in natural environments which increase their retention and survival (Kantani *et al.*, 2003). Given the strong association of pathogens with sediments, the delivery of pathogens will be mediated by the structure and transport dynamics of the sediment particles. As such, it is critical that microbial tests designed to evaluate source, fate and effect of pathogen pollution, consider the sediment (bed and suspended) as a source and vector for pathogen erosion, transport and delivery within aquatic environments. By improving our knowledge of sediment pathogen interactions we will be better able to protect and predict microbial threats to our water resources.

Conclusions

Pathogens have been shown to be highly associated with suspended sediment and, as such, the structure of these sediments (flocs) will dictate the transport and delivery of the associated pathogens. Pathogens settled to the bed of a river or lake can survive for extend periods of time, and if eroded can represent a significant source/reservoir of pathogenic pollutants to downstream water bodies and communities with potential detrimental effects. The flocs examined in the South Nation River were small but still possessed a significant interaction of pathogens in association with EPS. All components of EPS were found to be present in both the bed and suspended sediment, although the DNA content was low. Acid polysaccharides (measured as uronic acid residues), protein and humic acid were the dominant EPS components. While a minimal component of the total organic content of the sediment, these colloidal particles are critical in the development and settling behaviour of the flocs and their associated pathogens. As the sediment can represent a significant reservoir of pathogens, water quality sampling and models need to incorporate sediment-pathogen interactions for more accurate assessments.

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Figure Captions

Fig. 1 (a) Transmission electron micrograph of a floc exhibiting bacteria (species unknown) with EPS binding bioorganic and inorganic (clays) components of the floc. (b) Conventional optical micrograph of flocculated material.

Fig. 2 Representative (a) floc and (b) sonicated grain size distributions by number and volume. Labels at top of bars represent the number of particles in each size class.

Fig. 3 Representative (a) settling velocity distribution of resuspended SFGL flocs and (b) density and porosity distribution of the same flocs.

Fig. 4 (a) phase contrast micrograph of a bed sediment sample. (b) - (e) CLSM images following staining with lectin-conjugates. (b) Con A, (c) WGA, (d) SBA, (e) composite of all three CLSM images. All images acquired using 63x objective.

