# Improving the assessment of polluted sites using an integrated bio-physico-chemical monitoring framework

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Anuradha Garg[a], Brijesh K Yadav[a,*], Diganta B Das[b,*], Paul J Wood[c,]

[a] *Department of Hydrology, Indian Institute of Technology Roorkee, Uttarakhand, India*

[b] *Department of Chemical Engineering,* Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK

[c] *Geography and Environment, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK*

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* *Corresponding authors (Emails: brijesh.yadav@hy.iitr.ac.in; d.b.das@lboro.ac.uk)*

*Abstract*

*Soil – water pollution resulting from anthropogenic activities is a growing concern internationally. Effective monitoring techniques play a crucial role in the detection, prevention, and remediation of polluted sites. Current pollution monitoring practices in many geographical locations are primarily based on physico–chemical assessments which do not always reflect the potential toxicity of contaminant 'cocktails' and harmful chemicals not screened for routinely. Biomonitoring provides a range of sensitive techniques to characterise the eco–toxicological effects of chemical contamination. The bioavailability of contaminants, in addition to their effects on organisms at the molecular, cellular, individual, and community level allows the characterisation of the overall health status of polluted sites and ecosystems. Quantifying bioaccumulation, changes to community structure, faunal morphology, behavioural, and biochemical responses are standard procedures employed in biomonitoring studies in many High-Income Countries (HICs). This review highlights the need to integrate biomonitoring tools alongside physico–chemical monitoring techniques by using 'effect–based' tools to provide more holistic information on the ecological impairment of soil-water systems. This paper considers the wider implementation of biomonitoring methods in Low to Middle Income Countries (LMICs) and their significance in pollution investigations and proposes an integrated monitoring framework that can identify toxicity drivers by utilising 'effect–based' and 'risk–based' monitoring approaches.*

**Keywords:** Biomonitoring, organisms, contaminant, polluted sites, integrated monitoring

## 1. Introduction

The quality of soil and water resources has declined significantly during the post-industrial period due to pollution from agricultural, urban, and industrial sources (Delmail, 2014; Chandrappa and Das, 2021). The soil and water resources of polluted sites may contain highly elevated concentrations of multiple contaminants (e.g., pesticides, petroleum based compounds, dyes, toxic organics, suspended solids and heavy metals) compared to the natural surrounding environment (Mohammadzadeh Pakdel and Peighambardoust, 2018; Ali *et al.*, 2020). The unregulated and uncontrolled discharge of waste into the environment in many regions of the world is largely responsible for the high levels of freshwater and soil-water contamination (Debén et al. 2017; García-Seoane et al. 2018; Barnett-Itzhaki et al. 2018; Mangadze, Dalu, and William Froneman 2019; Ighalo and Adeniyi 2020). Increasing contamination levels disturb and disrupt the ecological functioning of polluted ecosystems. For example, elevated nutrient availability and production in the soil/water environment with resulting effects on inter- and intra-specific interactions among soil microbial communities and freshwater organisms (Beniah Obinna and Ebere, 2019). Depending on the concentration and bioavailability of pollutants, this may result in adverse effects on human health observed via irritation and allergic reactions upon contact, through to chronic diseases or organ failure as a result of long term exposure and, in extreme instances death (Martin and Griswold, 2009; Beniah Obinna and Ebere, 2019). These human health outcomes may occur more frequently and present significant greater challenges within low-income countries due to their limited ability to reduce pollution exposure and limited access to public health facilities for the majority of the population (Brainerd and Menon, 2015; Lavaine, 2015; Wang and Yang, 2016a). In addition, the contamination diminishes the aesthetic quality of the environment through its impact on the odour, colour and, transparency of water as a result of its contact, transport, and deposition of debris, tar, plastic, and other waste (WHO, 2003; T. Zhang *et al.*, 2020). Consequently, effective monitoring represents significant challenges to the management of polluted sites (Behmel *et al.*, 2016; Ali *et al.*, 2020). Effective monitoring requires resource managers to characterise the environmental status and baseline conditions for determining future management, remediation, and restoration activities. Soil-water quality monitoring is undertaken drawing on both long-term records (where available) and the application of standardized measurements to define the quality / health status and temporal dynamics of the site (Bartram and Ballance 1996; Behmel *et al.*, 2016; Bo et al. 2017; Chandrappa and Das, 2021). The aim of these activities is to develop a standardized long-term monitoring strategy that is spatially

81  distributed and that is able to assess the effects of ongoing / current activities that influence the current
82  soil-water quality status (Bartram and Ballance 1996; Bo et al. 2017).

83      The assessment of polluted sites includes analysing the quality status of land (including soil) and
84  water resources. Water quality testing is the most widely undertaken approach, although soil monitoring
85  is rarely undertaken in association with this due to financial constraints (especially in low-to-middle
86  income countries), and to avoid potential duplication of effort (Duarte *et al.*, 2018; Huang *et al.*, 2019).
87  Water quality monitoring (WQM) programmes are typically designed to provide site specific, relevant,
88  precise, and reliable information regarding the status of a site over space and time. A common challenge
89  in soil and water monitoring programmes is the poor spatial coverage which frequently results in the
90  extrapolation of results  (Bartram and Ballance 1996; Harmancioglu et al. 1999; Behmel *et al.*, 2016).
91  Other challenges include the inappropriate selection of monitoring sites, inadequate sampling frequency
92  and the limited number of parameters considered. In order to address these challenges, alongside
93  ongoing scientific advances, there is a need to consider new monitoring approaches, technologies, and
94  sensors (Winkler et al. 2008; Winkelbauer et al. 2014; Altenburger *et al.*, 2019) to accurately
95  characterise the overall health status of a site. The current monitoring 'toolbox' available to scientists
96  and regulators needs to be updated to minimize the inaccuracy associated with the pressures and effects
97  of soil-water pollution of individual and networks of sites at the river basin scale (Carvalho *et al.*, 2019).

98      Physico-chemical analysis represents the foundation of historic soil and water quality monitoring.
99  The majority of low-to-middle income countries (LMIC) in Asia, Africa, Latin America and Middle-
100 East still follow this conventional practise (Mangadze *et al.*, 2019; El Sayed et al. 2020). Although
101 chemical assessments are essential, they are limited by not considering the effects of 1) emerging
102 contaminants 2) 'contaminant cocktails' or pollution mixtures 3) different bioavailability or
103 concentrations of contaminants in soil, water, sediments or biota, and 4) eco-toxicity of chemical
104 substances not routinely screened (Villares et al. 2001; Gosavi *et al.*, 2004; Amiard-Triquet et al. 2015;
105 Schöne & Krause, 2016; Prabhakaran *et al.*, 2017). It also does not take in account the impact of
106 chemical pollution on the functioning and survival of biological communities, which are potentially
107 important as early warning indicators for human health risks (Gosavi *et al.*, 2004; Milinkovitch *et al.*,
108 2019). Chemical analysis alone, cannot characterise the ecological health of a system at different spatial
109 and temporal resolutions, and may lead to inadequate screening of polluted sites (USEPA 2005; Zhou
110 et al. 2008; Schöne & Krause, 2016).

111     In view of these challenges, this review examines the opportunities for improving current practices
112 within LMICs through incorporation of biomonitoring into conventional environmental monitoring
113 approaches (Delmail, 2014; Altenburger *et al.*, 2019). This paper proposes utilizing available state of
114 the art monitoring technologies but also emphasises the importance of fully integrated monitoring
115 frameworks for thorough assessment of polluted sites (specifically integrating biological, chemical, and
116 physical approached). An integrated approach to characterise the physical status, chemical
117 concentrations, and biological effects of pollution will maximise benefits, reduce the risks to human
118 health and ultimately make most effective use of the resources available. The authors propose that such
119 an integrated programme should be employed globally, with appropriate adjustments based on the local
120 geographical conditions and constraints. The highlighted biomonitoring techniques are not proposed as
121 a substitute for physico-chemical monitoring, but to complement the existing tools for identification
122 and confirmation of the contaminants of interest. Scientists have emphasized the need for integrated
123 monitoring tools in order to establish cause-effect relationships over many years (e.g., Reineke *et al.*,
124 2002; Delmail, 2014; Altenburger *et al.*, 2019; Brack *et al.*, 2017; Milinkovitch *et al.*, 2019), however,
125 the lack of standardised frameworks and clearly-defined methodologies has impeded their wider
126 application in the field.

