PLOS SUSTAINABILITY AND TRANSFORMATION

RESEARCH ARTICLE

Reducing environmental impacts of marine biotoxin monitoring: A laboratory report

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Abstract

Laboratories globally contribute significantly to consumption of resources, greenhouse gas emissions, and generation of waste. Shellfish destined for human consumption are required to be tested for the presence of regulated marine biotoxins, that can be harmful to human health. Whilst running the national monitoring program for the detection of biotoxins in shellfish, efforts were made to increase resource efficiencies by reducing waste and energy consumption leading to reduced environmental and financial costs. Methods were verified to allow transitions to more sustainable and environmentally-friendly consumables, replacing plastics with paperboard and glass alternatives, leading to a reduction in the consumption of single-use plastics by 69%. A shift to polystyrene recycling and composting non-toxic shellfish waste led to an overall reduction in non-chemical waste of >95%. Adoption of green analytical chemistry principles to procurement and preparation of chemical solutions led to a reduction in hazardous chemical waste by ~23%. A further reduction in printing (~81%) was achieved by transitioning to digital document control. Strategies to reduce energy consumption through 'switch off' campaigns and improved fume hood and cold storage equipment management were also implemented. Fume hood and cold storage equipment energy consumption was reduced by 30%. The strategies implemented could be adopted by other laboratories e.g., monitoring and research laboratories dealing with pharmaceutical, biological, and environmental samples.

Author summary

Transitioning to more sustainable methods for the monitoring of marine biotoxins in shellfish reducing single-use plastics by 69%, waste to landfill/incineration by >95%, hazardous chemical waste by ~23%, printing by ~81%, and fume hood and cold storage equipment energy consumption by 30%.

Introduction

Current consumption of earth's resources and generation of waste by humans is leading to ecosystem collapse and dire predictions for the future [1]. Contributing to this consumption

are scientific laboratories. The worldwide scientific laboratory sector is huge—globally, there are an estimated 20,500 laboratories involved in medical, biological, or agricultural research alone [2].

Laboratories are high consumers of plastics. The average Irish person consumes ~59 kg of plastic per year [3], in the USA it is 106 kg [4], while the average bench scientist uses ~1,000 kg per year [2]. The mass of plastic globally (8 Gt) is estimated to be twice the mass of all land animal and sea creatures combined (4 Gt) [5]. These plastics are ending up in the soil and in the oceans (more than 80% of marine litter is plastic [6]) contributing to serious environmental damage and impacting on human health [7,8]. It is estimated that 19 to 23 million metric tons, or 11%, of plastic waste generated globally in 2016 entered aquatic ecosystems, which may reach up to 53 million metric tons per year by 2030 [9]. Recent studies reported the presence of microplastics in Arctic waters [10] and levels of plastic present in the Atlantic Ocean are estimated to be 10 times higher than previously thought [11]. Global emissions from plastics in 2015 were equivalent to nearly 1.8 billion metric tons of CO_2, and if current trends continue it is expected that emissions will reach 17% of the global carbon budget by 2050 [12].

Analytical laboratories use large amounts of solvents for sample extraction and analysis (liquid chromatography mobile phases and cleaning solutions). In 2019, 46,813 tons of solvent (non-halogenated) waste was generated in Ireland, primarily from pharmaceutical and chemical industries [13], the majority of which was exported for treatment (recovery or incineration) [14]. Indigenous treatment and recycling of solvent waste [15] reduces transport costs and emissions, saves resources, enhances security of supply, and contributes to a circular economy [13].

Paper (for printing) consumption is also high. The environmental impact of paper consumption includes deforestation, air, water, and land pollution. The paper industry is among the world's largest generators of air and water pollutants, waste products, and the gases that cause climate change [16]. Hazardous chemicals (organic solvents) are used in the production of printing inks emitting volatile organic compounds and air pollutants during manufacture and printing. Further impacts arise from handling and waste disposal of ink cartridges [17].

Laboratories are additionally high consumers of energy, using five to ten times more energy per square meter than office buildings [18]. Equipment such as fume hoods [19], ultra-low temperature (ULT) freezers [20], and freeze driers [21] are among the highest energy consumers.

It is seen as critical for all laboratories to adopt good environmental practices [22]. One way of achieving this is through green certification e.g., via My Green Lab or ISO 14001. Many laboratories are recognising the need to operate in more sustainable ways and have implemented changes to working practices to reduce their waste and energy consumption [23–25]. Successful transitioning to such work practices is achieved through staff engagement. Regular feedback to laboratory users on their behaviour and the impact of that behaviour on energy use and cost has been shown to be effective in instilling behavioural change [26,27].