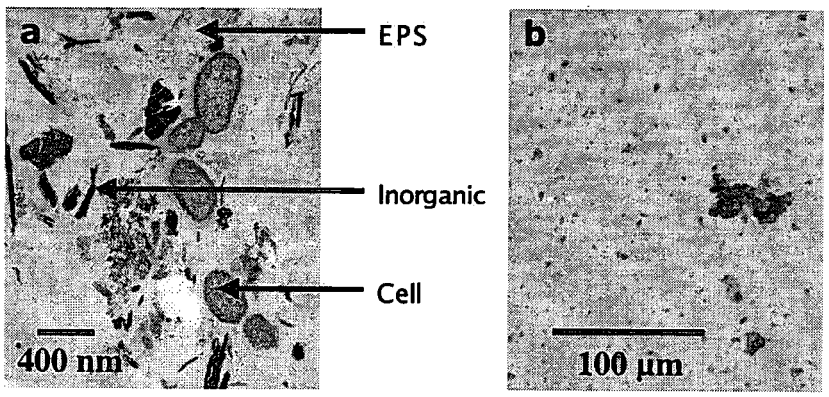

Fig. 1

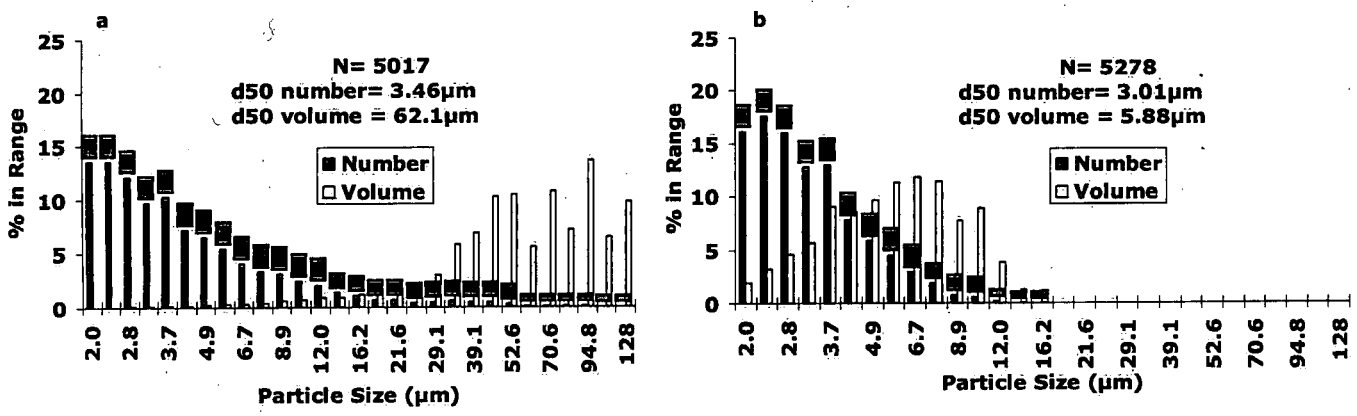

Fig. 2

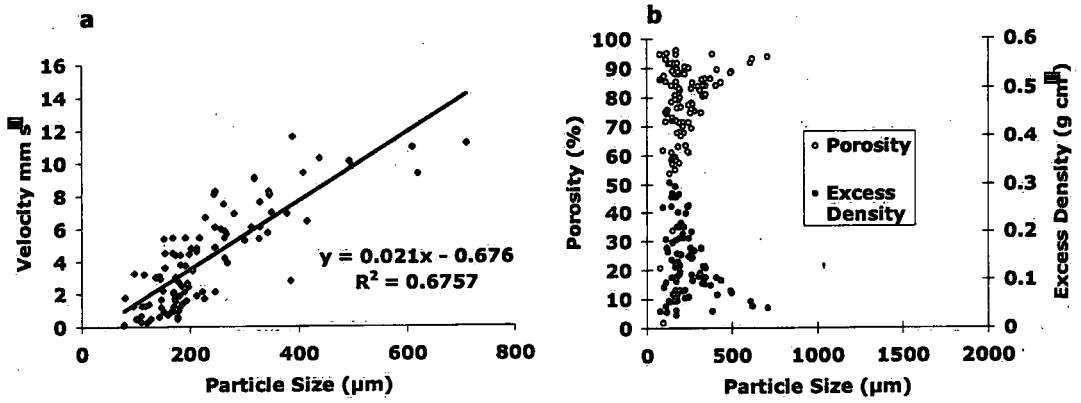

Fig. 3

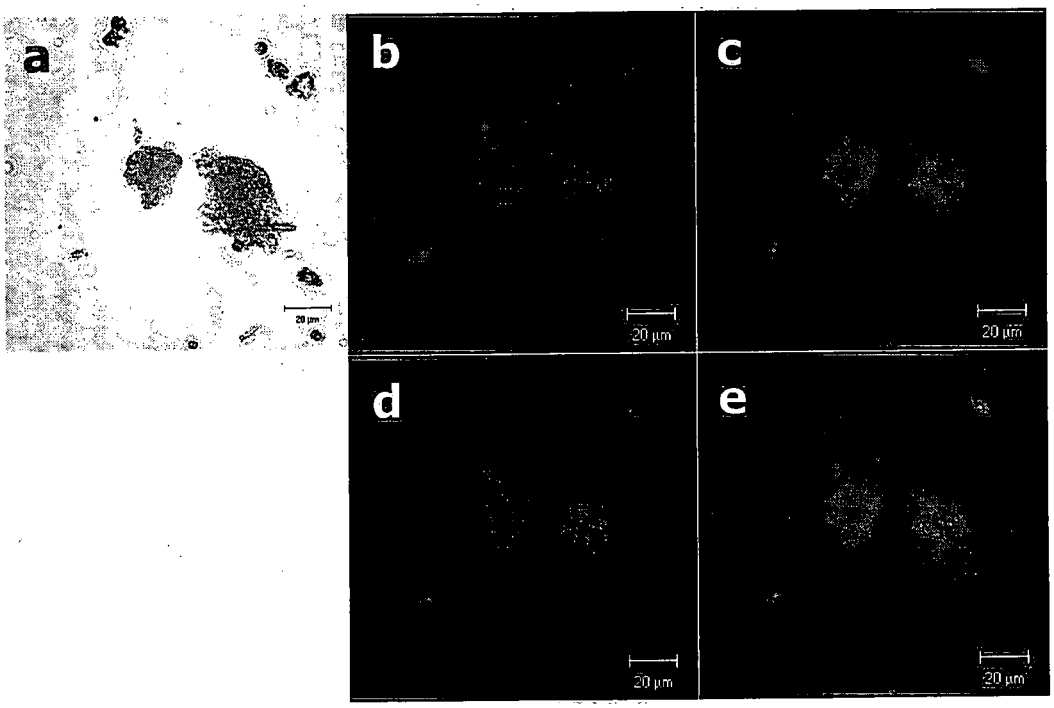