127     Specific contaminants, like hydrophobic substances, are typically persistent, bioaccumulative and
128 toxic in water and are poorly monitored in most localities due to their high spatial variability in water

bodies (Brack *et al.*, 2017). In such cases, the toxicity to organisms associated with the chemical exposure provides the best assessment approach rather than a complete physical and chemical analysis of the 'whole waterbody'. Similarly, polar molecular substances experience high temporal variations in concentrations in water, requiring frequent sampling to provide clear information on the contamination level (Brack *et al.*, 2017). Bioaccumulation and toxicity monitoring facilitates the detection and quantification of such chemicals (Booij *et al.*, 2016; Brack *et al.*, 2017). An integrated approach may therefore, potentially reduce the overall sampling frequency by focussing on organisms via passive sampling and toxicity profiling by prioritizing specific sampling locations, identifying hot spots and establishing 'cause and effect' relationships (Brack *et al.*, 2017). Thus, biomonitoring potentially provides cost-effective solutions, which may be especially beneficial within low-to-middle income countries (LMIC) where frequent sampling alongside highly sensitive (and economically costly) chemical analysis is challenging (Japitana *et al.*, 2018). The availability of sophisticated analytical instruments for a whole range of emerging compounds (e.g., surfactants, pesticides and anti-inflammatory drugs) and their active metabolites, is more challenging for LMICs (Schöne and Krause, 2016; Prabhakaran *et al.*, 2017; Calvo-Flores et al. 2018; Gogoi et al. 2018; Hybská et al. 2020). Analysing trace, yet toxic, concentrations of substances is a resource intensive and expensive procedure. The world is in indispensable need of monitoring approaches that are both scientifically sound and cost-effective in identifying and predicting the potential consequences to the ecosystem and human health (Zalewski, 2015; Brack *et al.*, 2017; Prabhakaran *et al.*, 2017).
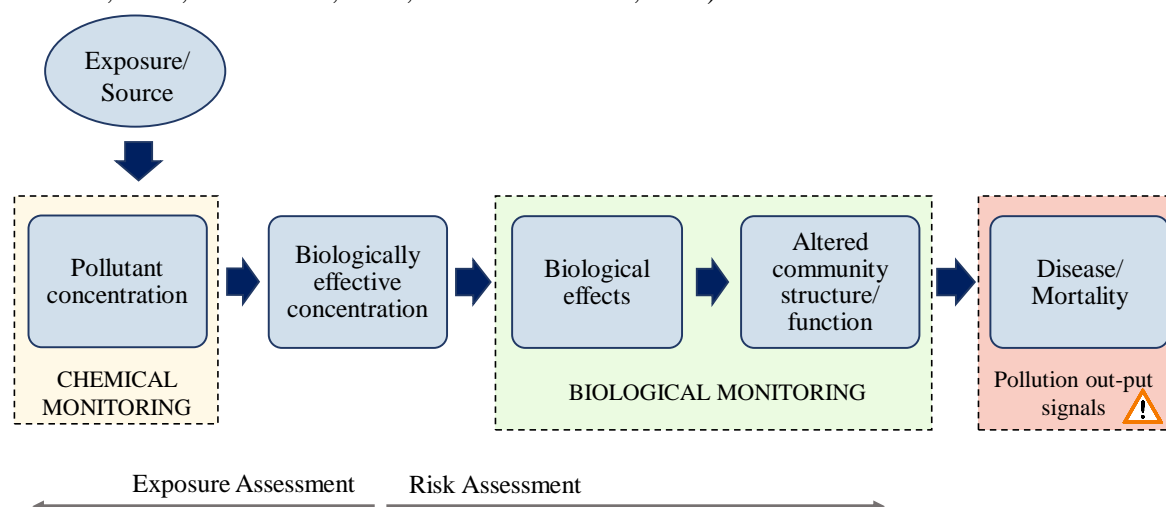


**Figure 1: Pollutant pathways from physical exposure, traditional chemical monitoring and biological monitoring of pollutants to identify potential biological effect and risks to human health via disease and mortality.**

The passage of pollutants through different components of the monitoring pathway after being released from their source are illustrated in Figure 1. Monitoring systems globally follow a receptor-oriented approach where pollution episodes may be directly related to environmental, ecological, or human health effects. Negative human and ecosystem responses to poor physico-chemical quality requires remediation actions within the affected area(s). Changes in the species composition of a biological community, diversity and mortality represent 'late indicators' that should be identified and addressed earlier wherever possible. The scientific community are consistently developing and advancing monitoring tools that can facilitate timely 'prevention' rather than 'remediation' plans (Milinkovitch *et al.*, 2019). Incorporating 'early-stage' biomonitoring (effects on species and community structure and function) within monitoring plans facilitates early intervention that will avoid future stress on the ecosystem and reduce risk to human health (Bolognesi 2003; Barnett-Itzhaki et al. 2018; Mangadze *et al.*, 2019). This will lead to the establishment of cost-effective 'prevention' plans rather than expensive 'remediation' measures.

164      Accumulation of pollutants into biomass via environmental pathways or the food chain, is critical
165 for the estimation of total pollution loading and lethal/sub-lethal effects (Lovett Doust et al. 1994; Adu-
166 manu *et al.*, 2017). Until now, biomonitoring experiments have primarily been conducted as part of
167 independent scientific research by biologists, ecologists, and/or environmental scientists and have not
168 been widely used in field studies within LMICs due to lack of standardised methods and clearly defined
169 protocols (Debén *et al.*, 2017; Altenburger *et al.*, 2019). European countries (e.g., Belgium, France,
170 Italy, UK, Germany and Portugal) along with the USA, Australia, New Zealand, and Canada regularly
171 undertake biomonitoring to assess and monitor the ecological status of surface water bodies
172 (Vanderpoorten 1999; Vincent, Lawlor, and Tipping 2001; Kapfer et al. 2012; Gecheva et al. 2015;
173 Guareschi, Laini, and Sánchez-Montoya 2017; Pratas et al. 2017; Favas et al. 2018). This approach has
174 been demonstrated to be especially effective if the wider ecosystem is also regularly monitored along
175 with regular physico-chemical analysis. Milinkovitch *et al.*, (2019) highlighted that the alterations in
176 ecological parameters (e.g., species abundance and diversity) may occur due to chemical stressors
177 and/or other factors including temperature, resource availability and salinity. Ecological evaluation
178 alone cannot identify the primary factors generating ecosystem level impacts (Schiedek *et al.*, 2007;
179 Thrush *et al.*, 2008; Moe *et al.*, 2013). As a result, an 'effects' based approach is necessary to address
180 pollution  (Brack *et al.*, 2017; Vethaak *et al.*, 2017; Altenburger *et al.*, 2019; Milinkovitch *et al.*, 2019).
181 The EU Water Framework Directive (WFD) requires biomonitoring and physio-chemical monitoring
182 techniques to be applied in all surface water quality monitoring programmes focussed at the ecosystem
183 level. This paper advocates the need to constantly reconsider and update existing programmes,
184 including those utilized as part of the WFD, and especially the establishment of new integrated
185 programmes for LMICs. The areas where the proposed framework outlined and advocated in this paper
186 may contribute in comparison to the existing approaches utilised within the EU WFD are outline in
187 Table 1. The approach will provide benefits to both more high income nations (in improving and
188 reviewing current strategies) and LMIC economies (in adopting new and cost-effective monitoring
189 tools).

190 **Table 1: The proposed framework for pollution monitoring of soil-water resources compared to**
191 **the European Union Water Framework Directive (EUWFD)**

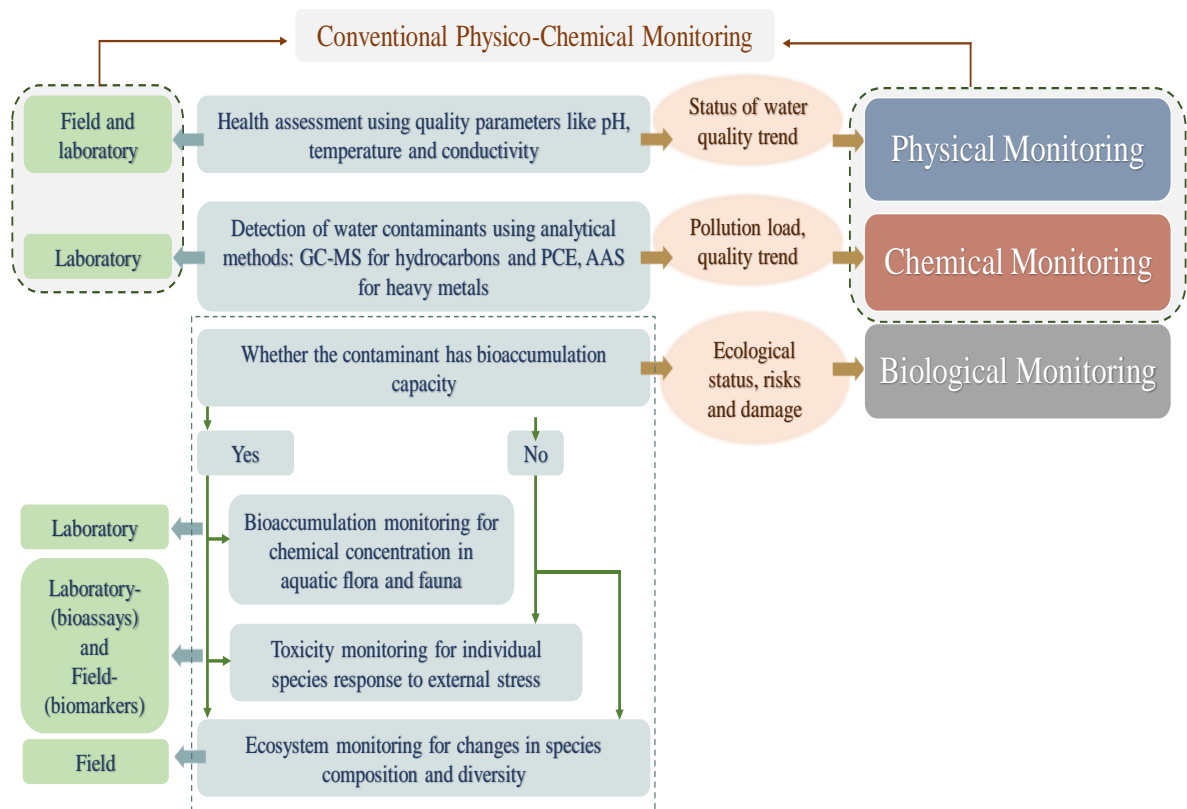|  | **Proposed Framework** | **EU WFD** |
|---|---|---|
| **Biomonitoring tool** | Ecosystem Bioaccumulation Toxicity | Ecosystem |
| **Target sites** | Water resources of polluted sites | All surface waters |
| **Application** | Internationally (especially LMICs) | European countries |