Governmental leadership in this area is critical to ensure the Intergovernmental Panel on Climate Change (IPCC) emissions targets for 2030 are met [28]. In 2009, the Irish Government set a national target to improve energy efficiency by 33% in the Public Sector by the end of 2020 [29]. The emphasis has now turned to the 2030 targets and the 50% improvement in efficiency being set for the Public Sector along with a 30% total CO_2 equivalent emissions reduction. In January 2019, the remit was broadened to include waste management and resource efficiency in conjunction with energy efficiency, with a view to reducing the proliferation of single-use plastics, the prevention of waste, and initiation of green public procurement policies [30]. More recent (2020) Irish Government [31] and European Union (EU) [32] strategy

documents highlight the importance of transitioning to a circular economy to minimise extraction of natural resources and disposal of waste.

With increasing pressure on terrestrial agriculture and wild fisheries, aquaculture, being the fastest growing food sector globally, is becoming increasingly significant as a source of sustainable food for growing populations [33]. Within aquaculture, the shellfish industry is considered to be one of the most sustainable, and ethical [34,35]. Shellfish aquaculture also provides ecosystem benefits through, for example, nutrient remediation and provision of habitat for other species [36]. In the EU, shellfish destined for human consumption are required to be tested for the presence of marine biotoxins. These toxins are produced by some species of microalgae and can accumulate in shellfish, rendering the food unfit for human consumption. The growth of this industry has been severely hindered by these toxin producing blooms, which may be increasing in intensity due to climate change [37]. Currently, in the EU, shellfish are regulated for six toxin classes (Table 1).

The Marine Institute run the national biotoxin monitoring program in Ireland, accredited to ISO 17025 standards. Here, we describe efforts made to reduce our laboratories environmental impact through energy saving and waste minimisation strategies, with a particular focus on reducing single-use plastics.

Results and discussion

In 2019, a typical year for the biotoxin chemistry laboratory, 3,183 samples for lipophilic and/or hydrophilic toxin testing were received. In that year ~5,700 analytical tests, including quality control samples, were performed for 23 analytes, using three different methods (Tables 1 and 2).

Over 60% of tests were performed for the lipophilic toxins, ~30% for DA, and 10% for STXs (Table 2). Historically, the OA group, AZAs, and DA toxins have been the most problematic for the Irish shellfish industry, with blooms of the producing organisms occurring annually leading to shellfish site closures [43–45]. In 2019, 7.5% of samples received in the laboratory were over the regulatory limit; 4.4% for the lipophilic toxins (98.6% OA group and 1.4% AZAs); 2.1% for DA; and 1% for the STXs (Tables 2 and A–C in S1 Text). Since the monitoring

Table 1. Classification, closure limits, and methods of analysis for EU regulated shellfish toxins.

Classification	Regulated toxins and closure limit	Method of analysis
Hydrophilic	Saxitoxin (STX), 800 μg kg^{-1} [38]	LC-FD [39]
	*Domoic acid (DA), 20 mg kg^{-1} [38]	LC-DAD [40]
Lipophilic	Azaspiracids (AZA), 160 μg kg^{-1} [38]	LC-MS/MS [41]
	Okadaic acid (OA) group, 160 μg kg^{-1} [38]	
	Pectenotoxins (PTX2), 160 μg kg^{-1} (including OA group) [38]	
	Yessotoxins (YTX), 3.75 mg kg^{-1} [42]	

* Samples also screened for DA using LC-MS/MS method.

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Table 2. Number of samples received and tests (including quality control samples) performed for the regulated biotoxins and % over the regulatory limit (>RL).

			Toxins		
			Lipophilic	**Domoic acid**	**Saxitoxins**
No. of samples to laboratory	3,183	**No. of tests**	3,432	1,671	564
		%	60.6	29.5	10.0
		% >RL	4.4	2.1	1.0

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program was established in 2001 closures due to STXs were limited to one site in the south of Ireland (Cork Harbour), however, since 2019 these toxins have been detected above the regulatory limit in samples from another location in the southwest (Castlemaine Harbour). Further, increased detection of the producing organism, *Alexandrium*, around the Irish coastline suggests changes in intensity and geographic distribution may be an issue in future years.

Polystyrene recycling and shellfish composting

In 2019, samples were harvested and sent, via the postal service, to the laboratory in polystyrene boxes. These boxes keep the samples cool during transport. Polystyrene is a petroleum-based non-biodegradable foam. It is considered to be a human carcinogen and can have serious impacts upon human health, wildlife, and the aquatic environment [46]. Previously, these boxes were sent straight for waste disposal (landfill and/or incineration). Landfills pose environmental risks to water, air, soil, and the natural environment [47] and EU directives have set targets to reduce the amount of waste going to landfill [48,49]. Although incineration is considered to be a better alternative to landfill, and is increasingly used as a means of dealing with waste disposal, it also has environmental impacts relating to CO_2 emissions, air pollution, and hazardous residues [50,51].