Fig. 4

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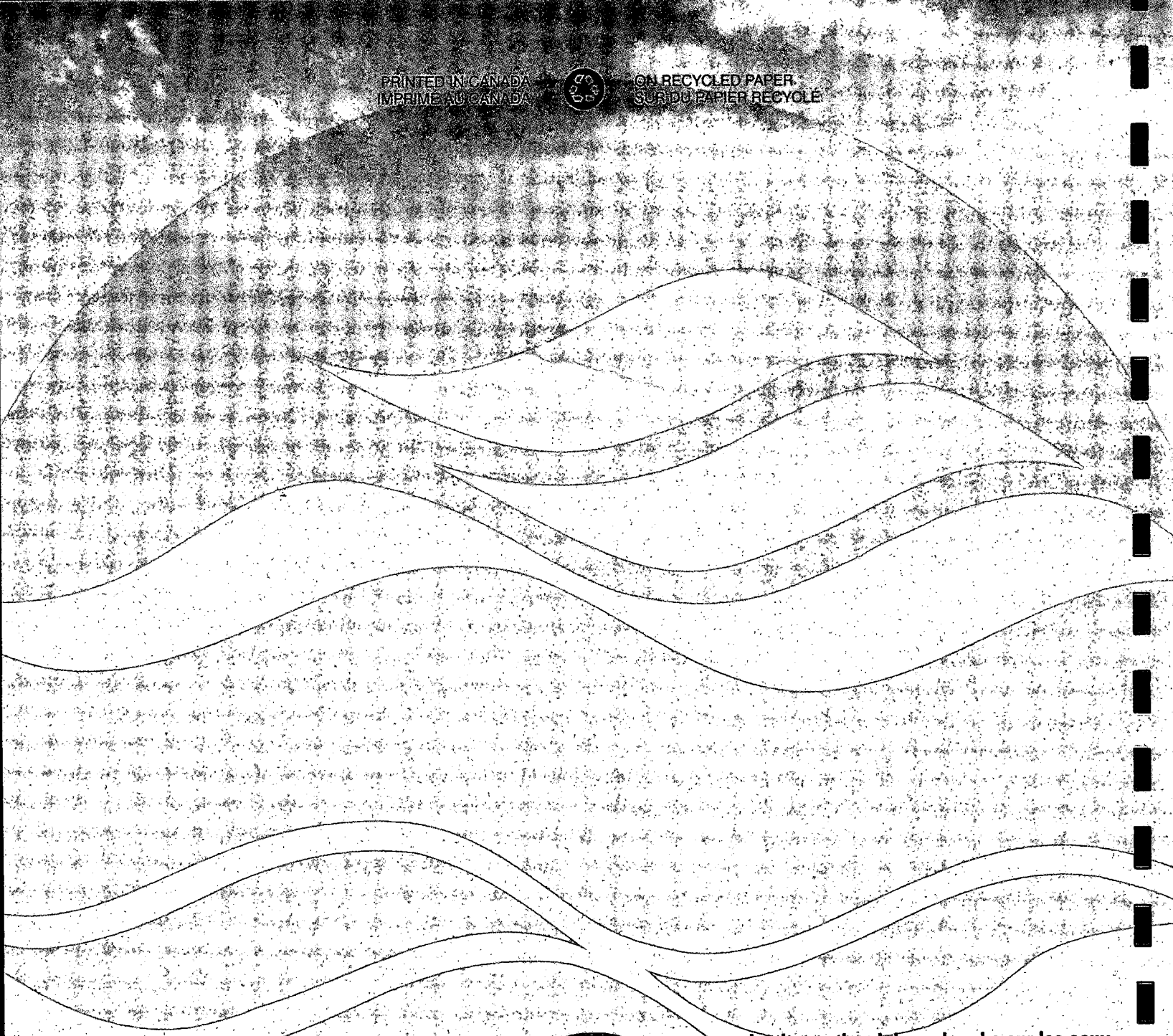

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