**Figure 2: Physical, chemical, and biological parameters and monitoring techniques used in characterizing polluted sites along with their target objectives**

A representation of the physical, chemical and biological monitoring objectives and measurements procedures advocated are outline in Figure 2. While physico-chemical monitoring combines approaches, biological parameters rarely form the part of the assessment methodology in most LMICs (Debén *et al.*, 2017). Practically, a single methodological approach cannot fulfil all monitoring requirements of impaired sites with a history of multiple pollution episodes (Altenburger *et al.*, 2019). Combining approaches and pathways will provide a mechanism for improving the overall quality and coverage of environmental monitoring programmes. Within LMICs, comprehensive soil and water quality monitoring is still at a relatively nascent stage of development (McBratney, Field and Koch, 2014; Bünemann *et al.*, 2018; Odountan *et al.*, 2019), and needs to be more cost-effective for its wider implementation. Generating reliable information within the confines of an economically feasible monitoring process is crucial for regions where financial constraints may limit the uptake of technological developments. Attempting to transfer monitoring frameworks from more economically developed regions (e.g. like those within the European Water Framework Directive), where environmental, social and economic conditions are different, may result in inadequately characterising data of local water quality issues (Behmel *et al.*, 2016). The process of data collection, analysis, and management of water quality data demands significant financial resources, professional expertise, equipment, and laboratories. These resources are limited or not widely available in LMICs and requires a greater focus on formulating recommendations for WQM pathways within the constraints of limited economic resources. Although biomonitoring is well established in some HICs, there is only an emerging body of research in LMICs, it has not been theoretically and practically adapted to addressing these limitations. This deficiency in literature from LMICs justifies the need to shift the focus towards a novel bio-integrated approach as proposed herein. This paper highlights the potential value and

significance of biomonitoring in addressing these issues with reference to polluted site subjected, but not limited to heavy metals, organics, hydrocarbon oils, and emerging pollutants. It advocates the use of different organizational levels of responses (e.g., biomolecular, morphological and diversity) of organisms and communities to complement physical and chemical analyses to be better prepared to manage and mitigate environmental perturbations / pollution where it occurs. This paper therefore attempts to improve knowledge on the application of standardized bio-integrated monitoring methodologies that can be applied across diverse geographical regions globally. The paper specifically contributes to the literature by advocating greater uptake of the approach within LMICs, where the advantages of biomonitoring are not currently explored to improve their conventional polluted site monitoring methods. While this paper is mainly focussed on targeting polluted water sites, similar approach can be applied and may be beneficial for polluted soil/land including brownfields where plants and soil microbial communities may act as biomonitors/bioindicators (Saunier *et al.*, 2013; Dadea *et al.*, 2017).

## 2. Biomonitoring

The term 'biomonitoring' can have different meanings depending on the disciplinary background of the individual(s). In the broadest sense, any biological measurement that aims to measure, protect and preserve natural ecosystems can be called biomonitoring (Zwart, 1995). The selection of biological monitoring approaches may be determined by the time, scale, stressor, and sensitivity of the measurement required. It can range from concentration measurement of pollutants within tissues to quantifying their large-scale ecological community impacts. Ecohydrology, which studies the two-way interaction between water and biota, can be fundamentally associated with biomonitoring as it can help in first characterising and subsequently achieving environmentally sustainable quality goals (Prabhakaran *et al.*, 2017). Biological responses depend on the magnitude, frequency and duration of exposure to stressors / contaminants. The reaction to pollutants may occur at three different levels: 1) interaction of stressor with organisms cells, 2) activation of cells responding to the stressors, and 3) adaptive response to maintain functioning (failure to adapt may result in death of the cell / organism) (Piña and Barata, 2011). The contaminant dose is an equally important factor as lower levels may result in adaptation while higher levels may result in acute toxicity and physiological responses by the biota, that may ultimately lead to death (Piña and Barata 2011; Amiard-Triquet et al. 2015).

### 2.1 Types of Biomonitoring

Biomonitoring of effects, and risk assessment in relation to chemical exposure to contaminants, follows a series of distinct methodologies developed over many years (Amiard-Triquet and Rainbow, 2009). The principal types of biomonitoring methods outlined here may be performed for specific purposes where the methods used will reflect the requirements in terms of sensitivity, organisms considered and the need to apply the results to the wider ecosystem. The results may enable environmental managers, regulators (government/private agencies), scientists, and the potential end-users to allocate resources to determine the most effective restoration and remediation strategies (Amiard-Triquet et al. 2015). Three types of biomonitoring are outlined below:

**2.1.1 Bioaccumulation monitoring**: This form of monitoring quantifies the concentration of pollutants measured within an organism, biological material or specific tissues (Zwart 1995; Schilderman et al. 1999; Salánki et al. 2003). The individual species should be examined for any accumulation of 'pollutants' or environmental markers within their tissues, biomolecules or DNA (Melville and Pulkownik, 2007; Baldantoni *et al.*, 2018; Favas *et al.*, 2018). Human biomonitoring (HBM) has also been widely applied for detecting the health effects of environmental pollutants, where chemicals and their metabolites are directly measured in human tissues and/or body fluids in medical research (e.g., Barnett-Itzhaki *et al.*, 2018). The approach is widely applied in public health studies by identifying

263 specific risk groups associated with particular contaminants (Bolognesi 2003; Barnett-Itzhaki et al.
264 2018; Vieira *et al.*, 2019; Zhang *et al.*, 2020).

265 **2.1.2 Toxicity monitoring:** This approach requires the organisms' response to external stressors to be
266 directly studied and quantified (Zwart, 1995). The measurements of an organisms' physiological,
267 morphological and biomolecular responses, such as lethal concentration, survivorship (Bonnail, Macías
268 and Osta, 2019), biomass, growth (Hybská *et al.*, 2020), damage to DNA and other genetic markers
269 (Vieira *et al.*, 2019), phytotoxicity, cytotoxicity, genotoxicity, mutagenity (Cavusoglu *et al.*, 2010; Bilal
270 *et al.*, 2016; Artico, Migita and Menezes, 2018; Olusola and Solomon, 2018) and locomotion responses
271 (Salánki *et al.*, 2003) have been utilised in previous research. Experiments may be conducted in
272 bioassays / mesocosms designed on simplifications of the natural environment, to control for the
273 complexities of real-world field conditions. Monitoring the modification to the organisms behaviour
274 provides an early warning of potentially significant ecological disturbances that may follow due to
275 increasing contaminant / pollution levels (Cavusoglu et al. 2010; Bilal et al. 2016; Li et al. 2018; Bonnail
276 et al. 2019; Hybská et al. 2020).

277 **2.1.3 Ecosystem monitoring:** Changes to community composition (taxonomic changes or community
278 functioning) due to environmental disturbances can be studied as part of ecosystem monitoring
279 approaches. These studies typically require long-term monitoring of the study area and specific sites.
280 Determining the ecosystem quality and health can be based on comparisons between variables such as
281 population density, species composition, abundance, diversity or may be based on specifically indices
282 / metrics developed to characterise individual stressors / contaminants (Zwart 1995; Clark and Clements
283 2006; Hering et al. 2006; Li et al. 2010; Delmail 2014; Niba and Sakwe 2018).

284 A comparative overview of the three principal forms of biomonitoring that may be performed by
285 regulatory, industrial, or academic organizations is outlined in the Figure 3. Bioaccumulation reflects
286 the interaction between the polluting compound and biota via its incorporation within tissue(s). Its
287 application typically depends on the pollutant's properties, bioaccumulation potential, and the biological
288 factors that determine the fate of pollutants within the food chain / web (Amiard-Triquet and Rainbow,
289 2009). Within this form of biomonitoring, it is crucial to analyse the differences between a
290 contaminants' concentration within both water and soil compared with the bioavailable concentrations
291 within the organism(s). The fraction of the available pollutant concentration entering the food chain
292 may result in significant changes to biological material (Yadav et al. 2011). Low sub-lethal
293 concentrations may cause chronic diseases, while lethal concentrations will kill most biota (Specziár
294 2002; Salánki et al. 2003).