To divert the polystyrene from these waste streams, and in collaboration with the INTERREG funded project 'Wise reduction of EPS marine litter in the North-East Atlantic Ocean' [52], an alternative management plan was introduced whereby the polystyrene boxes are cleaned and stored until onsite compacting (to remove the air) is performed by a company specialising in polystyrene recycling [53]. The remaining plastic is recycled for use in e.g., the production of recycled fish boxes [54] and the construction industry as thermal insulation [55]. In 2019, the European Parliament approved a directive that will ban some disposable plastics in the EU from 2021, including food containers made of polystyrene [6]. In response to this directive an alternative system is currently being trialled (ongoing) replacing the polystyrene boxes with corrugated plastic boxes, which can be flat-packed and reused.

Once the shellfish arrive in the laboratory they are shucked (flesh removed from shell), to give $\geq$100 g of meat which is homogenised. Previously the shellfish waste (shells and leftover meat) were sent for waste disposal. Landfilled biodegradable waste produces methane (28 times more potent than carbon dioxide as a greenhouse gas) many years after the waste has been deposited through anaerobic fermentation [56].

This practice has changed, such that now they are sent for composting (Fig 1) which complies with the requirement for EU member states to reduce the amount of biodegradable waste going to landfill [48]. Aerobic composting reduces methane production and offers a sustainable and low cost method of dealing with shellfish waste [57]. The addition of crushed oyster shell to soil (0.3 ton ha^{-1}) was found to double the number of nitrogen fixing bacteria [58]. More generally, shellfish waste is nutrient rich providing a 2:1:1 ratio of nitrogen:phosphate: potash that matches the nutritional requirements for agricultural purposes [57] and can be used as an effective fertiliser in organic farming [59].

Reducing use of plastics

The homogenised shellfish is transferred into 200 mL containers. Compostable paperboard pots (made from sustainable forest paperboard with a natural polylactic cornstarch lining) were sourced to replace the 200 mL plastic pots used previously. The compostable pots are sturdy, freezer friendly, and leak-proof (Fig 2A), and can be used for storage of water and other biological samples. Using these pots ensures all non-toxic samples can go directly for composting following the required storage period (Fig 1).

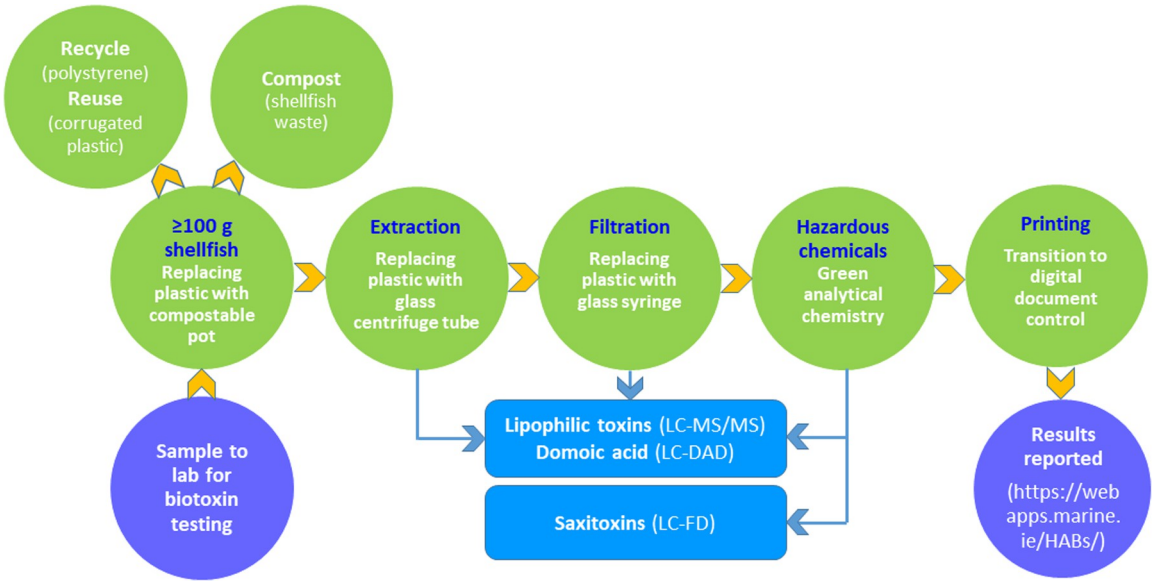

Fig 1. Schematic of procedure for biotoxin testing in shellfish, indicating where measures have been taken to reduce waste (green circles).

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Each sample is tested using one or more of the regulated methods listed in Table 1. The methods used for the analysis of the lipophilic (LC-MS/MS) and DA (LC-DAD) toxins use similar extraction procedures. However, the procedure for the analysis of STXs is significantly different, and for this method transitions to glass consumables were not feasible due to official method recommendations to avoid the use of glass [60]. Therefore, efforts to reduce plastics only focused on the methods used for detection of the lipophilic and DA toxins.