295 Toxicity monitoring is a growing area of eco-hydrological research, especially where accurate
296 monitoring of polluted waters is a major concern and where understanding temporal variability of
297 effects is required (e.g., under controlled laboratory exposure over set time-periods). Depending on the
298 type of pollution and biological variable(s) under investigation, monitoring can be performed in the
299 laboratory or *in-situ* in the field if appropriate control measure can be put in place. Experiments are
300 typically conducted in bioassays in the laboratory, while the field samples may be collected over space
301 and/or time with respect to clearly identified biomarkers. Laboratory based investigation may employ
302 smaller sample sizes than those collected in the field to observe responses to pollution stress compared
303 to non-polluted (control) bioassays under closely monitored experimental conditions.

304 Changes in the survivorship or growth of individuals within a population or community in response
305 to the input of pollutants forms the basis for ecosystem monitoring. The endpoints of pollution
306 monitoring may be based on the measures of survivorship, growth and reproduction potential, which
307 ultimately lead to changes in the community composition and population sizes. The biological
308 communities inhabiting polluted sites (compared to unpolluted / unstressed sites) provide evidence of
309 the contamination's effect at a polluted site. The biota act as continuous monitors (over their entire life
310 span) of the conditions they experience and can be directly related to instantaneous chemical analysis

(Zwart 1995; Dalu and Froneman 2016). Considering the wide range of pollutants, this approach enables the study of the 'cocktail effect' (additive, reductive or synergistic) of pollution mixtures (Fu *et al.*, 2018) although in many instances the nature of the 'cocktail' remains unknown.

While ecosystem monitoring facilitates the assessment of 'risks', bioaccumulation and toxicity monitoring allows the quantification of pollution 'effects'. The latter two methodological approaches provide early indicators that bridge the information obtained by chemical analysis and ecosystem monitoring. The three levels of biomonitoring together deliver maximum benefits when undertaken in an integrated solution-oriented framework of polluted sites.
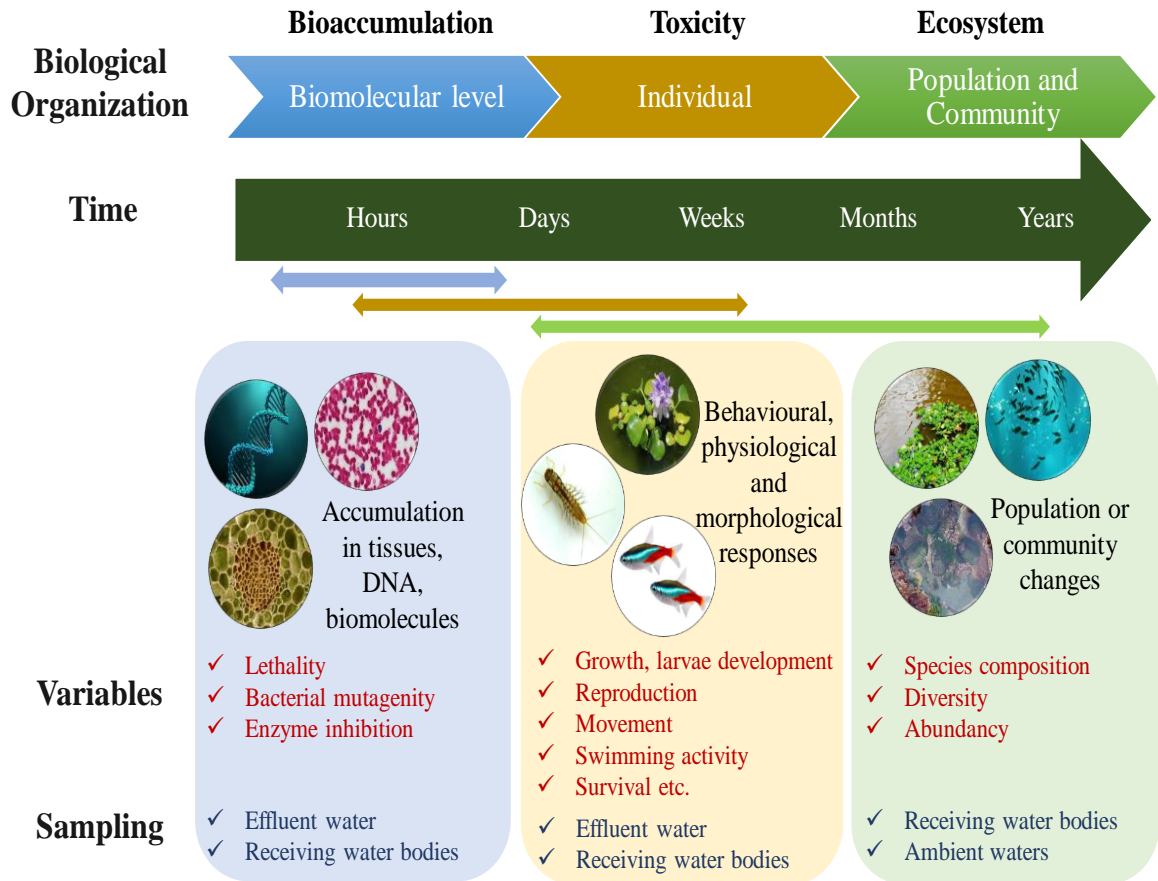


**Figure 3: Schematic representation of the three types of biomonitoring techniques widely employed internationally: Bioaccumulation, Toxicity and Ecosystem monitoring. Indicating the level of biological organization, time required for study / analysis, typical types of variables considered, and sampling approach required to implement monitoring/**

## 2.2 Biomonitors, Biomarkers and Bioindicators

Many scientists describe 'biomonitors' and 'bioindicators' as the same entity, i.e., a species or group of organisms reflecting the surrounding environment / abiotic conditions, including stressors and pollutants (Rainbow, 1995; Prabhakaran *et al.*, 2017; Samsi *et al.*, 2017). However, the definitions have evolved over time. The biotic species studied can be classified into two types: a) indicator organisms - which display specific tolerances to the environmental/abiotic conditions, and b) bio-accumulators of pollutants. Indicator organisms are typically referred as bioindicators, and accumulators as biomonitors (Phillips and Rainbow 1994; Li et al. 2010). Another term used in the literature, 'biomarkers', refers to the biological response/characteristics or the presence of (bio)chemical markers whose presence indicates environmental perturbations associated with pollution loading (Celander 2011; Hamza-

Chaffai 2014). The three terms, however, are often used interchangeably despite the differences that are acknowledged by those working directly in the field.

## 2.3 Selection of species and biomonitoring variables

The success of contaminant biomonitoring depends on the selection of suitable species that form the basis of specific criteria (Hamza-Chaffai 2014; Amiard-Triquet et al. 2015). The choice of variables (both the organisms used and contaminants studied) depends on the context specific circumstances associated with pollutants. The essential features for selection of the most suitable species for biomonitoring have been considered by several authors (Table 1) – Metcalfe-Smith (1994), Zwart (1995), and Amiard-Triquet et al. (2015). The species are primarily selected based on the scientific requirements of the study while the selection of abiotic (including chemicals) variables (parameters) depends on administrative and policy decisions too.

The selection criteria outlined in Table 2 identifies 'sentinel species' (indicator taxa / groups of organisms) ideal for the biomonitoring studies. For instance, a species may be selected from a range of 'sentinel species' having experienced serious effects (survivorship, growth or population size) due to pollution (Chapman, 2002; Hale and Koprowski, 2018). In aquatic systems, benthic taxa like amphipod Crustacea (e.g., Gammaridae) are important 'keystone species'. They play a crucial role in detritus processing and comprise an essential part of the aquatic food webs as both shredders of detritus and prey for higher secondary consumers (Amiard-Triquet et al. 2015). These species are sensitive to a wide range of chemical stressors, including heavy metals, organic pollution and hydrocarbons (Von Der Ohe and Liess 2004; Dauvin and Ruellet 2007). Their life cycle and readily recorded responses to abiotic conditions are significant for the scientific understanding of wider community structure (Kunz et al. 2010) and ecosystem functioning. The selection of biomarkers may vary according to the type of contaminant and the environmental setting. Amiard-Triquet *et al.* (2013) utilized several species of micro-algae, nematode worms, bivalves, crustacea, insects, and fishes as biomarkers of tolerance and as early indicators of heavy metal pollution. The list can be extended further by adding phytoplankton, annelid worms, and amphibian species as biomarkers of organic contaminants, including PCBs, PAHs, as well as a range of pharmaceuticals and pesticides (Amiard-Triquet *et al.*, 2013). However, careful selection of biomarkers is required to ensure clear demarcation of the contamination stressor and the influence of confounding factors (non-chemical stressors) (Jemec *et al.* 2010).