From the homogenised sample, 2 g is weighed for extraction of biotoxins into a 50 mL centrifuge tube (one each for LC-MS/MS and/or LC-DAD). A 50 mL glass centrifuge tube was

Fig 2. Transition from A) 200 mL plastic pots to compostable paperboard pots for storage of shellfish samples, B) transition from 50 mL plastic to glass centrifuge tubes and C) transition from 5 mL plastic to glass syringes.

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Table 3. Comparison of okadaic acid (OA), yessotoxin (YTX), azaspiracid (AZA), pectenotoxin-2 (PTX2), and domoic acid (DA) liquid chromatography-mass spectrometry (LC-MS/MS) results for a certified reference material extraction (n = 5) using plastic centrifuge tubes and syringes and replacing with glass alternatives[a].

		OA equiv. ($\mu g\ g^{-1}$)	YTX equiv. ($\mu g\ g^{-1}$)	AZA equiv. ($\mu g\ g^{-1}$)	PTX2 equiv. ($\mu g\ g^{-1}$)	DA ($\mu g\ g^{-1}$)
Plastic	Average	0.48	1.25	0.91	0.06	14.10
	stdev	0.02	0.03	0.02	0.00	0.12
Glass	Average	0.50	1.16	0.92	0.06	14.33
	stdev	0.04	0.08	0.04	0.01	0.28

[a]Equivalents of total regulated toxins calculated following application of the toxic equivalence factors.

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sourced as an alternative to the plastic centrifuge tube used previously (Fig 2B). For the glass centrifuge tubes, a lower centrifugal force was applied, with no significant impact on pellet formation. The methods were changed, such that the second extraction step, which previously required the sample to be ultra turraxed for 1 min, was replaced by a vortex step. This reduced the sample extraction time significantly and had no impact on the results (Tables 3 and 4). Once extracted the sample is filtered using a 5 mL syringe. Glass syringes were sourced to replace the previously used plastic versions (Fig 2C). Glass also has an environmental impact, but when used at least >8 times, its environmental impact is significantly reduced compared with plastic [61].

To ensure compliance with our quality system (ISO 17025) a verification of the methods using the glass centrifuges and syringes was performed. For the lipophilic toxins a certified reference material (CRM) and a laboratory reference material (LRM) were extracted using both methods (plastic and glass), with no significant difference ($p>0.05$) observed (Tables 3 and D in S1 Text). LRM control chart data ($n>30$) further showed no significant differences in results post-transition (data not shown).

Biotoxin carryover was further assessed to ensure appropriate cleaning procedures were applied between use. In addition to the samples detailed in Tables 3 and D in S1 Text, a naturally contaminated mussel (*M. edulis*) sample with a high concentration of OA group toxins (~11-times over the regulatory limit), was extracted (Table E in S1 Text) and subsequently tested for carryover. No carryover was detected for any of the samples tested (all rinses were <LOD) neither at verification stage nor since the transition.

For the DA method a LRM and contaminated scallop (*P. maximus*) tissues (adductor muscle and gonad) were extracted (n = 5) using both methods (plastic and glass), with no significant difference ($p>0.05$) observed (Table 4). LRM control chart data ($n>30$) further showed no significant differences in results post-transition (data not shown).

Biotoxin carryover was also assessed for this method to ensure appropriate cleaning procedures were applied between use. Similar to the lipophilic toxin method, no carryover was

Table 4. Comparison of domoic acid (DA) liquid chromatography-diode array detection (LC-DAD) results for laboratory reference material (LRM), *P. maximus* adductor muscle, and *P. maximus* gonad extractions (n = 5) using plastic centrifuge tubes and syringes and replacing with glass alternatives.

		DA (LRM) ($\mu g\ g^{-1}$)	DA (adductor muscle) ($\mu g\ g^{-1}$)	DA (gonad) ($\mu g\ g^{-1}$)
Plastic	Average	36.6	2.5	5.3
	stdev	1.4	0.7	1.4
Glass	Average	36.5	2.3	4.8
	stdev	0.4	0.7	1.5

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Table 5. Weights (kg) of consumables used in 2019 in the testing of samples for lipophilic toxins, domoic acid, and saxitoxins pre- and post-transitioning from plastic to glass and paperboard alternatives.

	Plastic pots Wt.	*Consumables Wt.			Total Wt.
		Lipophilic	Domoic acid	Saxitoxins	
Pre-transition	90.0	87.6	41.5	34.0	253.1
Post-transition	0	31.2	14.0	34.0	79.2
% Reduction	**100**	**64**	**66**	**0**	**69**

*See also Tables F–H in S1 Text.

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detected for any of the samples tested (all rinses were <LOD) neither at verification stage nor since the transition.

For the lipophilic and DA toxin methods a reduction in single-use plastics (used for toxin extraction) of ~64 and 66%, respectively was achieved (Table 5). For all three methods plastic consumption was reduced by 69%, from 253 to 79 kg (Fig 1 and Table 5).