**Table 2: Criteria for the selection of species and variables for the establishments of biomonitoring programmes. Source: Metcalfe-Smith (1994), Zwart (1995), and Amiard-Triquet et al. (2015)**

| Criteria for species selection | Criteria for variables selection |
|---|---|
| ▪ Ease of identification | ▪ Ecological and environmental value of the information |
| ▪ Sedentary nature for representativeness of spatial extent of pollution at the site | ▪ Representativeness of response in the studied and other species |
| ▪ Sufficient population size | ▪ Cause (contamination) – effect (variable) relationship specificity |
| ▪ Ease of sampling | |
| ▪ Longevity as compared to other aquatic species for recording temporal changes in the response | ▪ Sensitivity to the pollution stress |
| | ▪ Quantifiable response range and rate |
| ▪ Availability for capture throughout the year | ▪ Standardization procedure of the measurement method |
| ▪ Sensitivity to dose-effect and cause-effect relationships | ▪ Applicability to similar sites for comparison |
| ▪ Representativeness towards the environmental conditions | ▪ Cost-effectiveness |
| | ▪ Retrospection |

10

## 3. Global history and current research trends in Biomonitoring

Despite the wide application of biomonitoring methods in several HIC regions of the world, it is a relatively novel concept for some low- and middle-income countries (LMICs). European countries including Czech Republic, Italy, the Netherlands, Germany, Sweden, UK, France, Poland, Slovakia, Denmark, Italy Canada, the US, Australia and Asian countries like South Korea, Japan and China have been at the forefront of using biomonitoring techniques and methodologies to define the ecological health status of aquatic waterbodies (Clarke *et al.*, 2006; Hering *et al.*, 2006; Buss *et al.*, 2015). Some regions (e.g., European Union member countries) have developed standardized protocols to monitor and assess the biological status, patterns, and trends in the heath / quality of surface waters. These protocols define standard procedures for sampling, collection and identification of biological taxa (Birk *et al.*, 2012a). The regions with a longest history on biomonitoring methods (e.g. Europe and the USA) also have some locally developed small scale biomonitoring programs specific to their geographical location or ecosystem type (Birk *et al.*, 2012b; Buss *et al.*, 2015). Legislation like the EU Water Framework Directive (WFD) in Europe aims to maintain the ecological health of surface water bodies. Similarly, programmes like Australian River Assessment System (AUSRIVAS), Canadian Aquatic Biomonitoring Network (CABIN) and USEPA's National Aquatic Resources Survey (NARS; previously called EMAP) undertake routine biological monitoring to help maintain aquatic ecosystem health. The most commonly and widely used biological indicator group of organisms are macroinvertebrates, followed by fish, bacteria, and algae (Morse *et al.*, 2007; Buss *et al.*, 2015). While the methods have been widely explored and applied in these areas, some global regions such as Latin America, Africa and parts of Asia (encompassing low and middle income countries LMIC) lack a history of biomonitoring of freshwater ecosystems (Buss *et al.*, 2015). In addition, in many tropical nations around the world, a small number of pilot studies have been undertaken, e.g., National River Health Monitoring Program in South Africa like SASS and MIRAI (Dallas, 1997, 2004; Ollis *et al.*, 2006) and thus, it is imperative to develop standardised methods for biomonitoring in these regions. Field applications of biomonitoring focus only on ecosystem monitoring for the assessment of ecological and human health risks and such practices mean that significant knowledge gaps persist (Brack *et al.*, 2017; Milinkovitch *et al.*, 2019).

Generic surface water monitoring (primarily using chemical and physical approaches) may help identify potential pollution sources within the ecosystems and wider catchments. However, this review highlights the need to incorporate biomonitoring of the effects recorded at polluted sites (e.g., industrial area) on soil-water resources. We emphasize the values of integrated approaches, where chemical monitoring may identify specific pollutants, and biomonitoring quantifies the ecological damage and effects caused by pollutants. Starting from the point of effluent discharge and working downstream to receiving water bodies in a systematic manner would potentially save time and reduce costs of monitoring the entire network. The framework in this paper proposes monitoring (bioaccumulation, toxicity, and ecosystem monitoring of specific waters) which tracks the extent and temporal duration of biological degradation (fate and transport). It proposes a focus on measuring the extent of degradation caused from clearly identified pollution sources, rather than plotting deterioration in ecosystem quality. It also emphasizes the importance of the wider field application of bioaccumulation and toxicity monitoring along with ecosystem monitoring. Global regions with a limited history of biomonitoring applications (especially many LMICs) would gain from the establishment of credible integrated monitoring programmes (encompassing chemical, physical and biological approaches). This would be particularly beneficial for areas with finite economic resources, where nation-wide application of one standard method is not feasible

## 4. Biomonitoring applications

With the increasing occurrence of environmentally persistent toxic compounds, new emerging contaminants, and mixed 'cocktails' of pollutants discharging into waterways, the need for robust, readily available and inexpensive pollution assessment methods has grown. Biomonitoring has provided reliable, efficient, accurate and cost effective information over many years of use (Zwart 1995; De Bisthoven et al. 1998; Jemec et al. 2008; Oost et al. 2016; Prabhakaran et al. 2017). The biotic responses of 'sentinel species' or 'taxa' in the form of pollutant uptake, (Lovett-doust et al. 1994; Meador et al. 1995; Zuykov et al. 2013; Prabhakaran et al. 2017), accumulation (Lee and Wang 2001; Lovett-doust and Lovett-doust 2001; Liu et al. 2016; Nascimento et al. 2018; Vieira et al. 2019), and lethality (Altinok and Capkin, 2007; Paulino *et al.*, 2014; Vieira *et al.*, 2019) have been used to quantify and characterise the wider health of pollutes sites and ecosystems. Examination of the literature indicates that biomonitoring methods are categorized in two types: active and passive. Passive monitoring is performed by collecting 'resident species', those organisms naturally inhabiting and growing in the area. In contrast active monitoring, may require the study of organisms transported to the laboratory-although some studies have be undertaken in the field (García-Seoane, Fernández, et al. 2018; Vieira et al. 2019). Passive techniques are more frequently utilized as they provide greater insights to the spatial distribution and extent of bioavailable pollutants under real world conditions (García-Seoane, Aboal, *et al.*, 2018). In active monitoring, a well-studied organism can be exposed to the conditions present or to the specific compound / chemical present at the target (polluted) site for specified periods of time. Comparisons between multiple bio-monitors or bio-indicators can produce statistically powerful results. Comparisons can also be made by using the same species for a range of different pollutants to analyse their bioavailable concentrations or interactions when multiple compounds are present in the environment (Rainbow 1995; Lee and Wang 2001; Nascimento et al. 2018). Examples of previous studies utilising biomonitoring techniques to a range of pollutants present in the natural environment are presented in Table 2.

**Table 3: Recent studies on biomonitoring techniques using plants and animal biological indicator species.**

| Biomonitoring Technique | Bioindicator Species | Pollutant(s) | Biological/Ecological parameter | Reference | Coupled with Physical/chemical monitoring |
|---|---|---|---|---|---|
| Bioaccumulation | *Konosirus punctatus, Mugil cephalus* | Microplastics | Indigestion by fish | Zhang et al. (2020) | Yes |
| Toxicity | *Daphnia magna, Allium cepa, Lemna minor* | Waste Water Treatment Plant | Immobilisation in *Daphnia*, Biomass, growth, and leaf count in *Allium cepa* and *Lemna minor* | Hybská et al. (2020) | No |
| Bioaccumulation and Toxicity | *Corbicula fluminea* | As, Cd, Cu, Cr, Co, Fe, Mn, Pb, Ni, Sb, Se, Zn | Lethality, Survival, Accumulation in soft tissue | Bonnail et al. (2019) | Yes |

| | | | | | |
|---|---|---|---|---|---|
| Bioaccumulation and Toxicity (Active Monitoring) | *Prochilodus lineatus* | Organochlorine pesticides (OCPs), Trace metals (Cu, Cr, Cd, Pb, Ni, Zn), Pesticides | Biotransformation and antioxidant enzymes, oxidative damages, DNA damages and liver histopathology | Vieira et al. (2019) | Yes |
| Bioaccumulation | *Apium nodiflorum, Potamogeton pectinatus* | Macronutrients, micronutrients and toxic elements | Bioconcentration in roots and shoots | Baldantoni et al. (2018) | Yes |
| Toxicity | *Allium cepa* | Coal contaminants | Phytotoxicity, cytotoxicity and genotoxicity | Artico et al. (2018) | Yes |
| Toxicity | *Allium cepa* | Pharmaceutical effluents | Phytotoxicity, cytotoxicity and genotoxicity | Olusola and Solomon (2018) | Yes |
| Toxicity | Zebrafish Larvae species | Waste Water Treatment Plant | Change in heartbeat rate, Survival response etc. | Li et al. (2018) | No |
| Bioaccumulation | *Fontinalis squamosa, Brachythecium rivulare, Platyhypnidium riparioides, Thamnobryum alopecurum, Lemanea fluviatilis* (Bryophytes) | 46 elements including heavy metals | Phytoaccumulation | Favas et al. (2018) | Yes |
| Ecosystem | Benthic macroinvertebrates (multiple species) | - | Species abundance, richness, trend | Niba and Sakwe (2018) | Yes |
| Bioaccumulation | *Corbicula fluminea* | Lanthanides | Accumulation in soft tissue | Bonnail et al. (2017) | No |
| Bioaccumulation | *Posidonia oceanica, Cymodocea nodosa, Phragmites australis, Arundo donax, Typha domingensis, Apium nodiflorum,* and *Nasturtium officinale* | Heavy metals | Bioconcentration in roots and leaves | Bonanno et al. (2017) | No |
| Toxicity | *Triticum aestivum* | Textile effluent | Phytotoxicity and cytotoxicity | Bilal et al. (2016) | No |