Further potential to reduce plastics exist through use of Grenova's TipNovus benchtop pipette tip washing device, that allows pipette tips to be washed for reuse [62]. More generally, much of the plastic used in laboratories is high grade and has the potential to be washed/decontaminated and reused, or at the very least recycled [63]. In our laboratory we are washing the 200 mL plastic (polypropylene) pots, used to store samples (prior to transitioning to the paperboard pots), for recycling.

Similar efforts to reduce and reuse plastics were made in a microbiology laboratory (team of 7). The laboratory transitioned to sustainable materials, such as reusable wooden sticks for patch plating and metal loops for inoculation. Plastic tubes were reused following chemical decontamination and autoclaving. The adopted strategies resulted in 516 kg of plastic being diverted from incineration each year [25].

Overall, transitioning to paperboard and glass alternatives, recycling/reusing sample boxes (polystyrene and plastic), and composting shellfish waste has led to >95% (from ~4,000 kg to 130 kg) of non-chemical waste generated by our laboratory being diverted from landfill and/or incineration (Table 6).

Reducing hazardous chemical waste

Our monitoring program operates multiple analytical instruments (two LC-MS/MS, two LC-DAD, and one LC-FD). We attempted to adopt green analytical chemistry principles [64]

Table 6. Approximate reduction in non-chemical waste to landfill/incineration.

Waste diverted from landfill/incineration	**Wt. (kg)**
Sample boxes (polystyrene)	*982
Shellfish waste (composted 2019)	2,780
Plastics	173.9
Waste to landfill/incineration	
Toxic shellfish waste	*48
Plastics	79.2
Total waste	**4,063.1**
% Reduction to landfill/incineration	**96.8**

*Approximate weight. Note: data does not include more general laboratory waste e.g., nitrile gloves, tissue, non-recyclable plastic packaging, etc.

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in the laboratory i.e., ordering and preparing only what is required. Additionally, extending expiry dates of solutions and mobile phases from one week to one month led to reduced consumption of solvent by ~23% (~300 L, mostly comprising acetonitrile, methanol, and water) and generation of hazardous chemical waste, with no impact on quality and instrument performance. The reduced use of hazardous solvents and chemicals not only results in environmental protection and significant financial savings but additionally has health and safety benefits by protecting staff from unnecessary exposure [65]. Further measures to reduce hazardous waste could be adopted, for example, by replacing methanol with ethanol (which has ~half the hazard value of methanol [66]) in instrument cleaning solutions and as the extraction solvent (for both the lipophilic and DA methods). This solvent was previously shown to act as an effective alternative to methanol in the extraction of toxins from shellfish [67]. Opportunities also exist in replacing acetonitrile as the organic solvent in mobile phases, and some greener and safer alternatives have been reported in pharmaceutical [65,68] and biomedical applications [69].

Reducing printing

We estimated that 113 reams of paper (A4) are printed annually in our laboratory for the monitoring program, primarily consisting of laboratory worksheets and chromatographic results, that are stored for up to 10 years. Transitioning to digital document control offers multiple benefits: records can be backed up; require no physical space; can be used by multiple team members at the same time from different locations; and financial savings. To date, transitioning from paper to digital document control has led to a reduction in printing of ~81%—from ~113 to 21 reams per year. Additionally, the Marine Institute ISO 17025 quality system (that covers multiple laboratories) has transitioned to full electronic document control using a document control management system (Paradigm 3 compliance management software). Such strategies lead to a significant reduction in paper consumption, use of printing ink, printer maintenance, electricity, and the requirement for storage space [16]. Other ways to reduce paper consumption include: use of recycled paper; using the blank sides of unneeded single-sided copies (scrap paper) for printing drafts or writing notes; printing on both sides; and using FollowMe printers [16].

Reducing energy consumption

In our laboratory we have additionally adopted practices to save energy including turning off equipment (including PCs) when not in use and keeping fume hood sashes down whilst in operation but not in use. Each fume hood is estimated to use up to 3.5 times the energy of an average USA home [19]. Keeping the sashes shut when not in use is not only the safest practice but additionally results in energy savings of up to 75% [70].

A number of fume hoods in our laboratory (Table I in S1 Text) also act as extraction systems for chemical storage units located underneath. Installation of Chemtrap^{TM} filtration systems (Table I in S1 Text) allowed this function to be performed (using significantly less energy), enabling fume hoods to be powered off and switched on only when required. A 40% reduction in energy consumption was achieved through improved fume hood management (Table I in S1 Text).

Fridges, freezers, and freeze driers are not only high consumers of electricity, the refrigerants used for cooling (that are also present in air conditioning systems) release highly potent greenhouse gases (hydrofluorocarbons) [71]. Energy consumption can be reduced by introducing cold storage maintenance schedules whereby cold storage equipment is regularly defrosted to prevent ice build-up and maintain efficiency (in addition to improving equipment lifetimes). Often, when older samples can be disposed of and/or better organised some cold

storage equipment may be taken out of use. ULT (−80˚C) freezers can potentially be operated at higher temperatures. An increase to −70˚C leads to a 28.6% reduction in energy, while an increase to −60˚C leads to a 42% reduction [20]. To date, no evidence exists to show there is any impact on sample integrity when the storage temperature is increased to −70˚C [72]. Further studies have reported strategies to reduce costs and energy consumption of freeze driers regularly used in pharmaceutical, food, and research laboratories [21,73,74].

In our laboratory, 11 freezers were taken out of use (equivalent to powering an average EU household per year) (Table J in S1 Text), leading to a 10% reduction in cold storage energy consumption. Overall, energy consumption (for fume hoods and cold storage equipment) in our laboratory was reduced by 30%. Specific energy savings data relating to powering down equipment after use and closing fume hood sashes in our laboratory were unavailable, however, total electricity consumption for the Marine Institute building reduced by 26% compared with baseline levels (set in 2016).

Cost savings

Whilst some investment was required for some of the equipment and services employed in this study, payback was reached after ~2 years, at which point significant savings were achieved.

The cost to have the polystyrene compacted on-site and removed for recycling is ~€600 per year. This is an ongoing cost justified by environmental protection and is a good example of a circular economy. In 2019, 2.78 tons of shellfish waste were sent for composting. The cost of composting and recycling (€100 per ton) was slightly lower than landfill/incineration disposal (€125 per ton), resulting in small savings (Table 7). The compostable paperboard pots were 2.2-times cheaper than the plastic pots and resulted in a ~€600 saving per year.

The initial outlay for the purchase of the glass centrifuge tubes and syringes was higher relative to the plastic alternatives, however, the continual reuse makes them more economical in the long run and the costs are recouped after ~2.4 years. The transition to the glass alternatives led to adoption of more efficient practices with respect to use of water (using recycled water for soaking glassware) and dishwasher operation (only inserting items that require dishwasher cleaning and operating with a full load). Additional analyst time required for such cleaning

Table 7. Approximate cost savings (€) per year achieved through implementation of more sustainable strategies.

Action	Saving	Cost
Polystyrene recycling		597.3
Composting shellfish	69.5	
Plastic recycling	14.2	
Transition from plastic to compostable pots	598.4	
Transition from plastic to glassware	*1,725.5	
Solvents/chemicals	6,468.6	
Solvents/chemicals disposal	1,146.9	
Printing paper	344.0	
Chemtrap™ filters		940.0
Fume hood power down	#6,055.6	
Freezers energy	720.4	
Freezers calibration	154.0	
Total	**17,297.1**	**1,537.3**
Overall cost savings	**15,759.8**	

*Savings after ~2.4 years.

#Savings after ~1.5 years.

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was offset by time saving efficiencies achieved in eliminating the ultra turrax step and reduced requirement for preparation of mobile phases for analytical instrumentation. Regardless, the cleaning time was not so significant as to impact on overall laboratory operations.

While costs were incurred for the installation of the Chemtrap^{TM} filtration units, reductions in fume hood operations meant that costs were recouped after ~1.5 years. The Chemtrap^{TM} units require an annual filter change resulting in an ongoing cost of €940 per year. The reductions in fume hood operations resulted in a 40% decline in energy consumption (Table I in S1 Text) and a significant (~€6,000 per year) cost saving. Taking 11 freezers out of use resulted in a 10% decline in energy consumption (Table J in S1 Text), and with no requirement for freezer calibrations a cost saving of ~€870 per year was achieved.

Taking an economic approach (green analytical chemistry) to ordering and preparing chemical solutions led to significant savings of ~€7,600 per year due to reduced requirements for solvents and chemicals, and disposal costs. Further transitioning from paper to digital document control has so far led to a reduction in printing of ~81% (from ~113 to 21 reams per year), with a saving of ~€344 per year.

Overall, cost savings achieved in our laboratory through implementation of these strategies are estimated to be ~€15,800 per year (Table 7). However, this is likely to be an underestimate as the calculations do not account for additional cost saving behavioural changes e.g., powering down equipment after use, shutting fume hood sashes, etc.

Summary and conclusions

Reducing resource and energy consumption is critical for environmental protection. Scientific laboratories can contribute significantly to meeting CO_2 emissions [28] and waste reduction [30–32] targets through the implementation of procedural and staff behavioural changes. The challenges and opportunities associated with the introduction of an environmental management system have been described [75].

Adoption of simple, effective, and cost reducing transitions in our laboratory has led to reductions in single-use plastics, waste, and energy, without compromising scientific standards. Although this study applies specifically to monitoring of marine biotoxins in shellfish, the strategies adopted (Table 8) could be implemented in any laboratory. Increasing awareness and altering mindsets, that are prone to habitual action, is crucial. A key component of the success achieved to date has been through staff engagement and behavioural changes. In addition to environmental protection and financial savings, these strategies promote environmental awareness, innovation, and greater staff engagement [76,77].