| Bioaccumulation | *Fontinalis antipyretica, Sphagnum denticulatum* | Heavy metals | Accumulation in moss | Debén et al. (2016) | No |
|---|---|---|---|---|---|
| Toxicity | *Vicia faba* | Petroleum refinery effluent | Cytotoxicity, genotoxicity and mutagenicity | Cavusoglu et al. (2010) | Yes |
| Bioaccumulation | *Caloglossa leprieurii, Catenella nipae, Bostrychia* sp. | Heavy metals | Concentration factors in algae | Melville and Pulkownik (2007) | Yes |
| Bioaccumulation and toxicity | *Lymnaea stagnalis L.* | Heavy metals | Accumulation in gills, muscles, liver, locomotion pattern, effect on resting behaviour | Salánki et al. (2003) | No |
| Bioaccumulation | *Orconectus limosus* (Crayfish) | Heavy metals (Cd, Zn, Cu, Pb) PCBs, PAHs, and Organochlorine pesticides (DDT, DDE) | Accumulation in hepatopancreatic tissue | Schilderman et al. (1999) | Yes |

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Early research on bio-accumulation and monitoring largely focussed on quantifying heavy metals in plants and animals (Mathur and Yadav, 2009). Macrophytes are popular taxa for bio-monitoring purposes because they can accumulate significant pollutant concentrations in various body parts (e.g., roots, shoots and leaves). Baldantoni et al. (2018), for example, observed bio-concentration of micro-nutrients (Ca, K, Mg, P), micronutrients (Cu, Fe, Mn, Na, Ni, Zn), and toxic elements (Cd, Cr, Pb, V) in the roots and shoots of the aquatic macrophytes, *Apium nodiflorum* and *Potamogeton pectinatus*. The variation in concentrations of elements at different sites helped to establish a correlation with potential agricultural, urban, or industrial pollution sources. The concentrations of heavy metals within five different species of bryophytes was also studied by Favas et al. (2018) to highlight species specific bioaccumulation characteristics. Further research by Bonanno et al. (2017), calculated the bio-concentration of heavy metals in the roots and leaves of two seagrasses *Posidonia oceanica* and *Cymodocea nodosa*, and in five wetland macrophytes *Phragmites australis*, *Arundo donax*, *Typha domingensis*, *Apium nodiflorum*, and *Nasturtium officinale*. While heavy metal accumulation is mainly studied in macrophytes, oils and organic chemicals bio-accumulation has been studied in faunal species. Contemporary research is using biological indicators to examine microplastics, pesticides and a wide range of heavy metals (e.g., Bonnail et al. 2019; Vieira et al. 2019; Zhang et al. 2020).

Toxicity monitoring is widely applied using fish, macroinvertebrates, bryophytes and macrophytes (Li et al. 2018; Bonnail et al. 2019; Hybská et al. 2020; Zhang et al. 2020). Ecosystem monitoring is comparatively less popular in terms of toxicity and sensitivity but has the ability to demonstrate the ecological significance and effects in the natural environment. The technique requires sampling of multiple sites simultaneously (Delmail, 2014) or periodic sampling of the same polluted sites in association with appropriate non-polluted control sites (Niba and Sakwe, 2018) for ongoing tracking of changes in the ecosystem properties (e.g., community structure or biodiversity). Biomonitoring of freshwater aquatic ecosystems widely utilizes benthic macroinvertebrates (Carter et al. 2006; Mathers et al. 2016; Bo et al. 2017). Amphipod crustaceans from the family Gammaridae are among the most widely studied and utilized group of macroinvertebrates used to investigate pollution for more than 90 years (e.g., Amiard-Triquet et al. 2015). More than 20 species of *Gammarus* have been utilised in eco-

toxicological studies of aquatic systems. The species are suitable for both laboratory (bioassays) and field toxicity (biomarkers) studies (Kunz et al. 2010); with freshwater species *Gammarus pulex* being the most widely utilised (Kunz et al. 2010; Gerhardt et al. 2012; Besse et al. 2013; Lebrun et al. 2015; Ciliberti et al. 2017; Fu et al. 2018; Shahid et al. 2018; Tatar et al. 2018; Serdar 2019; Serdar et al. 2019; Lebrun and Gismondi 2020). The group of amphipod shrimp (*Gammarus spp.*) are also notable as they have also been used to assess the effects of varying concentrations of chemicals associated with sediments (e.g., Costa et al. 2005; Neuparth et al. 2005; Gaskell et al. 2007). In eco-toxicological studies, population endpoints can be measured in terms of survivorship (mortality), the population structure (age / size classes), density, and interaction with other species (Kunz et al. 2010). Their feeding activities, growth, size, fecundity, locomotion, and survival can all be effectively recorded and used as indicators of chemical / abiotic stressors (Gerhardt 1995; Kunz et al. 2010). Among plant species, macrophytes such as *Eichhornia crassipes* (water hyacinth), bryophytes such as *Brachythecium rivulare* and *Thamnobryum alopecurum*, and seagrass species including *Posidonia oceanica* and *Cymodocea nodosa* have all been widely used to monitor the accumulation of chemical compounds and their effects on morphology, growth, size, density and physiological functioning (Romero et al. 2006; Bonanno, Borg, and Di Martino 2017; Favas et al. 2018).

## 5. Example of Biomonitoring Method

In order to demonstrate the principles of biomonitoring, we measured the effects of a species respond to a low-level exposure of a model contaminant. The response of benthic macroinvertebrates *Gammarus pulex* (Crustacea, Amphipoda) under the presence of mild non-aqueous phase liquid (mineral oil) were measured in terms of changes to movement and survivorship. *G. pulex* were collected from Burleigh Brook on the campus of Loughborough University (UK) in a pond net (mesh size = 1mm) using the kick-sampling method. The collected specimens were carefully transferred into containers of stream water and transferred to the laboratory for acclimatization in an aerated mesocosm at 23ºC prior to the experiment. This bioassay study used a sandy soil medium with a particle size range of 100 – 250 μm with bulk porosity of 0.25 and hydraulic conductivity of 0.023 cm/s. Mesocosms were set up by taking varying volumetric proportions of soil – water – mineral oil mixture. A bulk density of 1.59 g/ml was maintained initially in all experimental units, which were later supplemented with 150 ml water in order to create the media for the species to be observed within. The treatment mesocosms were spiked with mineral oil to achieve 0 to 25% oil – water mixture. Each mesocosm contained five *G. pulex* and were run simultaneously with four replicates.

Observations of the behaviour and movement of individuals during the experiment indicated that when individuals came into contact with the mineral oil their buoyancy was increased. The results representing the average number of *Gammarus* individuals floating near the water surface was plotted at the end of 24 h study period for each of the treatments (Figure 4). The results and response observed clearly indicated an increasing number of individuals close to the water surface with an increasing ratio of mineral oil – water emulsion (Treatment 1 - 0% mineral oil / control – treatment 7 - 25% mineral oil). The greater number of individuals recorded displaying modified movement due to increased buoyancy were recorded for treatment 6 (20% Mineral Oil: 80% Water). Within Treatment 6, an average of 50% of individuals displayed changes in their movement pattern and were located close to the water surface at end of the experiment. Almost all individuals were alive at the end of the experiment (survivorship was > 90%). These observations illustrate the effects of an oil-based contaminant and change in the movement and behaviour of *G. pulex*. This species typically inhabits river bed sediments (benthic sediments) where it seeks refuge between sediment clasts. The behavioural shift with the species being confined to the surface layers of the mesocosm would make individuals more vulnerable to predation by the higher-level organisms, especially predatory fish.
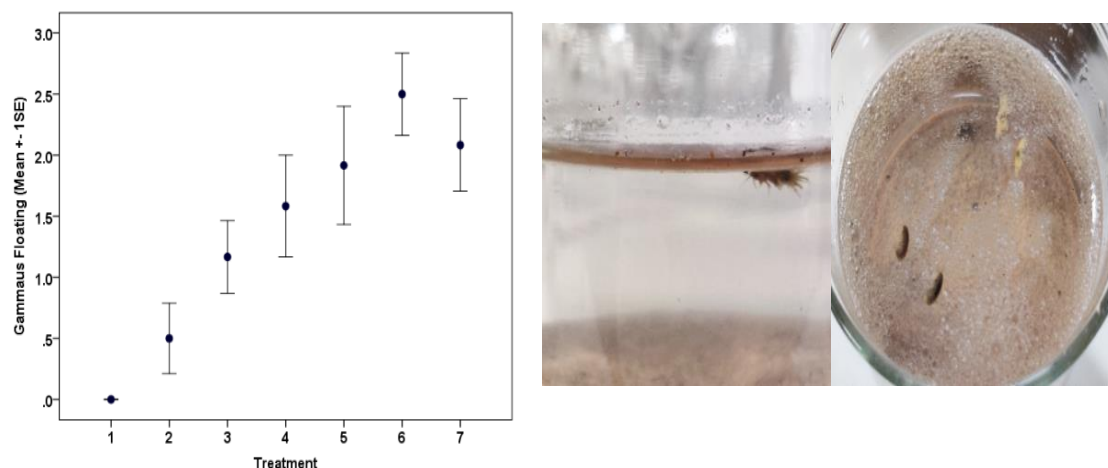
**Figure 4: Mean number of *Gammarus pulex* (+/- 1 standard error) displaying floating behaviour in different treatments of mineral oil. Treatments 1-7 are mesocosm with increasing volumetric ratio of oil and water, with no mineral oil in Treatment 1 (control treatment), and 25 % in Treatment 7. As the quantity of oil increased, more invertebrates were active in the top surface layer and were more buoyant. On average, 2.5 out of 5 *Gammarids* displayed this behaviour in Treatment 6 (20% Oil). *Gammarids* can be seen close to the interface of the oil-water emulsion in photos on the right**

This type of species response is significant in eco-toxicological studies where changes in behaviour are taken as endpoints of studies undertaking contaminant risk assessments (Peeters *et al*., 2009). Although mortality is more typically the endpoint considered, the exposure to contamination at below-lethal concentrations may disturb and disrupt individuals' overall performance, affecting their long-term survival. The response may reflect the direct effect of environmental pollution (toxicosis) or the species' response mechanism(s) when exposed to the pollutant(s) (Peeters *et al*., 2009). The response(s) recorded at the individual organism level are also important as they may help explain changes in the wider population and therefore, it is critical to establish associations between potential toxicity effects and ecosystem effects due to other factor such as behavioural change (Boyd *et al*., 2002). Mesocosm studies, as illustrated above, help provide evidence required for developing larger scale monitoring programmes. Such laboratory scale investigations are critical to identify the appropriate biomonitoring tools and suitable organisms within specific geographical regions. The responses may aid in the development of both laboratory and field techniques with a view for the development of standard monitoring systems in the future (Delmail, 2014).

## 6. Future prospects of Integrated Monitoring in Low to Middle Income Countries (LMICs).

Reviewing contemporary soil-water pollution literature, with direct consideration of LMICs, indicates that conventional physico-chemical monitoring does not align with the rapidly evolving patterns of contamination from industrial, domestic, and agricultural sources. The ever-increasing range of emerging contaminants, potentially posing serious threats to ecosystems and environmental health, require urgent academic and regulatory authority attention. Biomonitoring provides a mechanism and range of techniques to characterise and quantify the effects of these substances which would otherwise be excluded from the list of potential threats due to a lack of evidence and data (Brack *et al.*, 2017). Ecosystem monitoring provides useful coverage of ecological effects and risks associated with chemical contamination. However, it does not discriminate between specific chemical stressors and other abiotic factors (e.g., climatic variation) which may also cause ecological variability. Therefore, it is essential to investigate the biological effects at an early-stage in order establish an association with chemical exposure and direct 'effects' (Milinkovitch *et al.*, 2019). Compartment-wise investigation of pollution

548 (water → sub-individual →  individual →  population) will provide the data required to identify the
549 culpable contaminants and their environment fate and transport within the respective ecosystems.

550    The three principal types of monitoring methods applied in pollution investigations are illustrated
551 in Figure 5. Physico-chemical and biological monitoring approaches have largely been applied
552 independently from each other historically (Altenburger *et al.*, 2019). The authors argue that these
553 methods provide maximum information when employed together. An integrated site monitoring
554 framework as advocated in this paper would help address the current challenges and knowledge gaps.
555 Enhanced efforts to incorporate bioaccumulation and toxicity monitoring within existing monitoring
556 programmes will help identify the effects of non-targeted chemicals / emerging contaminants before
557 effects are identified at higher levels (ecosystem) (Depledge *et al.*, 1995; Milinkovitch *et al.*, 2019) or
558 they pose a threat to human health. The data generated via this holistic approach will provide greater
559 information about exiting environmental resource quality and ecosystem services degradation.
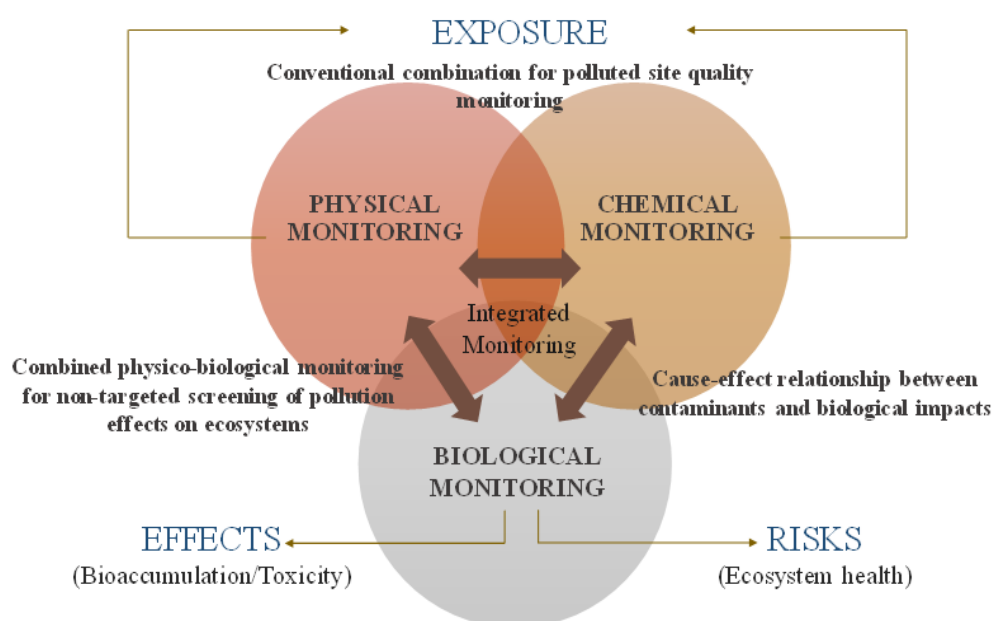


560

**Figure 5: Interrelationships among physical, chemical and biological monitoring**

562    A framework for integrated monitoring of polluted sites is outlined in Figure 6. The framework can
563 be applied to investigate anthropogenically polluted sites using physical, chemical and biological
564 parameters. It proposes a stage / longitudinal monitoring approach which progresses from the source
565 location to receiving water bodies. Biomonitoring allows the collection of time-integrated information
566 over extended period regarding pollutants which can account for seasonal and inter-annual variability
567 in many instances. Utilising a framework such as proposed would establish planned monitoring
568 programmes for the assessment of sites for chemical exposure, biological effects and wider risks to the
569 ecosystem(s) and human health and may represent significant economic cost savings for many LMICs
570 if implemented in a co-ordinated manner. Conventionally, the chemical and ecological status of polluted
571 sites has required two independent assessments, typically by different agencies in low- and middle-
572 income countries. This leads to duplication of effort and data as tests need to be completed for both
573 types of assessments, resulting in increased costs to characterize the pollution at the same site. The
574 integrated approach proposed would facilitate the identification of specific pollutants through chemical
575 monitoring and also quantifying ecological damage and effects using biomonitoring techniques.
576 However, instead of biologically monitoring each site subsequently, identification of key locations
577 allows streamlining and prioritization of effort and resources by identifying the hot spots through effect-

based monitoring. This potentially reduces the overall cost by removing the need to monitor the entire network. The integrated approach is especially beneficial for sites subject to pollution mixtures with high chemical concentrations but limited human health impacts or ecosystem degradation. In some instances, the ecosystem or human health risks may be less than indicated by chemical characterization alone. It should be noted that in the short-term, the combined approach may require more sampling, although, in the long-term, financial savings may be made when an appropriate integrated monitoring system is practiced. This new system may also help to prevent future pollution by identifying potential problems/locations that can be remediated earlier at less cost than the long-term remediation if not addressed early. Thus, the costs of acquiring additional information by 1) avoiding duplication of effort/sampling, 2) streamlining pollution monitoring effects, and 3) developing networks that serve as early warning monitoring sites may prevent large-scale damage and identify problems before they escalate. Incorporating biological parameters allows the comparison of effects in various compartments of the receiving ecosystem / sites and at different biological levels. This also facilitates pollutant tracing from source to sink and bioaccumulation within food-chains and organisms at different trophic levels respectively. Some existing monitoring programs do not consider water bodies where pollutant concentrations are typically below detectable limits and focus on the acutely polluted/degraded sites. The authors suggest that these locations may be ideal for ecosystem monitoring as they may represent ideal reference (control sites), especially if they are in the vicinity of known polluted sites.