State regulation and/or support to finance and incentivise such transitions, in addition to funding bodies requesting some form of green certification in order to access funding for research, etc., would advance progress in this area. Further solution focused funding to support projects that develop innovative technologies and solutions to this issue is urgently required e.g., production and use of bio-based plastics and transitions to renewable and/or energy efficient sources.

Efforts will continue to identify and implement further strategies to enhance sustainability in our laboratory, with the aim of achieving My Green Lab certification. The more laboratories that adopt such strategies, the greater the impact will be.

Materials and methods

Study approach

Shellfish destined for human consumption are required by EU regulations to be tested for the presence of marine biotoxins to prevent the placement of toxic shellfish on the market.

Table 8. Overview of solutions and strategies implemented in the biotoxin chemistry laboratory to reduce waste and energy consumption, outcomes, and cost savings.

Sustainability challenge	Source	Solution	Strategy	Outcome (reductions per year)	#Cost savings per year (€)
Waste	Polystyrene	Recycling (e.g., for use in construction and production of fish boxes) [53–55].	Procedural change	*Waste reduced by >95%	2,154
		Replacing with corrugated plastic containers that can be re-used.			
	Shellfish	Composting.			
	Plastics	Replacement of plastic storage containers with compostable pots.			
		Replacement of plastic centrifuge tubes and syringes with glass alternatives.			
	Chemicals	Adoption of green analytical chemistry principles [64] to procurement of chemicals and preparation of solutions.		Hazardous chemical waste reduced by ~23%	7,600
	Paper	Transition to digital document control.		Reams of paper reduced from 113 to 21.	344
Energy	Fume hoods	Use of Chemtrap^{TM} filtration units to enable fume hood power down.		Energy consumption reduced by 40%	5,990
	Cold storage equipment	Introduction of maintenance schedule for defrosting equipment and sample control. Improved organisation enabled 11 freezers to be taken out of use.		Energy consumption reduced by 10%	874
	Fume hoods	Shutting sashes when not in use (reducing energy consumption by ~75%) [70].	Instilling behavioural change	^Energy consumption reduced by 26%	^Data unavailable
	Equipment	Powering down (analytical equipment, PCs, etc.) after use.			
	Dishwasher	Only inserting items that require dishwasher cleaning and operating with a full load.			

#See also Table 7.

*Diversion from landfill/incineration.

^Specific laboratory energy savings data were unavailable, however, total electricity consumption for the Marine Institute building reduced by 26% compared with baseline levels (set in 2016).

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Samples are received in the laboratory, extracted, and analysed for the presence of marine biotoxins using analytical instrumentation (Fig 1). This study aimed to implement more efficient and environmentally-friendly practices (reducing waste and energy consumption), whilst maintaining quality control standards, into the monitoring program. The amount of waste generated and energy consumed by the laboratory was determined. Methods and procedures were reviewed, identifying areas where waste and energy usage could be reduced and subsequent resource reduction strategies verified (where appropriate) and implemented.

Reagents

Solvents (LC-MS/MS grade) were from Labscan (Dublin, Ireland). Distilled water was further purified using a Barnstead nanopure diamond UV (Thermo Scientific, IA, USA) purification system. Formic acid (≥98%), trifluoroacetic acid (99%), and ammonium formate (>98%) were from Sigma–Aldrich (Steinheim, Germany). CRMs were from the National Research Council (Halifax, NS, Canada). LRMs were prepared in-house.

Labware consumables

Plastic (polypropylene) pots (250 mL) were from CJK Packaging Ltd (Derbyshire, UK), Kraft heavy duty pots (8 oz, soup/ice cream containers, Cat.: GM-BB-BL-8-UK and GM-BB-BLL-

90-PAPER-UK) were from Greenman packaging (Dublin, Ireland). FORTUNA® Optima glass syringes (5 mL, Cat.: Z314544) and Whatman cellulose acetate filters (0.2 μm, Cat.: WHA10462700) were from Sigma–Aldrich (Steinheim, Germany). Kimax® glass centrifuge tubes (50 mL, Cat.: Z254878) were from Sigma–Aldrich (St. Louis, USA). Plastilab™ plastic (polypropylene) centrifuge tubes (50 mL, Cat.: ACF450.20X) were from Lennox (Dublin, Ireland). BD emerald™ plastic (polypropylene) syringes (5 mL, Cat.: BDAM307731) and plastic (polypropylene) pipette tips (1 mL, Cat.: 89041–370 and 200 μL, Cat.: 53508–810) were from VWR (Dublin, Ireland). HPLC vials (1.5 mL, Cat.: LAP11090519 and LAP09150869) were from Apex Scientific (Kildare, Ireland).