Sampling is a crucial step in accurately characterizing polluted sites. The optimal selection of sampling locations/sites, frequency, timing, sample size, volume, and pattern needs to reflect the investigation objectives and the properties of polluting substance and proposed sampling sites to be characterized (Namieśnik and Szefer, 2010). Water samples for physico-chemical analyses need to be taken from both upstream and downstream locations of effluent discharge points to provide a quantitative measure of the effect and a comparison with an unpolluted (control) site. Unrepresentative samples from the water body surface, bottom, boundaries/banks, and confluence zones of streams should be avoided (Namieśnik and Szefer, 2010). Samples from these locations present uncertainties due to their heterogeneity and the mixing properties of the water body. Biota sampling should be performed either through sampling specific locations or passive sampling devices (PSDs) depending on the contaminant type, habitat heterogeneity, and resource availability/sampling costs. Passive sampling should be undertaken for specific contaminants, including hydrophobic organic pollutants and polar substances, which display high concentration variability in aquatic environments (Brack *et al.*, 2017; Altenburger *et al.*, 2019). Integrating passive sampling methods in a time-integrated manner is suggested for quantification of hydrophobic/non-polar organic substances that frequently occur at trace level concentrations (El-Shenawy *et al.*, 2009; Miège *et al.*, 2015; Booij *et al.*, 2016). In addition, adsorption passive sampling should be undertaken to improve the representativeness of temporally variable hydrophilic/polar substances (Miège *et al.*, 2015; Brack *et al.*, 2017).

The proposed framework potentially provides multiple benefits if adopted more widely within LMICs, but also presents new challenges in the form of quantifying new and emerging pollutants and in the interpretation and analysis of the data. First, the effect(s) of confounding factors, including both intrinsic biotic (e.g., organisms size/weight/age, sex, and reproductive status) and extrinsic abiotic (e.g., pH, temperature, redox status, and salinity) factors needs to be considered to differentiate their effects from the pollutant stress under investigation. Second, the floral or faunal community level responses need to be undertaken on a long-term basis given that the effect of pollution may persist for multiple years and monitoring the post-event recovery may also be required. Third, the selection of biomarkers/bioindicators may be challenging in many LMIC regions where the taxonomy or many floral and faunal groups is not fully resolved, making implementation by inexperienced field practitioners even more challenging.
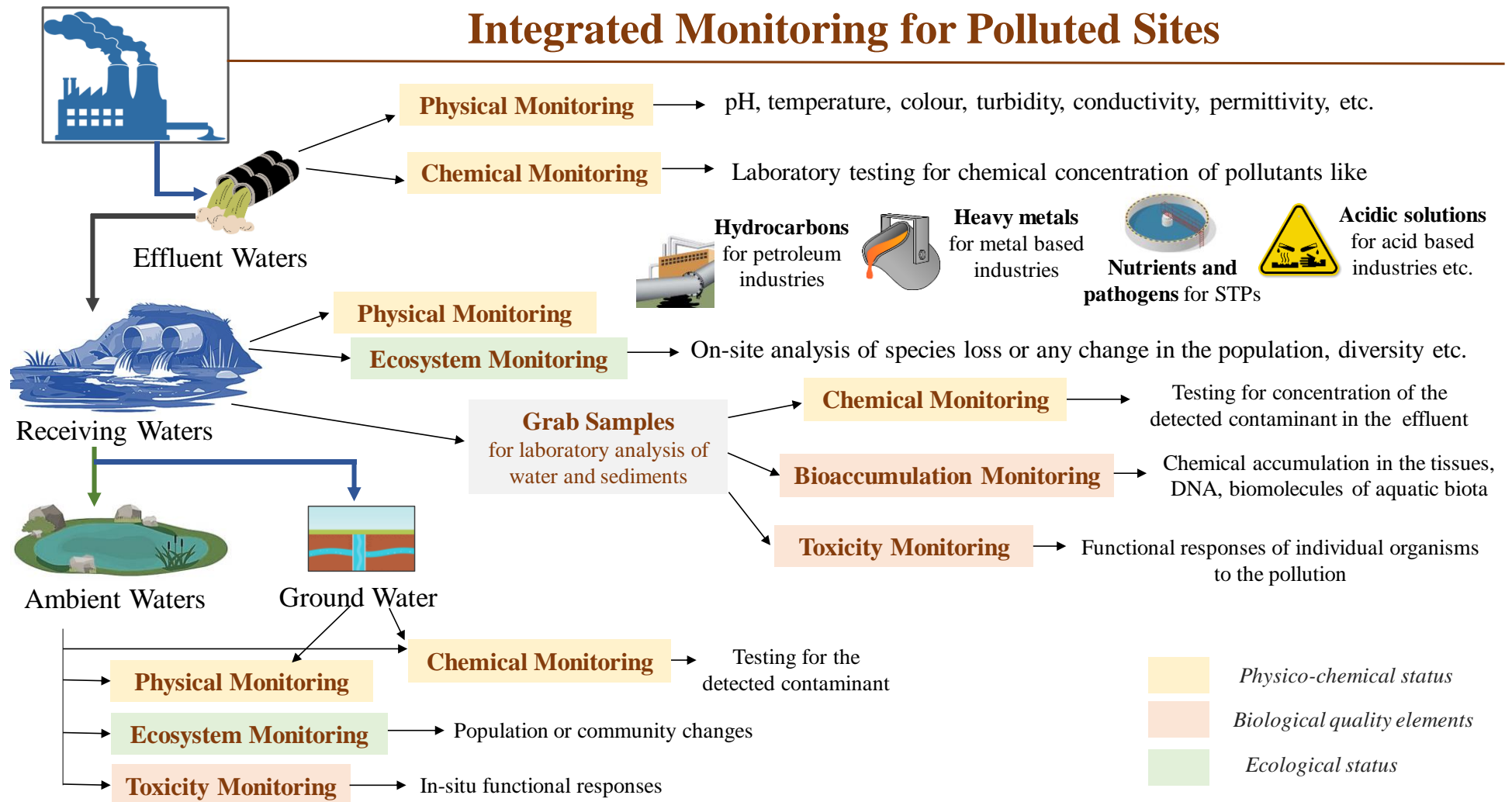
# Integrated Monitoring for Polluted Sites

**Physical Monitoring** → pH, temperature, colour, turbidity, conductivity, permittivity, etc.

**Chemical Monitoring** → Laboratory testing for chemical concentration of pollutants like

**Hydrocarbons** for petroleum industries

**Heavy metals** for metal based industries

**Nutrients and pathogens** for STPs

**Acidic solutions** for acid based industries etc.

Effluent Waters

**Physical Monitoring**

**Ecosystem Monitoring** → On-site analysis of species loss or any change in the population, diversity etc.

Receiving Waters

**Grab Samples** for laboratory analysis of water and sediments

**Chemical Monitoring** → Testing for concentration of the detected contaminant in the effluent

**Bioaccumulation Monitoring** → Chemical accumulation in the tissues, DNA, biomolecules of aquatic biota

**Toxicity Monitoring** → Functional responses of individual organisms to the pollution

Ambient Waters    Ground Water

**Chemical Monitoring** → Testing for the detected contaminant

**Physical Monitoring**

**Ecosystem Monitoring** → Population or community changes

**Toxicity Monitoring** → In-situ functional responses

*Physico-chemical status*

*Biological quality elements*

*Ecological status*

**Figure 6: Schematic diagram presenting the framework for integrated monitoring of anthropogenically polluted sites**

## 7. Conclusion

This review presents a framework for improving understanding and implementation of pollution monitoring and sampling strategies to reflect contemporary regulatory and research needs within Low to Middle Income Countries. The paper has identified knowledge gaps in the monitoring toolbox currently employed and has presented recommendations for comprehensive assessments within LMICs. Integration of biomonitoring approaches will help address and reduce the risks associated with transformation products, pollutant cocktails, and analytically undetected eco-toxic substances. This paper emphasizes the need to utilise information on chemical contamination and propagating its effects at various biological levels within the ecosystem and on human health. The effective integration of 'effect – based' and 'risk – based' monitoring will help improve monitoring of emerging and new (potentially unidentified) chemical and non-chemical stressors.

The review highlights the importance of the implementation and significance of the three biomonitoring methods that together form the basis for an integrated framework for monitoring anthropogenically polluted sites. Integrated monitoring, which is currently used to safeguard water resources (including hydrological, ecological, and societal resources) in many high-income countries, can be effectively applied for the assessment of polluted sites globally. While the current plans only consider ecosystem monitoring, the proposed integrated site monitoring framework should also incorporate biomarkers and bioassays to be most effective. This framework will help guide practitioners to adopt the most appropriate and cost – effective monitoring plans after selecting the ideal 'sentinel' (indicator) species and measurable parameters based on the local conditions.  For real field technological application, economic and policy factors also play a critical role, and thus, the proposed integrated site monitoring framework will provide great benefits to LMICs by being cost effective and providing a readily transferable approach that allows the results from a polluted site to be considered in a wider ecosystem context.

The integrated approach outlined in this paper and the resulting conceptual framework make an important contribution to the environmental literature in terms of understanding the role of biological monitoring in pollution assessment in LMICs. Although the material presented focusses on soil-water pollution, the approach and framework could be readily transferred to other environments. Future research and assessments are required, especially within polluted soils, urban locations, and industrial/post-industrial 'brownfield sites' where the validation of the methods and framework would deliver maximum benefits.

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