Extraction for lipophilic toxins

A LRM, CRM, and a naturally contaminated shellfish sample (*M*. *edulis*, harvested from the southwest coast of Ireland in August 2019) were extracted. The shellfish were shucked (≥100 g flesh), homogenized, and transferred into pots. Tissue samples were weighed (2 g) into 50 mL centrifuge tubes and extracted by vortex mixing for 1 min with 9 mL of methanol and centrifuged at 4,415 *g* (5 min) when using the plastic centrifuge tubes and at 3,029 *g* (5 min), when using the glass centrifuge tubes. The supernatants were decanted into 25 mL volumetric flasks. This step was repeated, with the vortexing time extended to 5 min (when using the glass centrifuge tubes), while the samples were ultra turraxed for 1 min when plastic centrifuge tubes were used. The supernatants were decanted into the same 25 mL volumetric flasks, which were brought to volume with methanol. The samples were filtered through Whatman 0.2 μm cellulose acetate filters into HPLC vials and analysed by LC-MS/MS.

Extraction for domoic acid

A LRM and a shellfish sample (*P*. *maximus*, harvested from the southwest coast of Ireland in April 2019) were extracted. The shellfish were shucked with adductor muscle and gonad tissue separated (≥100 g flesh each), homogenized, and transferred into pots. Tissue samples were weighed (2 g) into 50 mL centrifuge tubes and extracted by vortex mixing for 1 min with 9 mL of 50:50 methanol:water and centrifuged at 4,415 *g* (5 min) when using the plastic centrifuge tubes and at 3,029 *g* (5 min), when using the glass centrifuge tubes. The supernatants were decanted into 25 mL volumetric flasks. This step was repeated, with the vortexing time extended to 5 min (when using the glass centrifuge tubes), while the samples were ultra turraxed for 1 min when plastic centrifuge tubes were used. The supernatants were decanted into the same 25 mL volumetric flasks, which were brought to volume with 50:50 methanol:water. The samples were filtered through Whatman 0.2 μm cellulose acetate filters into HPLC vials for analysis by LC-DAD.

Glassware cleaning

The pellets in the glass centrifuge tubes were dislodged by a spray of water and disposed to general waste. The tubes were soaked in water prior to dishwasher (Lancer 815 LX model, acid-based detergent, and deionized water) transfer. The glass syringes were additionally washed in the dishwasher (contained in a Lancer stainless steel grid basket).

To check for carryover, 5 mL of extraction solvent was added to the washed glass centrifuge tube, shaken, and 1 mL passed through a washed syringe into a HPLC vial for analysis.

LC–MS/MS

Analysis was performed using a Waters Acquity UPLC coupled to a Xevo G2-S QToF monitoring in MS^e mode (100–1200 *m*/*z*), using leucine enkephalin as the reference compound.

The cone voltage was 40 V, collision energy was 50 eV, the cone and desolvation gas flows were set at 0 and 600 L h^{-1}, respectively, and the source temperature was 120˚C.

Chromatography was performed with an Acquity UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) column (Waters, Wexford, Ireland). Binary gradient elution was used, with mobile phase A consisting of water and mobile phase B of acetonitrile (95%) in water (both containing 2 mM ammonium formate and 50 mM formic acid). In negative ionisation mode the gradient was from 5–90% B over 2 min at 0.3 mL min^{-1}, held for 1 min, and returned to the initial conditions and held for 1 min to equilibrate the system (total run time 4 min). In positive ionization mode the gradient was from 30–90% B over 5 min at 0.3 mL min^{-1}, held for 0.5 min, and returned to the initial conditions and held for 1 min to equilibrate the system (total run time 6.5 min). The injection volume was 2 μL and the column and sample temperatures were 25˚C and 6˚C, respectively. Quantitation using CRMs was performed using Targetlynx software.

LC-DAD

Analysis was performed using a Shimadzu UPLC coupled to a DAD (190–370 nm, set λ = 242 nm). Chromatography was performed with an Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 μm) column (Waters, Wexford, Ireland). Binary gradient elution was used, with mobile phase A consisting of water (94.9%), acetonitrile (5%), and trifluoroacetic acid (0.1%) and mobile phase B consisting of water (4.9%), acetonitrile (90%), and trifluoroacetic acid (0.1%). Isocratic elution was performed at 7% B over 6 min at 0.4 mL min^{-1}. The column was flushed with 95% B over 4 min, and returned to the initial conditions and held for 3 min to equilibrate the system (total run time 13 min). The injection volume was 2 μL and the column and sample temperatures were 40˚C and 6˚C, respectively.

Statistical analysis

Statistical calculations were carried out using a t-test using Microsoft Excel (2016). The significance threshold (p-value) was set at 0.05 (95% confidence) for all experiments.

Supporting information

S1 Text. Contains supporting Tables A through J.
(DOCX)

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